

**MICROBIOLOGICAL AND MOLECULAR CHARACTERIZATION OF
PLANT DISEASE SUPPRESSIVE COMPOST**

BY

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DEDICATION

This work is dedicated to my better half Namasango Maluleka who has always supported me and shown the love and interest in my work; and our daughter Reitumetse born on 14 January 2012 whom when she sees this one day will be proud of her father.

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PREFACE

The use of composts for the management of plant diseases has increased considerably in recent years due to their contribution to both recycling waste and a reduction in the usage of non-renewable resources such as peat. Composts are reported to provide a cheap and effective management of plant diseases, and improve soil quality. Furthermore, with precautions taken against the use of certain chemicals for the control of plant diseases due to health concerns related to humans, animals and the environment, compost usage has increased significantly. The present study investigated the properties of compost which can be used for the management of plant diseases *i.e.* compost that can promote plant growth and suppress plant diseases while exhibiting no phytotoxic properties and harbouring no plant pathogens. The dissertation consists of four independent manuscripts based on research conducted over a period of two years in the laboratory and greenhouse. Thus some repetition and lack of continuity between chapters was unavoidable.

The first chapter is a literature review which addresses the most important characteristics of disease suppressive compost with the emphasis on plant diseases caused by *Rhizoctonia solani* Kühn. The mechanisms by which compost suppresses plant diseases are discussed. To put the potential use of compost into perspective as an alternative for chemical control measures of plant diseases, factors such as microbial diversity influencing compost suppression to diseases are also discussed.

Chapter two examines the possible phytotoxicity of composts to lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) and the suppressiveness of these composts to damping-off caused by *R. solani* in the greenhouse. Evaluation of the chemical and biological attributes of the compost was facilitated by using various microbiological, biochemical and molecular techniques.

In chapter 3, the study investigated the impact of genetic variation of the pathogen *R. solani* on disease suppressiveness by compost. The study evaluated the response of seven isolates from different anastomosis groups (some unknown) to composts in the greenhouse. The influence of various chemical and biological attributes of the composts was investigated using relevant laboratory technologies.

Most literature on disease suppressive compost is based on soilborne pathogens. However, there is mounting evidence that composts can suppress foliar diseases through the induction of systemic acquired resistance (SAR). In chapter four, various composts were examined in the glasshouse for their inhibitory effect on *Alternaria raphani* (Groves & Skolko) blight of radish seedlings. The levels of pathogenesis-related proteins β -1,3-glucanases and peroxidases, which are an indication of the induction of SAR, were therefore also studied in radish seedlings using suitable biochemical techniques.

CHAPTER 1

PLANT DISEASE SUPPRESSIVE COMPOSTS WITH SPECIFIC REFERENCE TO DISEASES CAUSED BY *RHIZOCTONIA SOLANI*

1.0. INTRODUCTION

With the prohibition of pesticides such as methyl bromide for the control of soilborne plant diseases, attention has shifted to alternative non-chemical control measures for controlling soilborne diseases. The use of composts for suppressing soilborne diseases in particular has increased in recent years due to their contribution to the recycling of waste. They are also reported to provide a cheap and effective means to suppress soilborne diseases when used as soil amendments (Hadar & Mandelbaum, 1992; Abawi & Widmer, 2000; Keener, Dick & Hoitink, 2000; Cotxarrera *et al.*, 2002; Diab, Hu & Benson, 2003; Escuadra & Amemiya, 2008; Pugliese *et al.*, 2008; Joshi *et al.*, 2009).

Various mechanisms are involved in the suppressive effect of compost on plant diseases. Disease suppressiveness results from a series of physicochemical and biological characteristics of compost attributed to compost inhabiting microorganisms (Boulter, Boland & Trevors, 2002). The mechanisms by which suppressive agents control diseases is through *competition* with pathogens for nutrients (Hoitink, Stone & Han, 1996; Tuitert, Szczech & Bollen, 1998; Garbeva, van Veen & van Elsas, 2004; Hoitink & Changa, 2004; Raviv, 2008), the production of *antibiotics* harmful to pathogens (Craft & Nelson, 1996; Hoitink *et al.*, 1996; Raviv, 2008), *parasitism* of suppressive agents on pathogens (Hoitink *et al.*, 1996; Hoitink & Boehm, 1999; Postma, Montanari & van den Boogert, 2003; Trillas *et al.*, 2006; Raviv, 2008; Joshi *et al.*, 2009), and the activation of a plant defence response, *i.e. systemic acquired resistance* (SAR) or *induced systemic resistance* (ISR) in plants (Zhang, Dick & Hoitink, 1996; Nelson &

Boehm, 2002). For example, in take-all disease of wheat, caused by the fungus *Gaeumannomyces graminis* var. *tritici* (Walker), 2,4-diacetylphloroglucinol produced by bacteria (mainly *Pseudomonas* spp.) in compost, proved to be an effective component that suppressed the pathogen (Weller *et al.*, 2002; Mazzola, Funnel & Raaijmakers, 2004). In another study, *Trichoderma* spp. was shown to cluster around hyphae of *Rhizoctonia solani* (Kühn) and suppress sclerotial bodies of the pathogen (Hoitink & Boehm, 1999). Zhang *et al.* (1996) and Zhang *et al.* (1998) reported that composts made from pine bark mix and spruce bark mix, and pine bark fortified with *Trichoderma hamatum* 382 Bainier and *Flavobacterium balustinum* 299 induced SAR to, cucumber *Pythium* root rot, *Colletotrichum* anthracnose of cucumber and *Pseudomonas syringae* bacterial speck of *Arabidopsis*.

The level of disease suppression by composts is typically related to the level of total microbial activity, e.g. bacteria, fungi and actinomycetes present in the compost. Thermophilic bacteria and fungi are reported to dominate in compost during the initial stage, while during the curing stage, mesophilic microorganisms recolonize the compost (Hoitink & Fahy, 1986). Craft & Nelson (1996) found that the level of microbial activity increased with the suppression of *Pythium* disease of creeping bentgrass. The addition of specific antagonists to compost has also been implicated in the enhancement of disease suppressiveness (Postma *et al.*, 2003; Fracchia *et al.*, 2006; Trillas *et al.*, 2006; Joshi *et al.*, 2009). For example, the addition of *Verticillium biguttatum* (Gams) (a mycoparasite of *R. solani*) to compost, increased its disease suppressiveness to pathogens of sugarbeet and potatoes. *Trichoderma asperellum* (Samuels, Lieckf & Nirenberg) added to compost also increased its suppressiveness to *R. solani* damping-off

of cucumber seedlings (Trillas *et al.*, 2006). Similar results were found when a non-pathogenic isolate of *Fusarium oxysporum* (Schlecht) was added to compost and then suppressed *Fusarium* wilt in carnations caused by pathogenic variants of *F. oxysporum* (Postma *et al.*, 2003).

The quality and quantity of compost applied to soil affects the growth of plants and its disease suppressive capability (Hoitink & Fahy, 1986; Gomez, 1998; Hoitink & Boehm, 1999). Compost maturity is the main determinant of compost quality and its disease suppressive capability. Maturation of the compost is defined as the state when: (i) most readily available nutrient sources have been used and (ii) recolonization by beneficial microorganisms has occurred. Maturity relates to the level of phytotoxic substances in compost and its suitability for plant growth (Aslam, Horworth & Van der Gheynst, 2008). Immature composts are said to impact negatively on seed germination, plant growth and development through the introduction of phytotoxic compounds such as heavy metals, certain phenolic compounds, ethylene, ammonia, and excess salts and organic acids (Manios, Tsikalas & Siminis, 1989; Tiquia, Tam & Hodgkiss, 1996). Phytotoxic compounds can also be produced by creating anaerobic and reducing conditions in soil or by means of various salts and toxins (Hoitink, Inbar & Boehm, 1991; Hoitink *et al.*, 1997).

This review will discuss the most important characteristics of compost that influence the suppression of plant diseases. Factors such as the level of microbial diversity in compost that affects plant disease suppression, are discussed. The

mechanisms by which compost suppresses plant disease will also be discussed with special emphasis devoted to the suppression of *R. solani*.

2.0. COMPOSTS AND DISEASE SUPPRESSION

Composts are made from organic waste that has been degraded by thermophilic and mesophilic microorganisms (Hoitink & Fahy, 1986; De Clercq *et al.*, 2003; Noble & Coventry, 2005). The impact of compost on soil characteristics varies according to the nature of the compost and the soil type. For example, mushroom composts alter biological parameters in clay and silty clay soil, while green waste compost does not (Pérez-Piqueres *et al.*, 2006). Composts are used to improve the physical structure of soils (Postma *et al.*, 2003), to boost plant development (Abawi & Widmer, 2000; Cotxarrera *et al.*, 2002; Escuadra & Amemiya, 2008), and to increase soil microflora (Crecchio *et al.*, 2004; Escuadra & Amemiya, 2008). Composts can therefore improve the biological, chemical and physical properties of amended soils (Abbasi *et al.*, 2002).

The use of composts to maintain soil quality and to produce healthy plants dates back to the 1930's. The first record of composted manure being used to control soilborne diseases was for cotton (Hoitink, 2006), where the compost significantly improved cotton yields. This finding led researchers to conclude that there were some beneficial microorganisms in the compost that were antagonistic to, or competed with, plant pathogens. To date, composted manures have been applied to many crops to control both soilborne diseases and foliar diseases across the globe (Hoitink, 2006). Composts have been shown to suppress numerous soilborne diseases (Abawi & Widmer, 2000; Cotxarrera *et al.*, 2002; Bailey & Lazarovits, 2003; Diab *et al.*, 2003;

Postma *et al.*, 2003; Escuadra & Amemiya, 2008; Joshi *et al.*, 2009) including diseases caused by *Pythium* spp., *Phytophthora* spp., *Fusarium* spp. and *R. solani* (Aryantha, Cross & Guest, 2000; Schönfeld *et al.*, 2003; Scheuerell, Sullivan & Mahaffee, 2005; Pérez-Piqueres *et al.*, 2006; Escuadra & Amemiya, 2008; Hagn *et al.*, 2008). Compost can reduce disease incidence by as much as 75% (Raj & Kapoor, 1997; Keener *et al.*, 2000). Most studies on the suppressive effect of composts on soilborne diseases have been documented in container mixes and far less under field conditions (Noble & Coventry, 2005; Scheuerell *et al.*, 2005). Although composts have been shown to suppress several diseases in the field, notably onion white rot (Coventry, Noble & Whipps, 2001), there is still a great lack of sound experimental data on the disease suppressive effects of compost amendments in field experiments. Disease suppressive effects of composts in the field are generally smaller and more variable than results obtained from container or microcosm experiments.

A list of diseases suppressed by compost in container and field experiments is given in Table 1 (as reviewed by Noble & Coventry, 2005; Noble, 2011). Although the level of disease suppression differs significantly between container and field experiments; certain results were similar for both container and field tests. For example, *Phytophthora nicotianae* (Breda de Haan) on citrus plants was found to be suppressed by composts amended to soil in pot experiments, although no suppressive effect occurred in field trials (Widmer, Graham & Mitchell, 1998). Anthracnose caused by *Collectotrichum coccodes* (Wallr) and *Xanthomonas campestris* pv. *vesicatoria* in tomato were suppressed in both field and container experiments (Abbasi *et al.*, 2002).

Table 1: Soilborne pathogens reported to be suppressed by composts in container versus field experiments using soil as medium.

Pathogens	Disease	Plant(s)	Container experiments	Field experiments	References
<i>Aphanomyces euteiches</i> (Drechsler)	root rot	pea	+	+	Lumsden, Lewis & Milner, 1983; Stone <i>et al.</i> , 2003
<i>Colletotrichum coccoides</i> (Wallr)	anthracnose	<i>Solanaceae</i>	+	+	Abbasi <i>et al.</i> , 2002
<i>Fusarium culmorum</i> (Sacc)	foot rot	winter wheat	+/-/#	-	Tilston, Pitt & Groenhof, 2002
<i>Fusarium oxysporum</i> (Schlecht)	wilt	basil, carnation, flax, melon	+	+/-	Lumsden <i>et al.</i> , 1983; Ferrera, Avataneo & Nappi, 1996; Cheuk <i>et al.</i> , 2003
<i>Fusarium solani</i> (Snyder & Hansen)	foot rot	pea	+	+	Lumsden <i>et al.</i> , 1983
<i>Phytophthora</i> spp.	root rot	various, tomato, etc	+/-	+/#	Lumsden <i>et al.</i> , 1983; Kim, Nemeč & Musson, 1997; Widmer <i>et al.</i> , 1998; Aryantha <i>et al.</i> , 2000
<i>Pythium ultimum</i> (Trow)	damping-off	various	+/-	+	Lumsden <i>et al.</i> , 1983; Fuchs, 1995; Dissanayake & Hoy, 1999; Stone <i>et al.</i> , 2003
<i>Rhizoctonia solani</i> (Kühn)	damping-off	general	+/-/#	+/#	Lumsden <i>et al.</i> , 1983; Lewis <i>et al.</i> , 1992; Fuchs, 1995;
<i>Sclerotinia</i> spp.	Watery soft rot	various	#	+	Merriman, 1976; Coventry <i>et al.</i> , 2002
	Southern blight	general	+	+/-	

+ suppressed - not suppressed # promoted disease

It is thought that differences in disease suppression are partly due to compost inclusion rate (v/v compost per soil), the type or characteristics of the soil used, the compost feedstock, and the degree of decomposition of the compost. The suppression of diseases has mostly been correlated with the activity of specific microbial antagonists present in the compost; particularly in the suppression of *Rhizoctonia* induced damping off (Tuitert *et al.*, 1998; Diab *et al.*, 2003; Joshi *et al.*, 2009).

Disease suppression can be general or specific. General suppression results from high microbial population diversity that creates unfavourable conditions for the development of certain pathogens. Specific suppression results from an organism directly suppressing a pathogen (Boulter, Boland & Trevors, 2000). For example, *R. solani* is not easily controlled by suppressive soils since their large propagules make them less reliant on nutrient sources and they are therefore not susceptible to microbial competition (Boulter *et al.*, 2000). Specific beneficial organisms can, however, colonize propagules and reduce the disease potential of *R. solani* (Hoitink & Boehm, 1999). Nutrition for antagonistic or beneficial organisms, is provided by organic matter or waste products released by other soil organisms (Haggag, 2002).

There are instances where composts can increase disease severity to one pathogen but not to another. Krebs (1990) found spruce bark compost enhanced the incidence of *Fusarium* wilt of cyclamen but in other studies, decreased the incidence of *Phytophthora* root rot of poinsettias (Lumsden *et al.*, 1983; Erhart *et al.*, 1999; Hoitink, Krause & Han, 2001a). In another example, highly saline composts enhanced diseases caused by *Pythium* spp. and *Phytophthora* spp., while municipal sewage sludge

compost and ammonium-nitrogen-releasing sludge compost enhanced *Fusarium* wilt due to their low C:N ratio (Hoitink *et al.*, 1996). Noble (2011) also reported that soil amended with compost promoted *Fusarium oxysporum* f.sp. *cepae* (Snyder), cause of onion basal rot, and *Pythium violae* (Chesters & Hickman), cause of carrot cavity spot, in container based experiments (Table 1). Similar results were also observed in root rot of bean caused by *R. solani* where dairy manure compost promoted disease.

2.1. COMPOST ASSAYS

Composts are made of organic substances which are partially mineralised and humified aerobically (Gomez, 1998). They are produced through biological decomposition and stabilization of organic substrates under high temperatures (Fracchia *et al.*, 2006). When fully matured, they require only agronomic analyses to evaluate their nutritional value (Gomez, 1998).

Composts may be evaluated by means of techniques that look at the raw materials used as feedstock, the sizing of materials during composting, the composting process, compost stabilization, maturity, and biological, chemical and physical properties of the product. Some techniques provide an easy and rapid method to predict stability parameters in compost such as carbon fractions, *i.e.* cellulose, lignin, humic substances and polysaccharides, which determine maturity and stability, carbon dioxide accumulation and compost age (Ben-Dor, Inbar & Chen, 1997; Soriano-Disla *et al.*, 2010).

2.2. MECHANISMS OF DISEASE SUPPRESSION BY COMPOST

The application of compost is said to increase the biomass and enzyme activity of resident beneficial microorganisms in soil. Beneficial microorganisms that suppress pathogens are known as antagonists and have been widely reported to do this via specific mechanisms discussed below. The mechanisms of disease suppression by composts depend on microbial composition, the composting process, compost maturity, available nutrients, time of application to soil, and loading rates (Hoitink *et al.*, 1996; Tuitert *et al.*, 1998; Hoitink & Boehm 1999; Smolinska, 2000; Coventry *et al.*, 2001; Quarles, 2001; Postma *et al.*, 2003; Garbeva *et al.*, 2004; Pérez-Piqueres *et al.*, 2006; Trillas *et al.*, 2006; Escudra & Amemiya, 2008; Gómez-Brandón, Lazano & Domínguez, 2008; Joshi *et al.*, 2009).

2.2.1. Competition

The suppression of plant disease by compost is associated with competition between beneficial microorganisms and pathogens for root exudates which are a valuable source of nutrients (Craft & Nelson, 1996; Hoitink & Boehm, 1999; Boulter *et al.*, 2000). If compost media are pre-inoculated with beneficial organisms, or if the organisms are already present in the growing medium (e.g. from compost amended soils or growing media), then pathogens are less likely to colonize the fresh organic matter (Hoitink, 2006). Many pathogens are weak saprophytes that readily lose this competition for nutrients in compost-amended soil, resulting in repression of pathogen spore germination and growth, a phenomenon called microbiostasis (Lockwood, 1990; Hoitink & Changa, 2004). *Trichoderma spp.* parasitize *R. solani* by curling around the

mycelium and preventing the formation of sclerotial bodies (Hoitink & Boehm, 1999). In situations where pathogens attack plant roots, beneficial organisms colonize plant roots before the pathogens do. For example, potato root is colonized by non-pathogenic strains of *Fusarium equiseti* (Corda) which can suppress *Verticillium* wilt (Stone, Scheuerell & Darby, 2004).

2.2.2. Antibiosis

Microbes in compost not only compete with pathogens for available resources but they also produce antibiotics that destroy pathogens (Quarles, 2001; Bailey & Lazarovits, 2003). For example, Take-all of wheat was suppressed by 2,4-diacetylphloroglucinol produced by *Pseudomonas spp.* in compost (Weller *et al.*, 2002; Mazzola *et al.*, 2004). The fungus *Gliocladium virens* (Miller, Giddens & Foster) produces enzymes and the antibiotic, gliotoxin that destroys the cuticle of *P. ultimum* (Howell & Stipanovic, 1983). High concentrations of acetic, propionic, isobutyric, butyric and isovaleric acids have been associated with disease suppressiveness in fresh compost (Cotxarrera *et al.*, 2002; Bailey & Lazarovits, 2003). Studies by McKellar & Nelson (2003) on the role of fatty-acid-metabolizing microbial communities revealed that communities of compost-inhabiting microorganisms that colonize cotton seeds suppressed germinating zoospores of *P. ultimum* by rapidly metabolizing linoleic acid. Populations of fatty-acid-metabolizing bacteria and actinobacteria were higher in suppressive composts and the authors concluded that they mediated suppression of the pathogen.

2.2.3. Parasitism

Organic amendments stimulate the growth of populations of beneficial organisms such as beneficial micro-arthropods, bacteria and fungi. These antagonistic organisms can attack and feed on pathogens reducing disease incidence in this manner. Other beneficial organisms cause hyphal lysis due to the excretion of extracellular hydrolytic enzymes such as chitinase and β -1,3-glucanase (Raviv, 2008). For example, *T. harzianum* and *T. hamatum* are parasitic biocontrol agents colonizing the sclerotia of *R. solani* (Sivasithamparam & Ghisalberti, 1998; Hoitink & Boehm, 1999; Haggag, 2002). In another example, increased abundance of arthropods after application of compost products to apple orchards significantly reduced the growth of *Monilinia fructicola* (Winter) brown rot in apple (Brown & Tworkoski, 2004).

2.2.4. Systemic resistance

Systemic acquired resistance is triggered when plants are exposed to foreign invaders such as virulent, avirulent, non-pathogenic microbes (e.g. growth promoting bacteria), or chemical agents such as salicylic acid, 2,6-dichloro-isonicotinic ester, benzo thiadiazole-7-carbothioic acid S-methyl ester, siderophores and lipopolysaccharides (Hoitink & Boehm, 1999; Vallad *et al.*, 2000; Nelson & Boehm, 2002). Boulter *et al.* (2000) reported that certain microorganisms in compost promote plant growth or induce plants to activate biochemical pathways (e.g. pathogenesis-related proteins such as chitinases, β -1,3-glucanase, and thymidine-like proteins with antifungal properties) leading to resistance. Zhang *et al.* (1996; 1998) found plants

grown in composts to have higher levels of β -1,3-glucanase and peroxidase enzymes than those growing in peat.

Another form of resistance induced by composts in plants is induced systemic resistance (ISR). ISR does not involve the accumulation of pathogenesis-related proteins and salicylic acid as seen with SAR. It relies on pathways regulated by jasmonate and ethylene (Van Loon, Bakker, & Pieterse, 1999). Unlike SAR, which is effective against a wide range of plant species, ISR is more specific in that rhizosphere microorganisms elicit ISR on certain plant species or genotypes only (Vallad & Goodman, 2004). For example, the suppression of *Fusarium* crown and root rot of tomato with composted paper mill sludge is said to be enhanced by ISR. ISR is induced by the biocontrol agent *Pythium oligandrum* (Dreschler) or *Trichoderma asperellum* (Lieckf) present in the roots (Cotxarrera *et al.*, 2002; Pharand, Carisse & Benhamou, 2002).

2.3. FACTORS AFFECTING COMPOST DISEASE SUPPRESSIVENESS AND THEIR RELATIONSHIP TO MICROBIAL DIVERSITY

2.3.1. Maturation effect

Mature compost is attained when most readily available nutrient sources have been used and beneficial microorganisms are present (Hoitink & Fahy, 1986). Maturity is also a term used to indicate the absence of phytotoxic (plant growth suppressive) substances in composts (Aslam *et al.*, 2008). When compost reaches a stage where most of the available nutrients have been utilized and most microorganisms are dormant, it is known as the humus stage (Quarles, 2001). Very stable, or fully

decomposed composts, do not support the growth of beneficial microorganisms that suppress pathogens (Hoitink, 2006). The maturity of compost influences the solubility of heavy metals, organic carbon and nitrogen. Since there is a relationship between solubility, transportation of trace elements by organic soluble carbon and bioavailability, maturation is an important factor in the uptake of heavy metals by plants (Tittarelli *et al.*, 2007). Maturity of compost also influences its microbial composition, and thereby the presence of soilborne pathogens and the survival of antagonists (Hoitink, 1996; Postma *et al.*, 2003; Trillas *et al.*, 2006).

Immature composts have a negative impact on seed germination, plant growth and development. The high microbial activity in immature compost reduces oxygen and blocks available nitrogen which can give rise to a serious N-deficiency (Tiquia *et al.*, 1996). Phytotoxic compounds such as heavy metals, phenolic compounds, ethylene, ammonia, excess salts and organic acids can also be present (Manios *et al.*, 1989; Tiquia *et al.*, 1996). Volatile organic acids (VOA) are also responsible for phytotoxicity in plants (Manios *et al.*, 1989). VOA's are metabolic by-products of anaerobic respiration and breakdown products of grease and fats in raw waste (Henefeld-Fourrier & Rebhum, 1980). Phytotoxic compounds can also be produced by creating anaerobic conditions in soil (Hoitink *et al.*, 1991; Hoitink, Stone & Han, 1997). These compounds lower the metabolic rate of the plant, thus reducing root respiration, and nutrient absorption while also slowing gibberellin and cytokinin synthesis and transport (Jiménez & Garcia, 1988).

Immature composts do not support biological control due to an abundance of readily available food for both beneficial microorganisms and pathogens. This is due to high glucose concentrations that repress the synthesis of lytic enzymes by beneficial organisms such as *Trichoderma spp.* (Hoitink, Krause & Stone, 2001b). Immature composts can thus increase disease incidence and severity. For example, *R. solani* and *Armillaria mellea* (Vahl) can grow on composted fresh straw and wood but fail to do so in composts fully colonized by microorganisms (Hoitink *et al.*, 2001b). Immature composts increase water retention in the soil and immobilize nitrogen if organic matter is not decayed adequately (Hoitink, 2006).

The importance of measuring compost maturity cannot be emphasized enough. However, there is no universal method for measuring compost maturity (Table 2). Most methods are usually based on assays using compost extracts or bioassays such as the germination index which is widely used to determine phytotoxicity. It can detect high levels of phytotoxicity, which reduces germination %, and also levels of organic toxic compounds, which will affect root growth (Helfrich *et al.*, 1998). Phytotoxicity of compost-amended soil is related to carbon mineralization associated with compost decomposition (Aslam *et al.*, 2008). Germination index reduces the time needed to evaluate phytotoxicity and maturity of composts compared to other tests which are lengthier (Helfrich *et al.*, 1998; Aslam *et al.*, 2008). Measurements of mineralizable carbon and the mineralization rate of composts in soil, and electrical conductivity are also used as indicators of compost maturity (Aslam *et al.*, 2008). Immature composts should be applied in the field at least weeks before planting to allow for compost stabilization (Hoitink *et al.*, 1997). However, other factors may still intervene with

Table 2: Different maturity tests conducted for composts

Maturity tests	Characteristics	References
1. Physical parameters	Evaluates colour, odour and structure of materials.	Sugahara, Harada & Inoko, 1979; Jiménez & Garcia, 1988
2. Microbial activity	Measures metabolic activity, biomass count, respiration, ATP, hydrolytic enzyme activity, the relationship between total carbon and soluble glucides, the ratio of carbon in reducing sugars to total carbon, and hydrolysable polysaccharide content.	Lossin 1971; Inoko <i>et al.</i> , 1979; Jiménez & Garcia, 1988
3. Humified organic matter	Paper chromatography and photolorimetric methods to determine humus richness and degree of polymerization.	Hertelendy 1974; Inoko <i>et al.</i> , 1979; Jiménez & Garcia, 1988
4. Chemical bonds	Determines C/N ratio in solid phase and water extracts, including pH, cation-exchange capacity and ammonia tests.	Jann, Howard & Salle, 1959; Spohn 1978; Harada & Inoko 1980a; Harada & Inoko, 1980b; Morel <i>et al.</i> , 1985
5. Phytotoxicity	Determines germination index of seeds incubated in compost.	Zucconi <i>et al.</i> , 1981a; Zucconi <i>et al.</i> , 1981b; Helfrich <i>et al.</i> , 1998; Aslam <i>et al.</i> , 2008

disease suppression; for example, high saline compost enhances disease caused by *Pythium* and *Phytophthora* spp., unless the compost is applied months ahead of planting to allow for leaching of salts by rain or irrigation (Hoitink *et al.*, 1997; Haggag, 2002).

2.3.2. The timing of application

Compost timing of application is another important factor in determining its disease suppressive ability. Compost applied in the field for longer period results in biodegradable substances that can be easily utilized by beneficial microorganisms (Tuitert *et al.*, 1998; Trillas *et al.*, 2006). Tuitert *et al.* (1998) demonstrated that compost applied to soil for a duration of 5-7 months suppressed *R. solani* growth in potting mixtures compared to compost applied for 1 month only, which instead stimulated pathogen growth. This finding is consistent with Trillas *et al.* (2006) who showed that compost application to soil 18-36 months before infection with *Rhizoctonia* spp. suppressed the pathogen far better than that applied 6-12 months prior to infection. The reason was that *T. asperellum* (T 34) established itself better in composts applied 18-36 months in the field before infection. Kuter, Hoitink & Chen (1988) revealed that composts cured for 4 months or more should be stored for 3-4 weeks before utilization to allow the disease suppressive effect to develop sufficiently and also to reduce problems associated with phytotoxicity.

2.3.3. Compost inclusion rates

Compost inclusion rates (v/v compost per soil) can also affect its disease suppressive ability. Inclusion rates of at least 20% (v/v) are normally required to consistently obtain a disease suppressive effect, particularly in peat-based media. Compost amended with soil at 25% v/v rate fully suppressed *R. solani* (Kuter *et al.*, 1988). Disease suppression increases with inclusion rates up to 50% v/v (Noble & Coventry, 2005). In other reports, compost at an inclusion rate of 10% v/v controlled *Rhizoctonia* root rot of bean, cotton and radish (Hoitink & Fahy, 1986). Low inclusion rates are said to be less likely to cause negative plant growth effects associated with high pH, electrical conductivity and phytotoxic compounds (Sullivan & Miller, 2001).

2.3.4. The decomposition process

The chemical makeup of organic matter and existing populations of microorganisms in compost are the two most important aspects of the decomposition process which affects compost particle size, maturity, nutrient content, salinity, aeration and microbial composition (Boulter *et al.*, 2000). Factors such as aeration, moisture, C:N ratio, pH, available nutrients and the physical state of the material also affect the decomposition process (Boulter *et al.*, 2000).

The degree of decomposition of compost is reported to affect its suppressiveness to *R. solani* (Tuitert *et al.*, 1998; Diab *et al.*, 2003). This is because decomposition of organic matter affects the composition of bacterial communities as well as the populations and activities of microorganisms, which are key elements in disease

suppression (Hoitink & Grebus, 1994; Hoitink *et al.*, 1996). Fully decomposed composts increase the levels of suppression of *R. solani* (Nelson, Kuter & Hoitink, 1983). This is because most fresh undecomposed composts release glucose and other sugars that support the growth of *R. solani* but do not support the growth of *Trichoderma spp.*, which is antagonistic to the pathogen (Chung, Hoitink & Lipps, 1988; Tuitert *et al.*, 1998). As organic matter decomposes further, the composition of the active microflora changes, the microbial carrying capacity of the compost declines and disease suppression is lost (De Brito, Gagne & Antoun, 1995; You & Sivasithamparam, 1995; Boehm *et al.*, 1997). Excessively stabilized organic matter (i.e. humus) does not support adequate levels of activity of biological agents, resulting in a lack of disease suppression.

2.3.5. The compost feedstock

Composts are traditionally made from wastes originating from food, animal feed, animal manure, biosolids and agricultural resources. Waste consists mostly of decomposable compounds and nutrients which release fulvic acids which act as chelating agents and bind micronutrients and organic compounds known to help in disease suppression (Hoitink *et al.*, 2001b). Composts prepared from different feedstock are therefore said to vary in their level of disease suppressiveness. Not only do composts prepared from different feedstocks vary in disease suppressiveness, but those prepared from different batches of the same feedstock are also variable. The reason for this variability is not well understood (Nelson & Craft, 1992; Craft & Nelson, 1996).

Composts prepared from cotton gin trash and sugar mill filter press cake were shown to reduce disease caused by *Pythium*, *Phytophthora*, and *Rhizoctonia* spp., and also stimulated plant growth in the process (Dissanayake & Hoy, 1999). Nelson & Craft (1992) reported that batches of sludge compost and compost made from biosolids suppressed damping-off and root rot of creeping bentgrass caused by *P. graminicola*. However, they also found that composts prepared from leaf, yard waste, food, spent mushroom, certain biosolids, cow manure, chicken-cow manure, and leaf-chicken manure were ineffective in suppressing *Pythium* damping-off. Wood and bark composts generally support higher populations of fungi compared to composts containing manure, biosolids and vegetable wastes which have greater populations of bacteria, which play an important role in disease suppression (Hoitink & Boehm, 1999; Ingham, 2005).

2.3.6. Compost stability

Stability in compost is defined as the degree to which organic fractions have been stabilized during the decomposition process (Kalamdhad, Pasha & Kazmi, 2008). Stable compost supports the growth of beneficial microorganisms that are capable of suppressing plant pathogens. It contains recalcitrant or humus like matter that cannot sustain microbial activity. Highly biodegradable substances which sustain microbial activity also lead to compost being unstable. Unstable compost can display phytotoxic behaviour (Kalamdhad *et al.*, 2008). Excessively stabilized compost however does not support high levels of activity of biocontrol agents, and therefore is unlikely to suppress disease organisms (Hoitink, 1996). It is therefore important to maintain a good balance between excessive levels of stability or instability.

2.3.7. Microbial diversity

The genetic and metabolic structure of microbial communities are often closely associated with disease suppression in composts (Chen, Hoitink & Madden, 1988a; Tuitert *et al.*, 1998; Cotxarrera *et al.*, 2002; Diab *et al.*, 2002; Noble & Coventry, 2005; Termorshuizen *et al.*, 2006). An increase in active microbial biomass in composts results in the availability of nutrients for beneficial microorganisms, thus lowering their availability for pathogens (Keener *et al.*, 2000; Bailey & Lazarovits, 2003; Escuadra & Amemiya, 2008; Joshi *et al.*, 2009). Competition for nutrients and the production of antibiotics by antagonistic microorganisms are high and the environment for pathogens is thus unfavourable.

The genotypes of microorganisms present in compost play an important role in disease suppression. Bacteria, fungi and actinomycetes are amongst the more common microorganisms associated with disease suppression in compost (van Bruggen, 1995; Boulter *et al.*, 2000). In the suppression of *Phytophthora* and *Pythium* diseases, bacteria were shown to be present throughout the process, while fungi were present 7-10 days after the onset of composting and actinomycetes in the final stages of composting (Boulter *et al.*, 2000). Therefore, the general microbial activity of compost determines its level of general disease suppressiveness and consequently its quality (Veeken *et al.*, 2005).

Pathogens such as *R. solani*, *Fusarium* wilt pathogens (e.g. *Fusarium oxysporum*) and *Sclerotium rolfsii* (Curzi) require specialized biocontrol agents (Boulter *et al.*, 2000). For example, *Rhizoctonia* is not inhibited by general competition and

suppression, but instead requires the presence of specific microbial antagonists in the compost such as *Trichoderma spp.*, *Bacillus spp.*, *Enterobacter spp.*, *Flavobacterium spp.*, *Pseudomonas spp.*, *Streptomyces spp.*, and *Xanthomonas spp.*, among others (Boulter *et al.*, 2000; Quarles, 2001; Joshi *et al.*, 2009). Diab *et al.* (2003) showed suppressiveness to *Rhizoctonia* damping-off in Impatiens to be associated with enhanced microbial activity, and the high functional and population diversity of a stable compost-amended mix. Thus, biomass of carbon and nitrogen, functional diversity, and population diversity are associated with disease suppression. The suppression of *R. solani* in long-matured compost has been shown to be associated with high population densities of cellulolytic and oligotrophic actinomycetes (Tuitert *et al.*, 1998). A combination of the above antagonists proved to be more effective than using single inoculants by themselves (Krause, Madden & Hoitink, 2001). For example, a combination of *F. balustinum* (strain 299) and *T. hamatum* (isolate 382) controlled *Rhizoctonia* damping-off in radishes (Kwok *et al.*, 1987). These antagonistic microorganisms eradicated the propagules of *R. solani* and thus reduced disease incidence. However, some compost possesses high levels of glucose which stimulate the growth of *R. solani* and also suppresses microorganisms antagonistic to this pathogen (Nelson & Hoitink, 1982).

Heating or sterilization of compost causes loss of disease suppressiveness, but the effect is restored by mixing heated compost with unsterilized compost (Zhang *et al.*, 1998). This suggests that disease suppression by compost is microbiological, and that the physiochemical and biological properties of compost influence its suppressive capacity (Zhang *et al.*, 1998; Boulter *et al.*, 2000). For example, Zhang *et al.* (1998)

reported that the suppression by compost of anthracnose caused by *Colletotrichum orbiculare* (Berk. & Mont.) in cucumber and bacterial speck caused by *Pseudomonas syringae* pv. *maculicola* KD4326 in Arabidopsis, was microbiologically based.

The addition of certain microorganisms to compost can enhance disease suppressiveness, although specific examples are limited (Keener *et al.*, 2000; Postma *et al.*, 2003; Fracchia *et al.*, 2006). Studies by Postma *et al.* (2003) indicated that the addition of *Verticillium biguttatum* (Gams) and a non-pathogenic isolate of *Fusarium oxysporum* (Schlecht) to the compost, enhanced suppression of *R. solani* stem rot of sugar beet and black scurf of potato. Trillas *et al.* (2006) inoculated *T. asperellum* (strain T-34) into compost, and found that the incidence of *R. solani* damping-off in cucumber was reduced. *Pseudomonas* spp. has also been reported to be antagonistic in compost to certain diseases (Stone *et al.*, 2004; Joshi *et al.*, 2009).

2.3.8. Other factors

Moisture content, nutrient or salt content, temperature, water-holding capacity and bulk density can affect suppressiveness of compost to disease (Chen *et al.*, 1988b; Hoitink *et al.*, 2001b; Hoitink, 2006). Moisture content is critical for bacterial mesophiles to colonise the substrate after peak heating. Dry composts (less than 34% moisture, w/w) become colonised by fungi which can be conducive to disease incidence. Moisture content must be 40-50% w/w for bacteria to colonise compost after peak heating, and thereby induce biological control of pathogens (Hoitink *et al.*, 1996).

High concentrations of salts in compost may cause plants to become stressed and thus more susceptible to pathogens such as *Pythium* and *Phytophthora spp.* Lack of nutrients in compost can also make plants more susceptible to diseases. Compost consists mainly of N or mineral forms of N as the primary nutrient. Lack of N may favour the development of diseases such as *Fusarium* wilt, as seen in immature composts due to high ammonium and low nitrate nutrition. Too much N in composts may on the other hand favour diseases such as bacterial leaf spots, fire blight, *Pythium* and *Phytophthora* diseases (Hoitink, 2006).

Temperatures attained during the decomposition process determine which microorganisms will survive and can thus indirectly determine disease suppressiveness (Hoitink, 2006). Growth media amended with low-temperature composts support microorganisms which suppress *R. solani* compared to those prepared from high-temperature composts which do not support suppression of the pathogen. At low temperatures, microorganisms antagonistic to *R. solani* are present in high numbers but slowly diminish as temperatures increase. Decrease in suppression capability however only appears four weeks after an increase in temperature due to the thermophilic bacterium *Flavobacterium balustinum*, an antagonist species of *R. solani*, which colonizes the compost (Tunlid *et al.*, 1989).

The genotype of certain pathogens can determine compost's suppressiveness to disease. Pathogens respond differently to composts, for example, Termorshuizen *et al.* (2006) tested 18 composts against 7 pathogens: *Phytophthora nicotinae* (Breda de Haan) on tomato, *P. cinnamomi* on lupin, *Cylindrocladium spathiphylli* (Uchida) on

Spathiphyllum spp., *Verticillium dahlia* (Kleb) on egg-plant, *F. oxysporum* f. sp. *lini* on flax, and *R. solani* on *Brassica oleracea* and *Pinus sylvestris*. The outcome was that each pathogen varied in terms of disease incidence, a phenomenon attributed to competition-sensitivity of pathogens such as *F. oxysporum* and *R. solani*. There is also the possibility that the specificity of genotypes of *R. solani* is variable.

3.0. CONCLUSIONS

Compost quality is reflected in its ability to promote plant growth, exhibit no phytotoxic properties, harbour no pathogens and suppress plant diseases. Compost maturity and stability are the main determinants of compost quality as determined by its physiochemical and biological properties (Gómez-Brandón *et al.*, 2008). Maturity and stability determine the safety and effectiveness of compost as a soil amendment for boosting nutrient content (Boulter *et al.*, 2000), as well as its disease suppressive ability. A clear definition of maturity and stability is important for a proper evaluation of compost quality. In general, highly stabilized composts with low organic matter content have lower disease suppressive capacity.

There is no single method which can predict compost maturity and stability. Any method should take into consideration the microbial community structure and dynamics. Studying the interactions between biotic and abiotic factors can help to understand the functioning of the compost ecosystem and thereby provide a way for improving the efficiency and quality of the composting process. Determining the quality of compost should therefore be focused on quantitative and qualitative aspects that pertain to microbial diversity.

Disease suppressiveness of compost is due to complexes of microbial populations, which invade the compost pile during the curing stage (Craft & Nelson, 1996; Raviv, 2008). One cannot, however, guarantee disease suppression under high levels of pathogen infestation. This is because different pathogens react differently to compost amendments and the genotypes of pathogens also differ. Selectivity of particular compost's disease suppressive ability is also due to differences in substrate and microbial composition.

Most reports on compost disease suppressiveness are based on a single pathogen, but in reality plants are exposed to multiple pathogens. Most composts tested to date protect plants against *Phytophthora* root rot, *Pythium* damping-off, *Fusarium* wilts and several diseases of turf grass but only a few can protect plants against *R. solani* (Lumsden *et al.*, 1981; Nelson *et al.*, 1983; Ben-Yephet & Nelson., 1999; Boulter *et al.*, 2002; Cotxarrera *et al.*, 2002; Diab *et al.*, 2003; Krause *et al.*, 2003; Hoitink & Changa, 2004). *R. solani* requires specific antagonistic microorganisms such as *Trichoderma spp.* to be present in compost. It can thus be concluded that there are general mechanisms of suppression of pathogens such as *P. ultimum* and more specific ones for *R. solani* (Hoitink & Boehm, 1999; Postma *et al.*, 2003; Trillas *et al.*, 2006; Joshi *et al.*, 2009).

The addition of effective antagonistic microorganisms to compost that inhibit growth of a broader range of pathogens will enhance its potential for disease suppression. In accordance with the full definition of compost quality, good compost must, in addition to suppressing diseases, also enhance plant growth and exhibit no

phytotoxic compounds or pathogens. Compost quality is therefore linked to its organic matter content and the presence of beneficial microorganisms. A multifaceted approach should therefore be directed to compost microbial dynamics, maturity and stability, and its potential for being disease suppressive, and this represents a very interesting topic for further research.

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CHAPTER 2

CHARACTERIZATION OF THE PHYTOTOXICITY AND DISEASE SUPPRESSIVENESS OF COMMERCIAL COMPOSTS

ABSTRACT

Composts of different composition, age and maturity and a growth medium consisting of finely ground soil from disused termitaria were evaluated for their phytotoxic impact on germination and biomass of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) seedlings respectively. The suppressive ability of composts on two isolates of *Rhizoctonia solani* (Kühn) was further evaluated in the glasshouse. Six batches of composts suppressed at least one isolate of *R. solani* and one batch was consistent in suppressing both isolates. Of the six disease suppressive composts, two batches did not significantly reduce germination and wet biomass of lettuce and radish plants while four batches were phytotoxic to one or both plants. On the other hand, of nine batches of composts that failed to suppress the isolates, four were consistently phytotoxic to the plants. Microbial diversity as measured by FDA, active carbon, bioluminescence, DGGE, PLFA and ergosterol content to predict phytotoxicity or disease suppressive ability of composts, was not consistent. However, termitaria soil, which exhibited the lowest microbial diversity compared to composts, was phytotoxic to radish plants and failed to suppress both isolates. The lack of consistent correspondence between phytotoxicity and disease suppressiveness in composts lead to the conclusion that the evaluation of various inherent, and highly variable, quantitative and qualitative aspects of composts pertaining to microbial diversity is an important criterion in predicting compost phytotoxicity and its disease suppressive ability.

INTRODUCTION

The use of alternative non-chemical control measures for controlling soilborne diseases has increased in recent years due to prohibition of soil sterilants such as methyl-bromide (Termoshuizen *et al.*, 2006; Pugliese *et al.*, 2008). Compost, in particular, is reported to provide a cheap and effective means of suppressing soilborne plant diseases when used as a soil amendment (Hadar, Mandelbaum & Gorodecki, 1992; Abawi & Widmer, 2000; Keener, Dick & Hoitink, 2000; Cotxarrera *et al.*, 2002; Diab, Hu & Benson, 2003; Escuadra & Amemiya, 2008; Pugliese *et al.*, 2008; Joshi *et al.*, 2009). Diseases such as damping-off and root rots, wilts, and several diseases of turf grass such as *Fusarium* patch can be significantly reduced by compost (Lumsden *et al.*, 1981; Nelson, Kuter, & Hoitink, 1983; Ben-Yephet & Nelson., 1999; Boulter, Boland & Trevors, 2000; Krause, Madden & Hoitink, 2001; Cotxarrera *et al.*, 2002; Diab *et al.*, 2003; Krause *et al.*, 2003; Noble & Coventry, 2005). Foliar diseases are not typically controlled by composts but there is evidence that they can also be suppressed by compost amendments (Tränkner, 1992; Abbassi *et al.*, 2002; Vallad, Cooperband & Goodman 2003; Ntougias *et al.*, 2008).

Disease suppressive compost provides an environment in which disease development is reduced despite pathogens being present (Hadar *et al.*, 1992; Mazzola, 2004). Suppression of disease can be specific where one organism (genus or species) directly suppresses a known pathogen. For example, suppression of *Rhizoctonia solani* (Kühn) by compost is said to be the result of *Trichoderma* spp. present in the compost which are antagonistic to the pathogen (Haggag, 2002; Diab *et al.*, 2003). General

suppression on the other hand results from a high diversity of microbes that create conditions unfavourable for disease development; for example, the suppression of *Phytophthora* spp. on tomato by composts derived from agro-industrial wastes (Ntougias *et al.*, 2008). Suppression of root-infecting pathogens has been reported to be typically related to the level of total microbial activity present in compost (Hoitink, Stone & Han, 1997; Abbasi *et al.*, 2002; Bailey & Lazarovits, 2003). Craft & Nelson (1996) found that as the level of microbial activity increased the level of damping-off and root rot of creeping bentgrass caused by *Pythium graminicola* (Subraman) decreased. Cellulolytic and hemicellulolytic actinomycetes present in high population numbers in soils amended with composts, have also been associated with disease suppression (van Bruggen, 1995).

There are several techniques used to measure the diversity of microorganisms in soil or compost. These techniques provide a means for understanding the impact of microbial processes (Hill *et al.*, 2000; Kirk *et al.*, 2004). They provide a perspective of microbial community structure under certain climatic, biotic and xenobiotic conditions. Disease suppressiveness can be correlated with high microbial activity in compost as measured by the hydrolysis of fluorescein diacetate (FDA) (Keener *et al.*, 2000; Escuadra & Amemiya, 2008), or changes in microbial community structure as evaluated using denaturing gradient gel electrophoresis (DGGE) and phospholipid fatty acid analysis (PLFA) (Tunlid *et al.*, 1989; Noble, 2011).

The quality and quantity of the organic matter in a batch of compost directly affects its chemical and biological properties which can, directly or indirectly, have a

drastic influence on the health and growth of associated crops (Hoitink & Fahy, 1986; Gomez, 1998; Hoitink & Boehm, 1999). Organic matter can increase the diversity and activity of resident microbial communities in compost and thereby, provide a climate more favourable to the natural biological control of soilborne plant pathogens (Abbasi *et al.*, 2002). Compost can however, contain compounds such as heavy metals, phenolic compounds, ethylene and ammonia, or excess salts and organic acids which can affect beneficial microorganisms, thereby reducing the disease suppressive ability of the compost and rendering it phytotoxic (Manios, Tsikalas & Simins, 1989; Bailey & Lazarovits, 2003; Albercht *et al.*, 2008; Joshi *et al.*, 2009).

Phytotoxic compost is defined as compost which contains substances that inhibit plant growth (Aslam & VanderGheynst, 2008). Phytotoxic compounds are usually produced by creating anaerobic conditions or by the presence of pathogens (Hoitink, Inbar & Boehm, 1991; Hoitink *et al.*, 1997). Compost stored in large compacted piles can sometimes create products with a pH of 2.5 which is also detrimental to plants (Hoitink & Fahy, 1986). Phytotoxic compounds rarely support the growth of antagonistic microbes and thus negatively affect the suppressive capabilities of the compost (Erhart *et al.*, 1999).

The objectives of the present study were firstly, to examine various commercial and homemade composts for possible phytotoxic effects on lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) plants, and secondly for their ability to suppress damping-off caused by *R. solani*. Various microbiological and molecular methods were

used to elucidate the inherent quantitative and qualitative microbial activities of the respective composts.

MATERIALS AND METHODS

Composts (homemade and commercial brands) (Table 1.) were obtained from various commercial nurseries and growers. A growth medium consisting of finely ground soil obtained from disused termitaria (termite mounds) was also included due to the reportedly high microbial activity in this substrate (Dauber & Wolters, 1999). The composts and their description are listed in Table 1. Composts were first tested for their phytotoxicity on radish and cucumber seedlings. Disease suppressiveness was tested in the greenhouse on radish seedlings using *R. solani* as a pathogen. Various techniques were then employed to examine possible relationships between phytotoxicity, disease suppressiveness and the chemical and biological attributes of the composts.

Phytotoxicity assay

Compost biological index: A test was performed according to the method of Fuchs & Bieri (2000) to determine the possible phytotoxic effect of compost on lettuce and radish seeds. The 15 composts and termitaria soil were each placed in four small plastic containers (5 x 5 x 3 cm), and 25 lettuce and radish seeds were randomly distributed into each respective container. Vermiculite served as the control treatment. The growth media were initially watered till moist and containers were incubated in a growth chamber at 22 °C for 10 days with a 12/12h light/dark cycle. After 16 days, germinated seedlings were counted in each container after which they were carefully removed,

washed in clean water, blotted dry and weighed to record their wet biomass. Analyses of variance (ANOVA) using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) were then performed on the data and means were separated using Fischer's least significant difference ($LSD_{0.05}$) test.

Pathogenicity bioassay

Inoculum preparation: Two isolates of *R. solani* (CCP 160 and CCP 159) obtained from unknown hosts, and deposited in the culture collection of the Department of Plant Pathology, University of the Free State; were grown on potato dextrose agar (PDA) containing 0.5mg/l of streptomycin and incubated for 7 days at 24 °C. Mycelial disks (5mm Ø) were taken from the colony margin of a 7-day-old culture and used to prepare inoculum as follows: Unrolled oats (100 g) was soaked in 200 ml distilled water in 1l Ehrlenmeyer flasks for 20 hours. Excess water was removed and the oats seed was autoclaved on two consecutive days for 1 hour at 121 °C, and then allowed to cool down to room temperature. Agar discs containing mycelium from each of the *R. solani* isolates were added to respective flasks and incubated at 25 °C for 2 weeks with intermittent shaking. The colonized seed was then removed from the flasks and air dried in a sterile environment at 30 °C for 72 hours. Dried inoculum was then ground into a fine powder using a coffee grinder and stored in sterile containers at room temperature.

Pathogen challenge: Compost mediated suppression of damping-off caused by the two isolates of *R. solani*, (isolate CPP160 and CPP159) was determined using radish seedlings in greenhouse. The trials were conducted on 15 composts and termitaria soil

(Table 1) according to the method of Krause *et al.* (2001) using inoculum prepared as described above. Pure compost and termitaria soil were amended with steam sterilized loam soil respectively in a ratio of 2:3 (40%: 60% compost: soil), shaken to mix thoroughly, and then placed in 500ml plastic pots. The mixture was allowed to stabilize for 7 days, and watered daily. Inoculum powder (25 g) was added to the mixed growth media and allowed to stabilize for a further 2 days. A positive control treatment, consisting of steam sterilized loam soil only, was similarly infested with inoculum of each pathogen isolate while a negative control treatment of steam sterilized soil was not infested. Five radish seeds were planted per pot. A randomized complete block design was used for the experiment with each treatment replicated four times and the experiment repeated. Pots were watered daily till moist and incubated for 3 weeks at 22-24 °C with a 12h/12h light/dark cycle. The numbers of germinated seedlings in each pot were counted after three weeks and the wet biomass of each seedling was determined. The combined data were subjected to analyses of variance (ANOVA) using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) and means were separated using Fischer's least significant difference ($LSD_{0.05}$) test.

Compost evaluation

Spectroscopy: The test was used to determine stability parameters in samples such as carbon fractions *i.e.* cellulose, lignin, humic substances and polysaccharides. The procedure was adapted from Albrecht *et al.* (2008) and Soriano-Disla *et al.* (2010). Twenty grams of each compost sample was placed in small petri dishes and oven dried

at 60 °C for 72h and ground with a coffee grinder to pass through a 2 mm diameter sieve. Each sample (5 g) was scanned in reflectance mode from 12,000 to 3800 cm^{-1} (equivalent to wavelength 830 to 2630 nm) using a Near Infrared (NIR) spectrophotometer (Bruker Inc.). Each sample was scanned in duplicate and shaken between scans to avoid settling of solid particles and the resulting spectra for each sample were averaged. The same ground samples (5 g) of each compost sample was scanned in the reflectance mode from 7,500 to 300 cm^{-1} using a Mid Infrared (MIR) spectrophotometer (Bruker Inc. Alpha Diffuse Reflection). Each sample was scanned 3x and shaken between scans to avoid the settling of solid particles and the resulting spectra for each sample were averaged.

Fluorescein diacetate analysis (FDA): General levels of microbial activity in each of the composts/growth media were determined by measuring the rate of FDA hydrolysis according to the procedure adapted from Green, Stott & Diack (2006). Two solutions were prepared, namely, 1) a substrate solution containing 0.1 g of FDA dissolved in 100 ml of acetone and stored at -20 °C, and 2) a buffer solution containing 8.7 g of K_2HPO_4 and 1.3 g of KH_2PO_4 , dissolved in 1l of deionized water (pH 7.6) and stored at 4 °C.

Samples of pure compost (1.5 g) were placed into 100 ml test tubes containing 50 ml of 60 mM potassium phosphate buffer (pH 7.6) followed by the addition of 0.50 ml of FDA. The test tubes were then placed on an orbital shaker for 2 h at 24 °C. Acetone (4 ml of 50%) was then added and the suspension swirled by hand for few seconds to terminate FDA hydrolysis. The composts and termitaria soil suspensions were then placed in 1.5 ml eppendorf tubes and centrifuged for 5 min at 6000 rpm to remove

debris whereafter the supernatant (1.2 ml) was removed with a pipette and placed in separate cuvettes. The spectral absorbance was measured with a spectrophotometer (Varian 8x6 Multicell Block Water Thermostatted Series II, Australia) set at a wavelength of 490 nm. The amount of FDA hydrolyzed by the respective compost and termitaria soil sample(s) was determined with reference to a standard curve and expressed as $\mu\text{g/ml}$ FDA hydrolyzed. A standard curve was prepared by adding 0.15 ml, 0.5 ml, 1.5 ml and 2.5 ml of FDA to 50 ml test tubes and brought to volume with potassium phosphate buffer. The solutions were placed in water bath for 60 min and then FDA hydrolysis was terminated by adding 2.5 ml of acetone. The absorbance was measured on a spectrophotometer set at a wavelength of 490 nm and plotted on a graph. Solutions that exceeded the limits of the standard curve were diluted with potassium phosphate buffer until the absorbance was within the limits of the standard curve. The data collected were subjected to analyses of variance (ANOVA) using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) and means were separated using Fischer's least significant difference ($\text{LSD}_{0.05}$) test.

Determination of active carbon: The procedure measures the fraction of organic matter in a form readily utilizable as energy source by microorganisms. The procedure was adapted from Weil *et al.* (2003) and involved two steps: 1) the oxidation of active (labile) carbon by 0.02 M KMnO_4 , and 2) measuring the absorbance of the reduced MnO^{-4} solution at 550nm. Pure compost and termitaria soil sample(s) (2.5 g) were oven dried for 24 h at 60 °C and shaken for 2 min in 0.02 M KMnO_4 solution. The suspension was allowed to settle for 8 min and then a 0.50 ml aliquot was collected, diluted 100x and measured for absorbance at 550 nm with a spectrophotometer (Varian 8x6 Multicell

Block Water Thermostatted Series II, Australia). Active carbon in mg/kg was determined by reference to a calibration curve prepared according to Weil *et al.* (2003). The data collected were subjected to ANOVA using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) and means were separated using Fischer's least significant difference ($LSD_{0.05}$) test.

Biolog Ecoplates®: The technique evaluates the community level physiological profiling (CLPP) of an environment where the physiological diversity of bacteria based on their utilization of specific carbon substrates can be determined using commercial Biolog (Biolog™, USA) Eco-plates, which each contain three replications of 31 carbon-sources and one control well per replication. Tetrazolium salt is used as an indicator dye that turns blue when the particular carbon source to which it is bound is metabolized (Hill *et al.*, 2000; Kirk *et al.*, 2004; Mazzola, 2004). The use of Biolog Eco-plates (Biolog™, USA) was adapted from Akmal *et al.* (2005) and Palmroth *et al.* (2005). Each of the pure samples (2 g) were suspended in 200 ml of distilled water and then placed into a 250 ml Erlenmeyer flask. The respective flasks were then shaken in a shaker for 30 min at 300 rpm and pipetted into each of the 96 wells on a Biolog Ecoplate. The Biolog Ecoplates were incubated at 25 °C for 6 days. The colour development in the Biolog Ecoplates was read using a microplate reader (BioTek EL X808, A.D.P, South Africa) at 595 nm. The average well colour development (AWCD) was calculated for each sample by dividing the sum of the optical density data by 31 and subjecting data to cluster and principal component analysis (PCA).

Denaturing Gradient Gel Electrophoresis (DGGE): DGGE is one of the fingerprinting methods used to describe and compare microbial communities in a given environment (Muyzer *et al.*, 1997; Green, Leigh & Neufeld, 2009). The technique involves the following steps:

DNA Extraction: PowerSoil[®] DNA isolation kit (MO BIO Laboratories, Inc.) was utilized for DNA extractions of the 16 substrate samples. A 0.25 g compost/termitaria soil sample was added to the PowerBead Tubes provided and gently vortexed for few seconds to mix. Then 60 µl of Solution C1 (SDS and other disruption agents required for complete cell lysis) was added and the tubes inverted several times. The PowerBead Tubes were then secured horizontally using the MO BIO Vortex Adapter tube holder and vortex for 10 min at maximum speed. Whereafter, they were centrifuged at 10,000 x g for 30 s at room temperature. The supernatant was transferred to a clean 2 ml collection tube (provided) and 250 µl of Solution C2 (Inhibitory Removal Technology^R for precipitating non-DNA organic and inorganic material) was added and vortexed for 5 s. The tubes were then incubated at 4 °C for 5 min and centrifuged hereafter for 1 min at 10, 000 x g. Following this, 600 µl of the supernatant was transferred to a clean 2 ml collection tube (provided) and 200 µl of Solution C3 (Inhibitory Removal Technology^R, an additional precipitating agent) added and vortexed briefly. The tubes were incubated at 4 °C for 5 min and centrifuged thereafter for 1 min at 10, 000 x g. The supernatant (750 µl) was transferred to a clean 2 ml collection tube (provided) and 1200 µl of Solution C4 (high concentration salt solution) added and vortexed for 5 sec. Approximately 675 µl of the supernatant was loaded onto a spin filter and centrifuged for 1 min at 10, 000 x g. The follow through

was discarded and an additional 675 µl of the supernatant added to the spin filter and centrifuged at 10, 000 x g for 1 min. The follow through was discarded and the remaining supernatant loaded onto the spin filter and centrifuged for 1 min at 10, 000 x g. 500 µl of Solution C5 (ethanol based wash solution) was added and centrifuged for 30 s at 10, 000 x g. The flow through was discarded and the tubes centrifuged again for 1min at 10, 000 x g. The spin filter was transferred into a clean 2 ml collection tube provide. 100 µl of Solution C6 (sterile elution buffer) was added to the centre of the white filter membrane and centrifuged at 10, 000 x g for 30 s. The spin filter was discarded and the DNA in the tube stored at -20 °C until further use. The DNA extracted was verified on a 1% agarose gel using electrophoresis and stained with ethidium bromide under UV.

DNA concentrations and purity: The concentrations and purity of genomic was determined using a Nano Drop spectrophotometer 3300 fluorospectrometer (Thermo Scientific) prior to 16S rDNA Polymerase Chain Reaction (PCR) amplification. The community rDNA's were PCR amplified from 1-50 ng of bulk DNA in reaction mixtures using a standard procedure (SOP_29) developed in the Metagenomics Platform laboratory of the University of the Free State (UFS), Bloemfontein. The PCR amplified fragments were verified with 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination. A nested PCR approach was applied where a fragment of the same 16S rDNA region was obtained for all specific bacterial groups using the target 16S rDNA PCR products to amplify DNA fragments suitable for DGGE analysis. The DNA fragments were verified with 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination and the amplified DNA fragments were exposed

to a gradient of denaturant (urea / formamide) at an elevated temperature (60 °C) within the polyacrylamide gel (Tzeneva *et al.*, 2008). The analysis was carried out according to the standard procedure (SOP_19b) developed in the Metagenomics Platform laboratory using a DCode™ Universal Mutation Detection System (BioRad).

PCR amplification of bacterial 16S rRNA gene: Bacterial-specific primers 27F and 1492R (Weisburg *et al.*, 1991) were used in the PCR amplification and the PCR reaction mixture contained 10 µM of each primer set, 10 mM of dNTPs, 1 – 50 ng of genomic DNA, 1.25U of Taq DNA polymerase (SuperTherm) and reaction buffer in a final volume of 50 µl. The reaction mixture was incubated at 95 °C for 2 min and this was followed by 30 cycles of amplification. Each cycle consisted of denaturation at 95 °C for 30 sec, annealing at 52 °C for 45 sec and extension at 72 °C for 1 min. While the final extension was at 72 °C for 10 min, this was followed by visualization with 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination. Nested PCR was performed using gel stabbed 16S rDNA products as templates and the same PCR program was used for PCR amplification with primers set 341F-GC and 517R (Muyzer *et al.*, 1997).

DGGE and analysis: DGGE analysis was carried out according to the standard procedure (SOP_19b) developed in the Metagenomics Platform laboratory using a DCode™ Universal Mutation Detection System (BioRad), followed by staining and visualization under UV transillumination. The DGGE banding profile of each sample was quantified and compared with Quantity One® 1- D Analysis imaging software

available from the BioRad Molecular Imager Gel Doc™ XR System to determine relatedness (SOP_66) (Green *et al.*, 2009) and PCA were performed.

Phospholipid Fatty Acid (PLFA) analysis: PLFA analysis evaluates total microbial composition in an environment. The procedure was conducted according to the method modified by White *et al.* (1979) as described by Marschner (2007) as follows:

Lipid Extraction: Compost and termitaria soil samples (2 g) were weighed in 25 ml glass centrifuge tubes, whereafter 1.5 ml citrate buffer, 1.9 ml chloroform, 3.8 ml methanol and 2 ml Blight and Dyer reagent were added to the tubes, and vortexed for 1 min each. The tubes were shaken for 2 h, followed by centrifuging at 3500 g for 10 min. The supernatant was transferred into a clean 25 ml glass centrifuge tube. The substrate pellet was again washed with 2.5 ml Blight and Dyer reagent (vortexed and centrifuged as previously described) and the supernatant combined with the previously obtained supernatant.

Phase Separation: The supernatant was then diluted with 3.1 ml chloroform and 3.1 ml citrate buffer, vortexed and centrifuged at 3500 g for 10 min. Following this procedure, 1–3 ml from the lower (organic) phase was transferred into a new 10-ml glass tube, and dried at 40 °C under a stream of N₂.

Lipid Fractionation: Dried samples (organic phase) were dissolved in 300 µl chloroform and then pipetted to the conditioned (2x 1 ml chloroform) silica-bonded column. The pipettes were rinsed with 300 µl chloroform between samples. Neutral lipids were eluted with 5 ml chloroform followed by elution of glycolipids with 10 ml acetone and

both were discarded. Phospholipids were then eluted with 5 ml methanol and transferred to a 10 ml centrifuge tube, dried at 40 °C under a stream of N₂.

Alkaline Methanolysis: A 30 µl internal standard (C19:0) was added to dried samples and then suspended in 1 ml methanol toluol solution (1:1, v/v). Thereafter, 1 ml 0.2 M KOH-MeOH was added to the suspensions, vortexed briefly and incubated for 15 min at 37 °C. A 2 ml hexane–chloroform solution, followed by 0.3 ml of 1 M acetic acid and 2 ml deionized water were added, vortexed for 1 min, and centrifuged for 5 min at 3500 g. The supernatant was transferred into 10 ml glass tubes and 2 ml hexane–chloroform solution then added, vortexed and the supernatant combined with the previously obtained one. The suspensions were dried at 40 °C under a stream of N₂.

Fatty Acid Determination and data analysis: Dried extract was dissolved in 130 µl isooctane. Fatty acid methyl esters (FAME) were quantified using a Varian 430 flame ionization gas chromatograph (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 µm ID, 0.2 µm film thickness). Column temperature was 40–230 °C (hold 2 minutes; 4 °C/minute; hold 10 minutes). Fatty acid methyl esters in isooctane (1µl) were injected into the column using a Varian CP 8400 autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19).

Peak identification of the fatty acids and the fatty acid profile were done with an external standard, Supelco 37 component FAME mix and the Sigma bacterial acid methyl ester (BAME) mix. Calculations were performed according to the equation listed by Marschner (2007), and data classified into different Bacterial Acid Methyl Esters (BAMEs) according to Whalen & Sampredo (2009). The BAMEs were then grouped in to their respective microbial groups and PCA was performed.

Ergosterol analysis: The procedure estimates fungal biomass in a substrate, and was conducted according to the method of Ruzicka *et al.* (2000) and Klamer & Bååth (2004). Compost and termitaria sample(s) (20 g) were added to 50 ml of methanol and mixed in a 100 ml beaker for 90 min, using a magnetic stirrer. The mixture was allowed to settle and 25 ml of the clean extract was added to a test tube with a screw cap, containing 3 g of KOH pellets. The mixture was stirred in a vortex mixer to dissolve the KOH crystals whereafter 10 ml of n-hexane was added and the mixture then incubated for 30 min in a 75 °C water bath. Distilled water (5 ml) was then added, mixed well and cooled to room temperature. The hexane supernatant was transferred to a 50 ml beaker, and n-hexane (10 ml) was added to the rest of the aliquot in the test tube and mixed well. The hexane layer was removed and added to the earlier aliquot. The last two steps were repeated. The hexane extract was evaporated in the beaker, until dry in a hot water bath. The residue was dissolved in 5 ml methanol and filtered through Acrodisc GHP membrane filter. The filtrate was analyzed on a HPLC (Flexar HPLC Detector, Perkin Elmer) machine. The amount of ergosterol content was determined with reference to a standard curve prepared according to Jambunathan, Kherdekar & Vaidya (1991), and expressed as µg/g ergosterol. The standard curve was prepared using the following

ergosterol standards concentrations: 0, 1, 5, 10, 15, 20 and 25 µg ergosterol /ml methanol. The results were subjected to an ANOVA using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA), and data and means separated using Fischer's least significant difference ($LSD_{0.05}$) test.

RESULTS

Phytotoxicity assay

Compost biological index: Germination of lettuce (mean germination of 40.13 %) and radish (mean germination of 36.25 %) seeds were reduced in most samples of compost compared to the control treatment (vermiculite) which had a mean of 71 and 77 % respectively (Figure 1). Trends were consistent with wet biomass (Figure 2). The different composts displayed a considerable variation in germination % for lettuce and radish seeds. For lettuce seeds, composts #2, #4 and #15 and termitaria soil (#11) resulted in higher germination (>71 %) than the control although were not significantly ($P<0.05$) different from each other. Composts #3 and #7 followed with a lower germination (>60 %) which was lower than the control treatment. For radish seeds, composts #2, #7, and #10 resulted in high germination (>75 %) although not significantly different ($P<0.05$) from control. Composts #1 and #8 had a germination >50%. Composts #5, #6, #9, #12, and #13 yielded a mean germination of 21 % and less for both lettuce and radish seeds. The correlation between mean germination % and mean biomass (g) was highly significant ($P<0.01$) for both lettuce ($r=0.843$) and radish ($r=0.979$) (Table 3).

Pathogenicity bioassay

Pathogen challenge: The mean germination % and biomass (g) of radish seedlings for both *R. solani* isolates was significantly reduced ($P<0.01$) compared to the control treatment (Figure 3). Composts #2 and #4 both yielded a significantly higher ($P<0.05$) mean germination % than the control (55 %) for isolate CPP160. However, only compost #2 (45 %) was most suppressive for isolate CPP159 and its germination was lower than that of the control (60 %). Compost #4 was ineffective in suppressing isolate CPP159 with no germination being recorded. Isolate CCP159 was the most pathogenic, with the mean germination ranging from 10-20 % for six composts and 0-5 % for the remaining nine composts. The mean germination for isolate CCP160 ranged from 0-20 % for nine composts and 20-40 % for four composts, while compost #16 recorded mean germination of 50 %. The correlation between mean germination % and mean biomass (g) was highly significant ($P<0.01$) for isolate CCP159 ($r=0.933$) and CCP160 ($r=0.912$) (Table 4). There was a positive correlation in terms of mean germination % and mean biomass (g) between pathogenicity and phytotoxicity. The correlation ($r=0.81$) was the highest between isolate CCP159 and lettuce in terms of wet biomass (g). This was followed by $r=0.76$ between isolate CCP159 and radish germination % and wet biomass (g), respectively (Table 2).

Compost evaluation

Spectroscopy: Results of cluster analyses conducted on NIR and MIR spectroscopy data were similar despite MIR being said to be more sensitive than NIR (Figure 5 and 7). Three clusters were identified with NIR (Figure 4) that were between 98.5-99.9 %

similar. In comparison, MIR (Figure 6) identified three clusters that were between 80-99 % similar. Commercial composts #13, #14, #15, #16 were in the same cluster (99.5 %) based on NIR data. These were also similar (97 %) based on MIR data (with the exception of #16). Composts #4, #5, #6, #10 and #11 were grouped in a single cluster with 99.5 % similarity based on NIR data and 94 % similarity based on MIR data respectively. There were no relationships that could be derived from the NIR and MIR spectroscopy with regard to phytotoxicity and pathogenicity.

Fluorescein diacetate (FDA): Compost #2 which displayed the highest germination % (78 % and 87 %) for lettuce and radish respectively also had the highest microbial activity (Figure 8). It was followed by compost #12 and #5 which both recorded low germination for both radish and lettuce. Compost #10 which had the highest germination % for radish seeds (88 %) had the 4th highest microbial activity. There were no discernible relationships between phytotoxicity and microbial activity for either lettuce or radish seedlings.

The relationship between microbial activity and disease suppressiveness of the 16 composts was mostly consistent for the two *R. solani* isolates (Table 3). The highest level of FDA hydrolysis was recorded for compost #2 which also had the highest germination % of radish seeds for both isolates. For isolate CCP159, of five substrates with highest microbial activity (#2, #5, #8, #10 and #12), four were also among the top five according to germination %. The one exception was #12 which inhibited germination by 100%. Of the twelve compost substrates in which a germination % of <10% was recorded, ten had the lowest microbial activity (<0.6). The two exceptions

were composts #8 and #12. There was a positive correlation ($r=0.79$; $p<0.05$) between microbial activity and biomass (Table 4) for isolate CPP159, the most pathogenic of the two isolates. There were however no significant correlations (Table 3) between microbial activity and phytotoxicity in any of the two crops.

Active carbon: There were significant differences ($P<0.05$) in active carbon for the different composts (Figure 9). Compost #2 was among composts (#1, #4-9 and #15) with the highest total carbon (ppm). There was a positive correlation between organic matter (as determined by active carbon), phytotoxicity and disease suppressiveness (Table 2 and 3), however not significant. Based on phytotoxicity, compost #2 and #7 recorded a high germination % for both lettuce and radish while compost #4 and #15 had a high % only for lettuce and compost #1 and #8, for radish. Compost #13 and #14, which recorded the lowest active carbon reading, also recorded a low germination %. Active carbon was only correlated with disease suppression for compost #2 for both isolates. For composts #4, #6, #7 and #8, active carbon was correlated with disease suppression for isolate CPP160 (Table 4).

Biolog Ecoplates®: The average well colour development (AWCD) of all/or most composts followed the same pattern, the exception being Sample 11 (termitaria soil) and compost #8 which had a low AWCD for all the sugars (Figure 10). In cluster analysis, termitaria soil was highly different from the other growth media (6 %), followed by compost #8 with 33 % similarity (Figure 11). Composts clustered in four distinct clusters (Figure 12) with two clusters containing a single entry each, viz. composts #8 and #11. The two remaining clusters did however not reflect many similarities with

regard to phytotoxicity or pathogenicity results. Thus although composts #2 and #7 were ranked 2nd and 3rd based on phytotoxicity results in radish, they shared a cluster with >80 % similarity with composts #3, #5 and #14 which were ranked 14th, 13th, and 10th respectively. Apart from the two most phytotoxic composts and least disease suppressive composts (#12 and #13) clustering together, no other inferences could therefore be drawn from Biolog results with regard to the relationship between community linked physiological profiles (CLPP) of composts and their inherent disease suppressive ability.

Denaturing Gradient Gel Electrophoresis (DGGE): The 16S rDNA DGGE profile of the composts and termitaria soil is presented in Figure 13. Compost #2 which had high germination % for phytotoxicity and disease suppressiveness, was most similar to compost #5 (56.3 %), #4 (55.7 %) and 16 (55 %) (Figure 14), that were closely associated in terms of ranking according to disease suppression and phytotoxicity. The commercial composts (#12-14 and #16) clustered more closely than those obtained from the private nursery (Figure 14). This trend was also consistent with cluster and PC analysis of NIR data. Compost #15 (commercial compost) which clustered with #1 and #10 according to DGGE results, were also closely associated (> 80% similar) based on results from Biolog Ecoplates. DGGE data corresponded well with MIR data, as both grouped composts #7 and #16; #2 and #3; #6 and #8; #5 and #11 and #1 and #10, while #15 was an outlier in both these techniques.

Phospholipid fatty acid analysis: The PLFA profiles of the composts were composed of saturated, unsaturated, methyl-branched and cyclopropane fatty acids (Figure 15). The

amount of phospholipid fatty acids (PLFA) varied between 4-8.3 % mole for general bacteria, 0-3.5 % for gram positive bacteria, 2.8-5.4 % for gram negative bacteria and 2.13-17.5 % for fungi (Table 5). The most common PLFAs were methyl hexadecanoate (C16:0), methyl cyclo octadecanoate (C18:1 ω 9), methyl octadecenoate (C18:0) and methyl cis-9,12-octadecadienoate (C18:2 ω 6) comprising between 30.47, 21.78, 12.97 and 9.59 mol % of the total amount of PLFAs respectively (Figure 16). Compost #3 exhibited the highest total PLFA (nmol/g) for these PLFAs groups. Of these common PLFAs, C16:0 and C18:0 are found in general bacteria while C18:1 ω 9 and C18:2 ω 6 in gram negative bacteria and fungi, respectively.

On average, the highest mol % of gram positive bacteria was recorded in composts #12, #6 and #10 (about or over 3 %) respectively. Gram negative bacteria on the other hand were the highest in compost #7 and #10 (5.44 % and 5.30 %). The mol % for fungi was high in composts #3, #9, #14, #16, #13, #11, #8 and #4 (ranged between 11-17 %). The ratio (Figure 18) of gram positive bacteria to gram negative bacteria was higher in composts #6 and 12 (1.11 and 1.10 respectively), the same composts that recorded low or no germination % in the phytotoxicity study. Only compost #6 recorded 40% germination for isolate CCP 160. Composts #1 and #3 recorded the lowest ratios, viz. 0.09 and 0.03 respectively. Compost #2 which was the best compost in terms of phytotoxicity and disease suppressiveness had a ratio of 0.65. The ratio of fungi to bacteria (Figure 18) was >1 for most composts with the highest recorded in compost #3 (1.78) followed by compost #9 (1.60), compost #14 (1.39) and compost #16 (1.35). The lowest ratio was recorded in compost #15 (0.17). Only compost #4 exhibited cyclo propyl however the ratio to mono-unsaturated precursor

was low (0.01). The homemade (#1, #2, #4-6, and #8) and commercial (#12, #13, #15 and #16) composts clustered more closely with termitaria soil (Figure 17). Compost #14 (commercial compost) clustered with #7 and #9 and composts #3 and #10 lied outside according to PLFA results. Correlations between PLFA, phytotoxiciy and pathogenicity were low (Table 3 and 4).

Ergosterol analysis: The amount of ergosterol found in composts varied significantly ($P < 0.05$) (Figure 19). Compost #7 recorded the highest ergosterol content (27648 $\mu\text{g/g}$), followed by compost #5 (18867 $\mu\text{g/g}$). Compost #7 recorded a high germination % in phytotoxicity results for both plant species but failed to suppress disease of either of the two isolates in the pathogenicity test. The latter was true for compost #5, which however, also recorded a low germination % for both crops in terms of phytotoxicity. Compost #5 recorded high microbial diversity in terms of its FDA and total carbon results. The best compost (#2) in terms of phytotoxicity and pathogenicity ranked 4th based on ergosterol content. On the other hand, compost #12 which allowed no germination in the phytotoxicity trial, and failed to suppress either of the two isolates, ranked 5th in terms of ergosterol content. Other composts that recorded a high ergosterol content were #3, #10 and #15. The relationship between ergosterol and phytotoxiciy or pathogenicity was low (Table 3 and 4).

DISCUSSION

Compost maturity as reflected by phytotoxicity is an important factor affecting its disease suppressive ability, and thus its quality. This is merely for the fact that mature composts support growth of antagonistic microbes whereas immature composts support

growth of pathogens and negatively affect plant growth (De Ceuster & Hoitink, 1999; Raviv, 2008). Mature (less phytotoxic) composts suppress pathogens such as *R. solani* more effectively than immature (phytotoxic) composts via a high density of antagonistic microorganisms such as *Pseudomonas* spp. and *Trichoderma* spp. (Tuitert, Szczech, & Bollen, 1998; Diab *et al.*, 2003).

In the present study, 15 different composts of different ages and soil from termitaria were tested for their impact on germination % of lettuce and radish seedlings, and their ability to suppress two isolates of *R. solani* (AG1 and unknown AG group). The majority of composts caused a reduction in the germination and wet biomass of lettuce and radish seeds compared to a vermiculite control treatment, but only composts #2, #4, #6-8 and #16 displayed a measure of disease suppression for isolate CCP 160. This was not the case for isolate CCP 159. Of the composts that suppressed isolate CCP 160, only compost #2 and #7 allowed a measure of germination for either of the two plant species in the phytotoxicity trial while composts #4, #8 and #16 allowed germination for one species only. Compost #6 did not allow germination in either of the plant species in the phytotoxicity trial. Composts #5, #9, #12 and #13 yielded a low germination% in both phytotoxicity and pathogenicity studies. Other composts (#1, #3, #10 and #15) in addition to the termitaria soil, which failed to suppress any of the isolates, were inconsistent in terms of a relationship between phytotoxicity and pathogenicity.

Phytotoxic compounds in composts are a result of anaerobic and reducing conditions (Hoitink *et al.*, 1991; 1997). Anaerobic conditions occur when less heat is

generated, such as after heavy rainfall or snowfall, resulting in reduced evaporation of water and fermentation. Phytotoxic conditions are also created when compost feedstock (e.g. fresh bark) is stored in large compacted piles creating products with a pH of 2.5 which is detrimental to plants (Hoitink & Fahy, 1986). All these factors need to be taken in consideration when using compost as a soil amendment. The impact of extreme environmental conditions such as rainfall, low temperature, etc. that could have had a negative impact on composts are not known. The composts in the present study were sourced from a private nursery and various commercial nurseries. Those from the private nursery (compost #1-10), including termitaria soil, were in compacted piles on the ground in different locations at the nursery. Of these, only composts #2 and #7 consistently exhibited no phytotoxicity to lettuce and radish plants, while composts #5, #6 and #9 were consistently phytotoxic to the two crops. Storage conditions of the commercial composts #12-16 are not known. They were, however sealed in plastic bags when purchased, which could have created anaerobic conditions. Compost stored in a sealed container can lead to the production of high concentrations of toxic metabolites within few hours of storage (Hoitink & Fahy, 1986). Another possible explanation of phytotoxicity in these composts could be the prior presence of pathogens. This was not however evaluated in the present study.

The maturity of composts used in the present study is important. Immature composts support the growth of pathogens and exhibit volatile organic acids (VOA) which are detrimental to plants (Manios *et al.*, 1989; Ianotti *et al.*, 1994). VOAs are the by products of anaerobic respiration (Manios *et al.*, 1989). VOAs may also be introduced into the composts through sources material such as waste water, biosolids

and raw manure (Kawamura, Ling & Kaplan *et al.*, 1985; Howgrave-Graham, Wallis & Steyn, 1991; Zahn *et al.*, 1997). The composts used in the present study were of different ages (range 3-36 months). The youngest compost (#7) did not reduce germination % of the plants significantly while the oldest (#10 and #11) gave variable results, suggesting that age did not have much impact on growth of the plants.

The sensitivity of different plants towards compost phytotoxicity differs. Cress is reported to exhibit greater sensitivity to phytotoxic compounds while lettuce and radish do not vary much but are less sensitive (Aslam & VanderGheynst, 2008), although lettuce was less affected in terms of germination and biomass than radish. This was because composts #3, #4 and #15 and termitaria soil did not allow high germination in radish as compared to lettuce. Besides this observation, germination results for both plants were consistent with the exception of composts #1 and #10 which recorded low germination in lettuce only. It appears that different crops will react differently to specific composts, depending on their level of sensitivity towards phytotoxic compounds.

Only 20 % of composts tested (>300) for disease suppression to *Rhizoctonia* damping-off have been positive (Kuter *et al.*, 1983; Nelson *et al.*, 1983; Hoitink & Boehm, 1999; Noble & Coventry, 2005; Scheuerells, Sullivan & Mahaffee 2005). Some composts are reported to increase the severity/aggressiveness of *R. solani*, especially if biological control agents were not present (Hoitink & Boehm, 1999; Noble & Coventry, 2005). It is therefore not surprising that the composts tested in this study gave variable results. *Rhizoctonia solani* Kühn suppression requires the presence of specific antagonistic microorganisms such as *Trichoderma* spp. Colonization of composts by

these specific antagonistic microorganisms can take months to occur. The inoculation of compost mixes with antagonistic microorganisms such as *Bacillus* spp., *Pseudomonas* spp., *Chryseobacterium gleum* (Holmes) and *T. hamatum* to suppress *R. solani* has been attempted. However, these antagonists must be applied during compost application to avoid competition with other low temperature microorganisms (Hoitink & Boehm, 1999; Krause *et al.*, 2001; Postma, Montanari & van den Boogert, 2003; Scheuerell *et al.*, 2005; Hoitink, 2006; Trillas *et al.*, 2006).

The ability of compost to suppress pathogens is attributed to the fact that compost microflora is able to prevent spore germination and vegetative growth due to competition for nutrient via lysis or hyperparasitism (Hadar *et al.*, 1992). The suppressive effect of composts in potting mixes to *R. solani* damping-off of radish is however highly variable in nature (Nelson *et al.*, 1983) and is reported to be a result of the specific composition of the microbial populations and their level of activity. Microbial activity measured by the rate of fluorescein diacetate (FDA) hydrolysis is an important criterion in determining the conduciveness or suppressiveness of composts to plant disease (Green *et al.*, 2006). Compost #2 had the highest microbial activity which could explain why it suppressed *Rhizoctonia* damping-off so effectively. Compost #8 which effectively suppressed isolate CCP160 of *R. solani*, also exhibited high microbial activity. Microbial activity was however not always consistent with the disease suppressive ability of composts. Composts #5, #10 and #12 for instance, had higher microbial activity but consistently failed to suppress damping-off by any of the two isolates, while composts #4, #6, #7 and #16 suppressed isolate CCP 160 but none had higher microbial activity. Microbial activity did not provide a good indication of compost

phytotoxicity and disease suppression. Composts that showed suppression to one or both the isolates recorded high microbial activity, however other composts that failed to suppress the two isolates also recorded high organic matter content. The organic and acid composts (#12-14) and soil from termitaria, which were phytotoxic to one or both plant species, recorded low activity. This however was not the case for all composts that were phytotoxic. Numerous studies report that microbial activity plays an important role in the suppression of pathogens such as *Fusarium* spp. and *Pythium* spp., but not so for pathogens such as *R. solani* which require specific antagonistic microorganisms (Lumsden *et al.*, 1976; Alabouvette, Couteaudier & Louvet, 1985). It is generally accepted that there is a broader range of antagonists for the suppression of pathogens such as *Pythium* spp. and more specific ones for *R. solani*.

The mechanisms of antagonistic microorganisms involved in disease suppression by compost are not fully understood. The use of physiological profiling such as CLPP and PLFA, and DNA-based techniques such as DGGE may lead to an improved understanding of the changes in microbial communities associated with disease control by composts mixes. Biolog Ecoplates® analysis evaluates the rate of which microorganisms utilize carbon sources. Biolog results provided no indications in terms of the relationship between the community linked physiological profiles (CLPP) of composts and their inherent disease suppressive ability.

DGGE of 16S rDNA fragments has frequently been used to profile the structure of bacterial communities in a given soil. However, the procedure has various types of intrinsic error and bias which often does not provide a good reflection of the relative

abundance of bacterial populations. Results obtained in the present study using DGGE showed that certain band patterns were present in all composts while other bands were strictly associated with certain composts. Despite a number of similarities that existed between composts with regards to their ranking in terms of phytotoxicity and/or pathogenicity and clustering based on the various molecular techniques used, there were many inconsistencies as well.

NIR and MIR spectroscopy yielded trends that were similar to DGGE results. However, these trends did not provide any indication of the disease suppressive ability of the composts, nor did they correlate with phytotoxicity of the composts. NIR and MIR spectroscopy merely provides stability parameters in compost such as carbon fractions (cellulose, lignin, etc.) which determine the maturity of compost and which is generally important for disease suppression (Ben-Dor, Inbar & Chen, 1997; Soriano-Disla *et al.*, 2010).

PLFA can be a good indicator of compost maturity (Klamer & Bååth, 1998; 2004), and bacterial fatty acid profiles can differ for disease suppressive and conducive composts (Tunlid *et al.*, 1989; Frostegård, Tunlid & Bååth, 2011). Low levels of polyunsaturated fatty acids and high levels of unsaturated fatty acids signal bacterial dominance such as PLFA profiles in the present study. Furthermore, trans/cis ratios of greater than 0.1 indicate starvation of bacterial isolates. The ratios of trans/cis of composts in the study were less than 0.05, which signals healthy bacterial populations (Piotrowska-Seget & Mzozik, 2003). Cyclo propyl fatty acids are important markers for gram negative bacteria, and can also be used to indicate anaerobic activity, however,

cyclo propyl were mostly not present in the composts. Some actinomycetes, which are important for compost disease suppression, are also reported to be profiled using PLFA technique as represented mostly by tuberculostearic acid (Tunlid *et al.*, 1989). The latter was however not detected in the composts. The technique also profiled fungal populations, although in low amounts. The low FAME of fungi was reflected during the fungal biomass determination as measured by the ergosterol analysis. There were no relationships that arose in terms of the fungal biomass, phytotoxicity and disease suppressiveness in composts

The present study showed that determining the quality (or biological profile) of a compost is an elusive task because of the many inconsistencies that exist between phytotoxicity, disease suppressiveness and inherent microbial activity. Specific compost can suppress one pathogen or enhance growth of a specific crop, however, the same compost can fail to suppress another pathogen or negatively impact growth of certain crops. There was thus a general lack of correspondence between the analytical techniques that were employed. Each technique should therefore be used for evaluating various inherent, and highly variable, quantitative and qualitative aspects pertaining to microbial diversity. Assays of microbial diversity should include integrating multiple holistic criteria at the total community level and partial approaches targeting structural and functional diversity. It therefore appears that a multifaceted approach to measuring biodiversity comprising both functional and taxonomic perspectives provides a better chance of detecting trends and patterns that are ecologically meaningful.

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Table 1: Characteristics of composts and termitaria soil used in the study

NO.	Composition/Brand	Abbreviation	Age (months)
1	Sawdust from <i>Eucalyptus grandis</i> + horse manure	SD+HM /12 mth	12
2	Sawdust from <i>E. grandis</i> + horse manure	SD+HM /6 mth	6
3	Sawdust from <i>E. grandis</i> + horse manure	SD+HM /4 mth	4
4	Garden refuse + horse manure	GR+HM /18	18
5	Garden refuse + horse manure	GR+HM /12	12
6	Garden refuse + horse manure	GR+HM /9	9
7	Garden refuse + horse manure	GR+HM /3	3
8	Garden refuse + <i>E. grandis</i> sawdust	GR+SD /18	18
9	Garden refuse + cattle manure + chicken manure	GR+Cat.M+Ch.M /12	18
10	<i>E. grandis</i> leaves	Euc leaves	>30
11	Termitaria soil	Termitaria soil	>36
12	Bloem organic compost	Bloem organic	>24
13	Culterra organic compost	Culterra organic	>24
14	Culterra acid compost	Culterra acid	>24
15	Terra microbe enriched compost	Microbe enriched	>6
16	Malan Seuns nursery	Malan seuns	>24

Table 2: Pearson correlation (r) between phytotoxicity and pathogenicity. $r > 0.75$ is highly significant ($P > 0.01$).

	Lettuce germ %	Radish germ %	Lettuce biomass	Radish biomass
Germ% CCP 159	0.18	0.76*	0.67	0.76*
Germ% CCP 160	0.12	0.41	0.39	0.36
Biomass CCP 159	0.24	0.58	0.81*	0.58
Biomass CCP 160	0.20	0.36	0.52	0.34

Table 3: Pearson correlation (r) between phytotoxicity, FDA, ctive C, Biolog, PLFA and ergosterol. r >0.75 is highly significant (P>0.01).

	Lettuce germ %	Radish germ %	Lettuce biomass	Radish biomass	FDA	Active C	AWCD	PLFA	Ergosterol
Lettuce germ %	1.00								
Radish germ %	0.21	1.00							
Lettuce biomass	0.84*	0.44	1.00						
Radish biomass	0.20	0.98*	0.45	1.00					
FDA	0.06	0.34	0.55	0.40	1.00				
Active C	0.05	0.34	0.15	0.33	0.33	1.00			
AWCD	0.05	0.07	0.15	0.17	0.06	0.44	1.00		
PLFA	0.17	0.30	0.02	0.28	0.30	0.11	0.31	1.00	
Ergosterol	0.14	0.23	0.09	0.23	0.16	0.33	0.19	0.32	1.00

Table 4: Pearson correlation (r) between pathogenicity, FDA, Active C, Biolog, PLFA and ergosterol. r >0.75 is highly significant (P>0.01).

	Germ% CCP 159	Germ% CCP 160	Biomass CCP 159	Biomass CCP 160	FDA	Active C	AWCD	PLFA	Ergosterol
Germ% CCP 159	1.00								
Germ% CCP 160	0.31	1.00							
Biomass CCP 159	0.93*	0.32	1.00						
Biomass CCP 160	0.32	0.92*	0.42	1.00					
FDA	0.71	0.27	0.79*	0.42	1.00				
Active C	0.30	0.47	0.24	0.38	0.33	1.00			
AWCD	0.15	0.18	0.14	0.18	0.06	0.44	1.00		
PLFA	0.28	0.07	0.24	0.06	0.30	0.11	0.31	1.00	
Ergosterol	0.40	0.01	0.25	0.17	0.16	0.33	0.19	0.32	1.00

Table 5: The mean phospholipid fatty acids (PLFAs) % of different microbial groups in composts and termitaria soil

Microbial groups	% total PLFA															
	Composts															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
General bacteria	8.31	6.29	5.30	5.82	7.44	6.31	4.13	6.22	5.64	4.29	6.56	6.01	6.51	5.61	6.04	6.56
Gram positive bacteria	0.26	2.12	0.13	0.59	1.33	3.44	2.63	1.13	0.46	2.99	0.71	3.50	0.56	0.42	1.89	0.31
Gram negative bacteria	2.82	3.28	4.39	4.35	3.01	3.10	5.44	3.61	4.04	5.30	3.54	3.18	3.63	4.30	4.46	3.54
Fungi	6.82	9.58	17.5	11.3	6.76	4.43	4.85	12.1	16.2	2.99	12.2	5.73	12.4	14.3	2.13	14.1
Ratio Gram positive/Gram negative	0.09	0.65	0.03	0.14	0.44	1.11	0.48	0.31	0.11	0.57	0.20	1.10	0.15	0.10	0.42	0.09
Ratio fungi/bacteria	0.60	0.82	1.78	1.05	0.57	0.34	0.40	1.10	1.60	0.24	1.13	0.45	1.16	1.39	0.17	1.35
Cyclo/mono-unsaturated	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

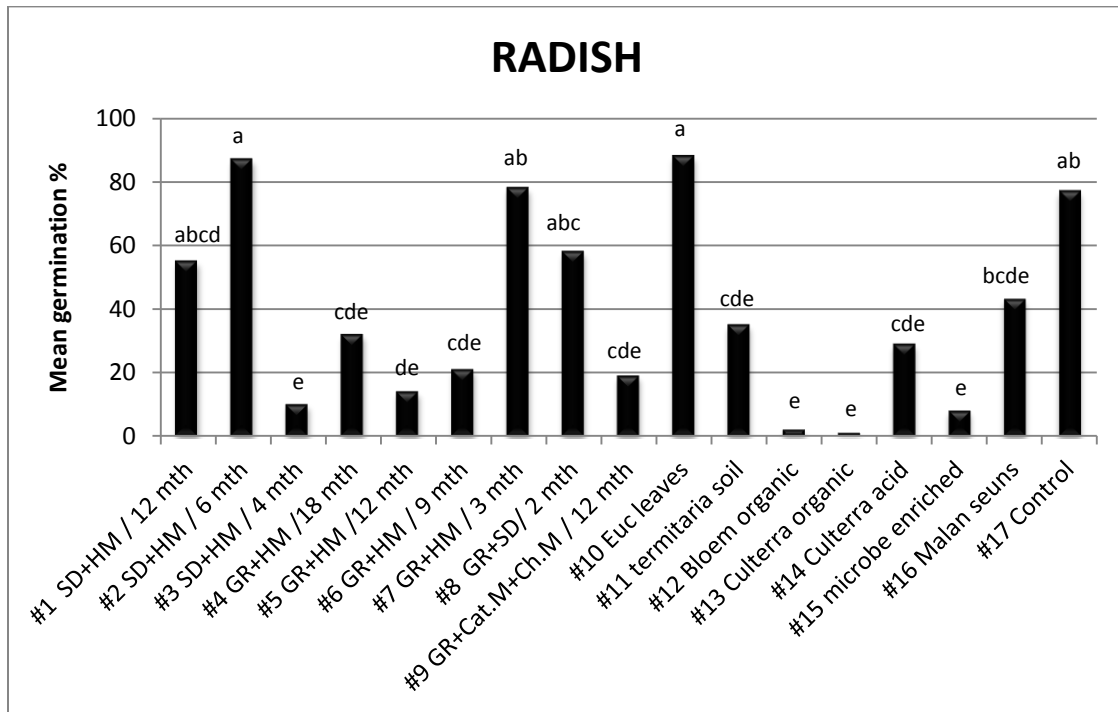
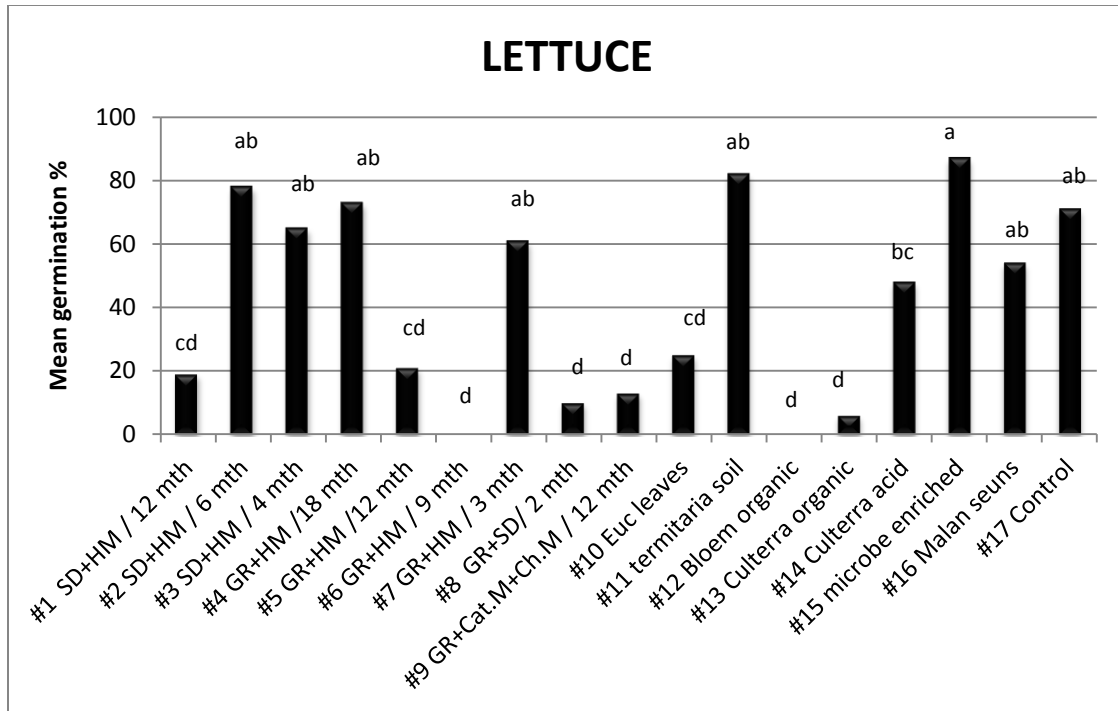


Figure 1: Mean percentage germination for lettuce and radish respectively (n=25, rep= 4) for different composts, termitaria soil and the control (#17 vermiculite). Bars not followed by the same letter are significantly different ($p < 0.05$) at $LSD_{0.05}$.

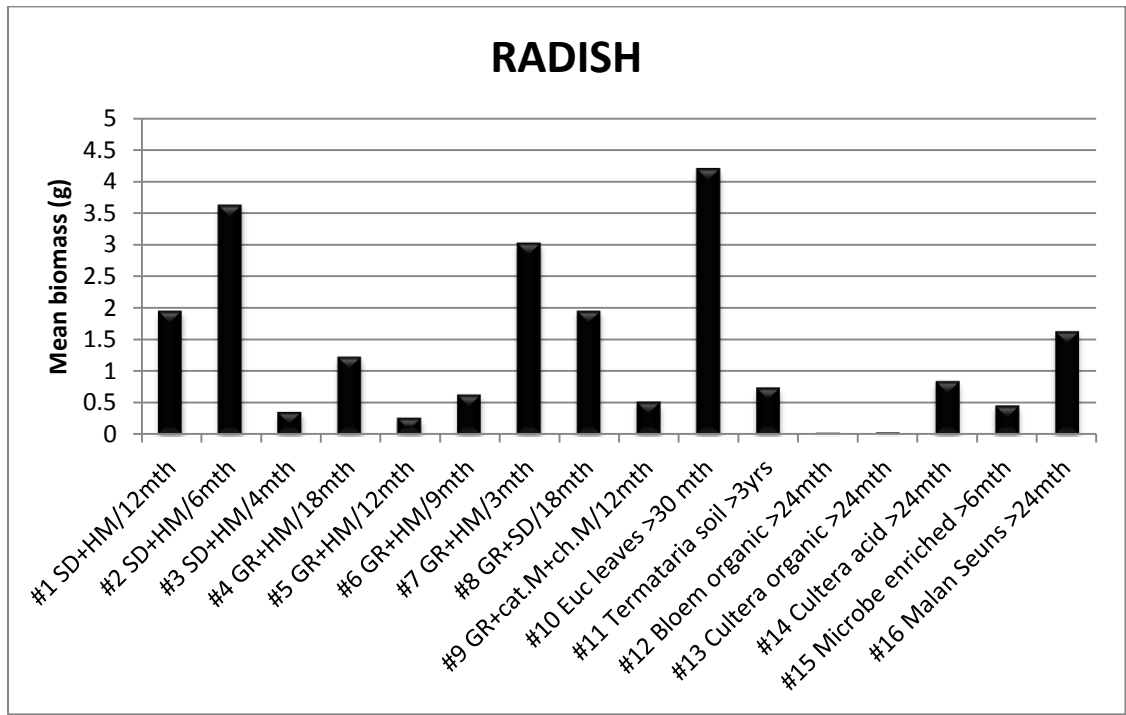
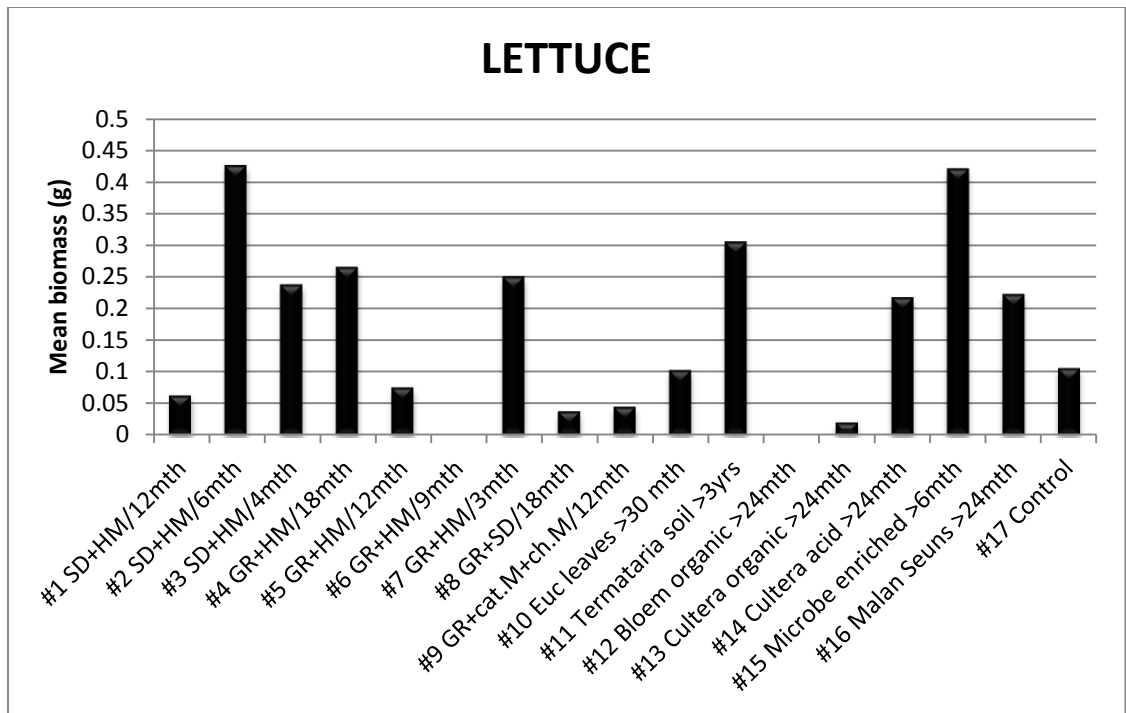


Figure 2: Total biomass for lettuce and radish respectively (n=25, rep= 4) for different composts, termitaria soil and the control (#17 vermiculite).

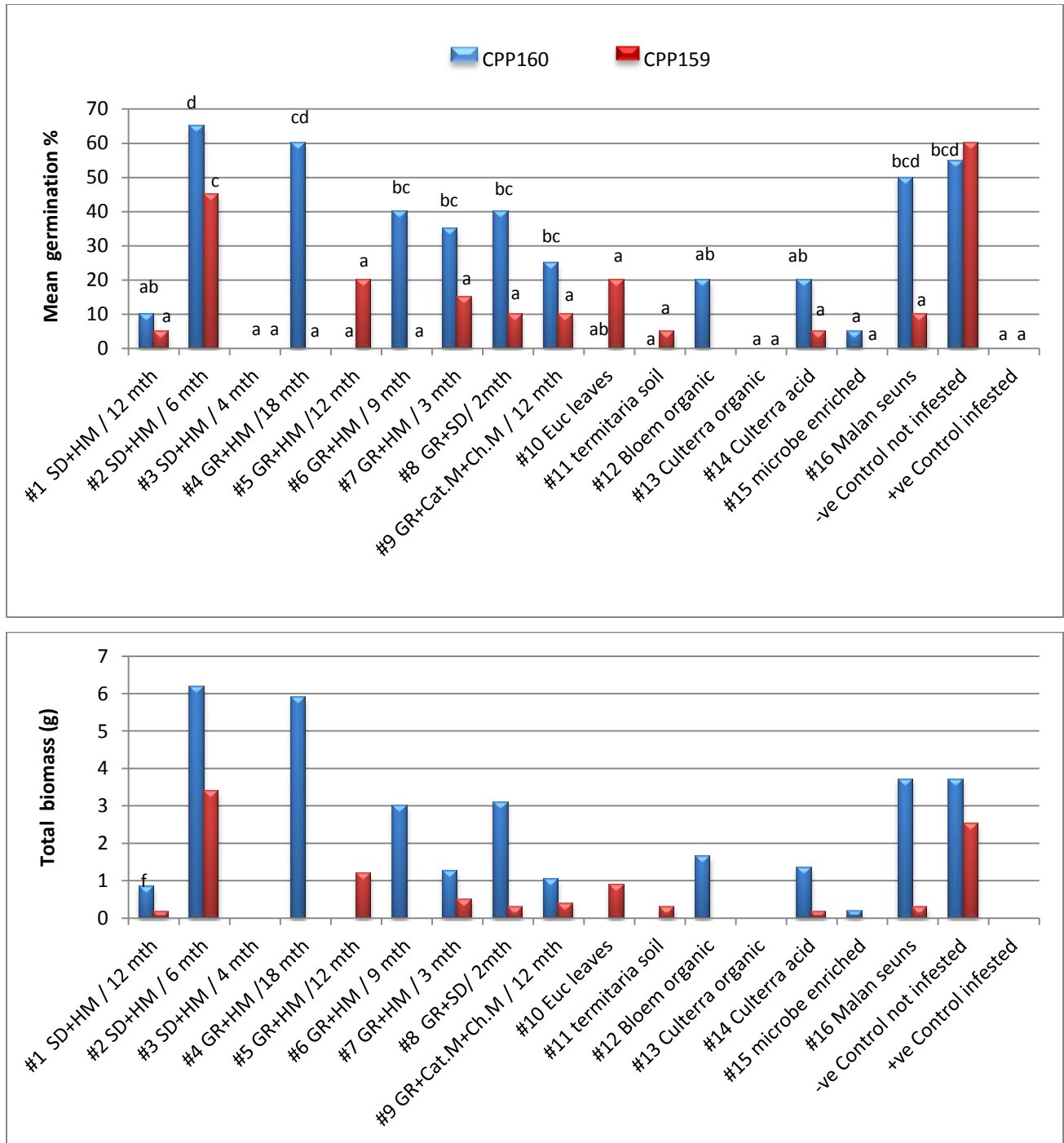


Figure 3: Mean germination percent and total biomass of radish (n=5, rep= 4) for different composts and termitaria soil in response to two isolates respectively; CCP 160 and CCP 150. The experiment included two controls; negative control (soil) and positive control (soil infested with the two isolates respectively). Bars not followed by the same letter are significantly different (LSD 19.995=0.05).

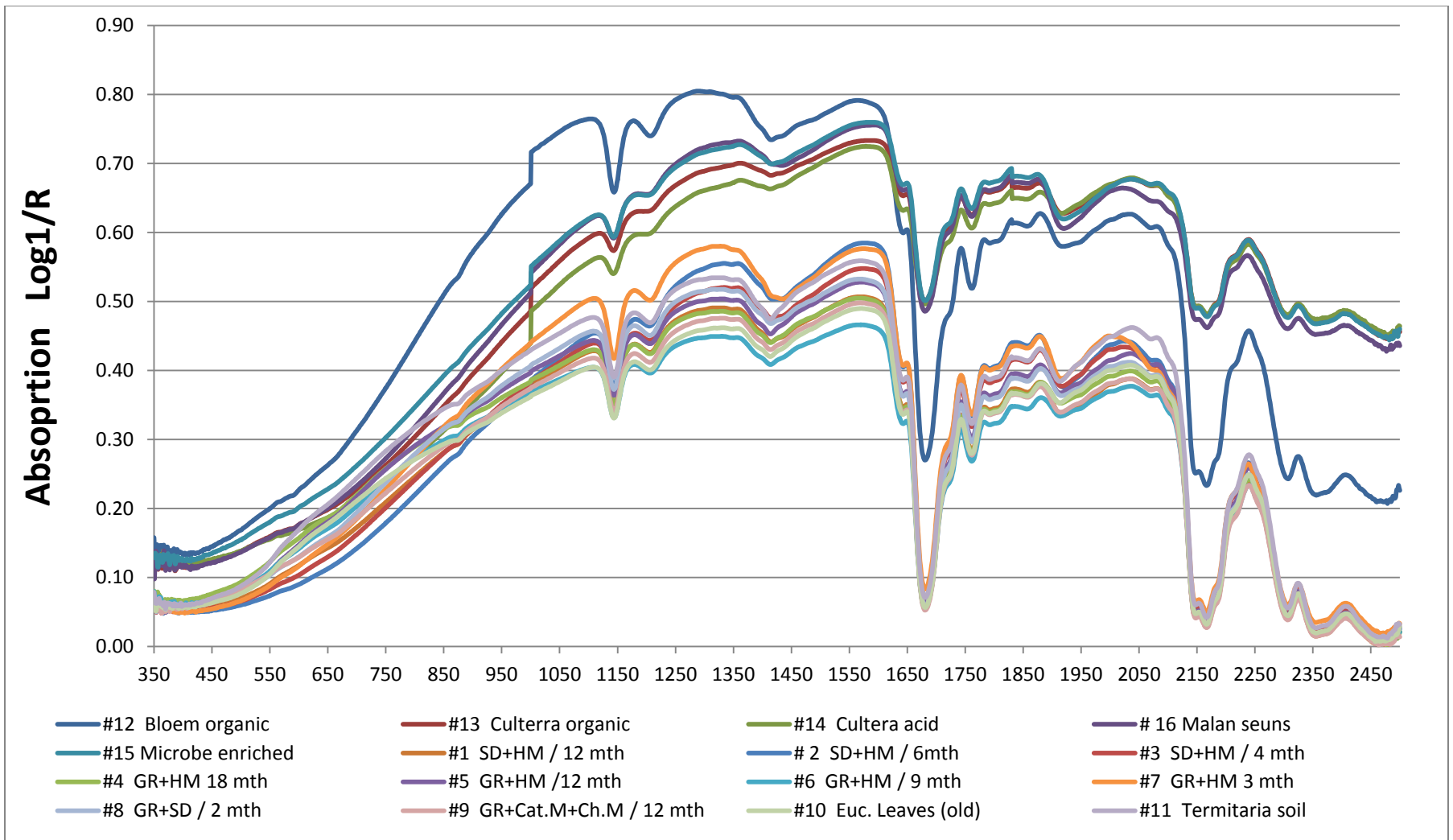


Figure 4: Near Infrared (NIR) Spectrophotometry for composts and termitaria soil

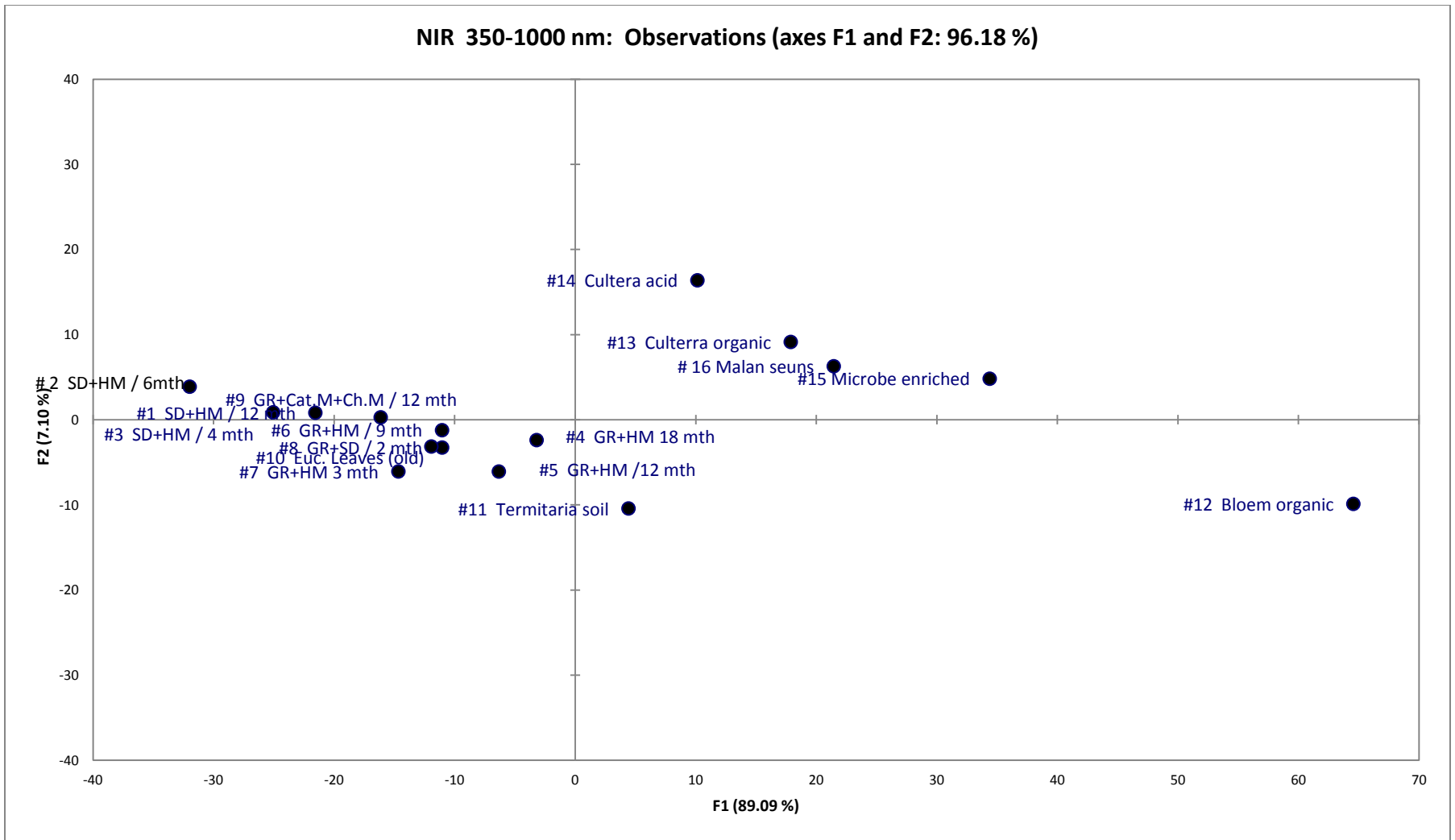


Figure 5: Principal Component Analysis showing the different clustering groups between the composts and termitaria soil based on NIR spectrophotometry.

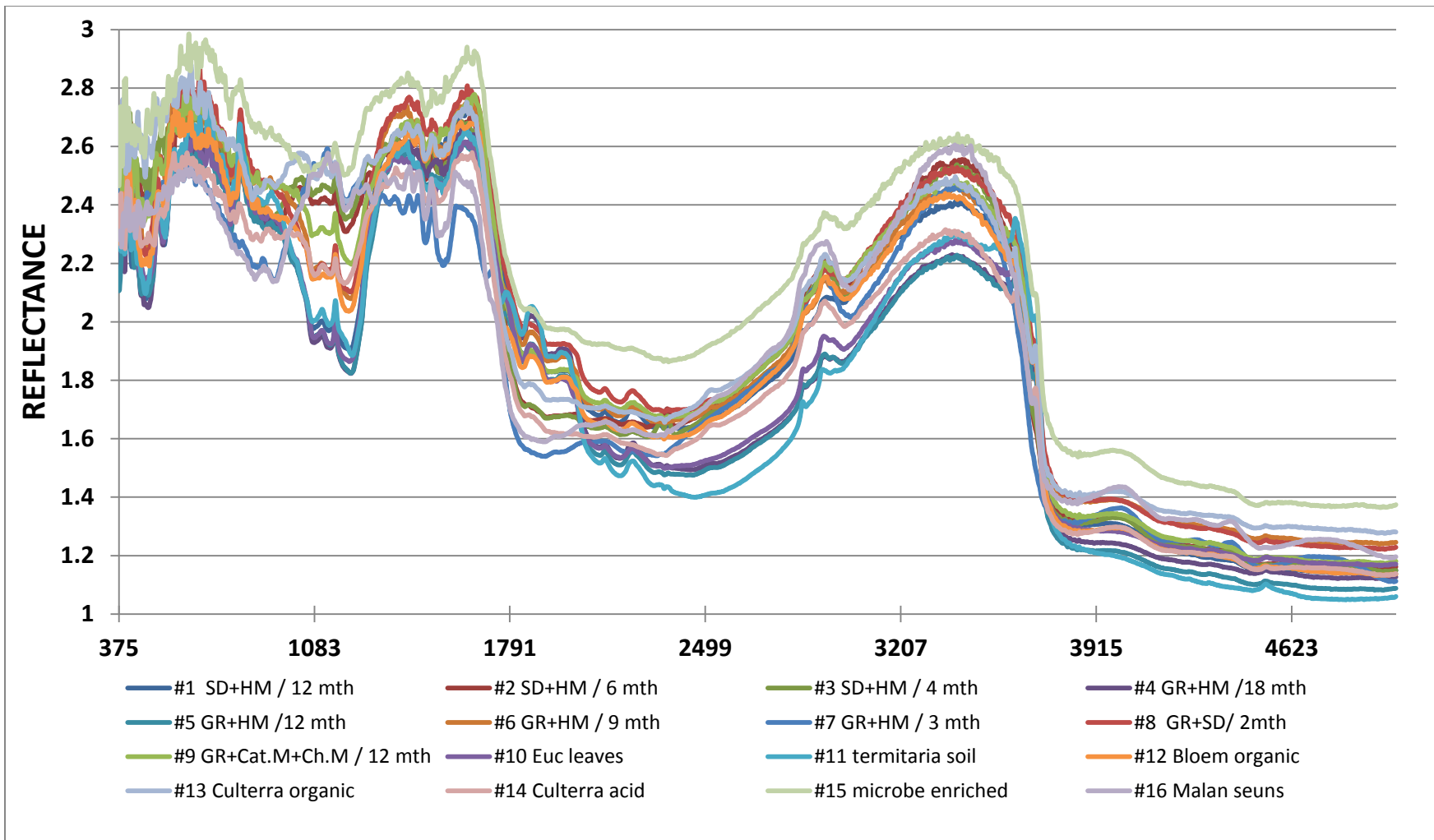


Figure 6: Mid Infrared (MIR) Spectrophotometry for composts and termitaria soil

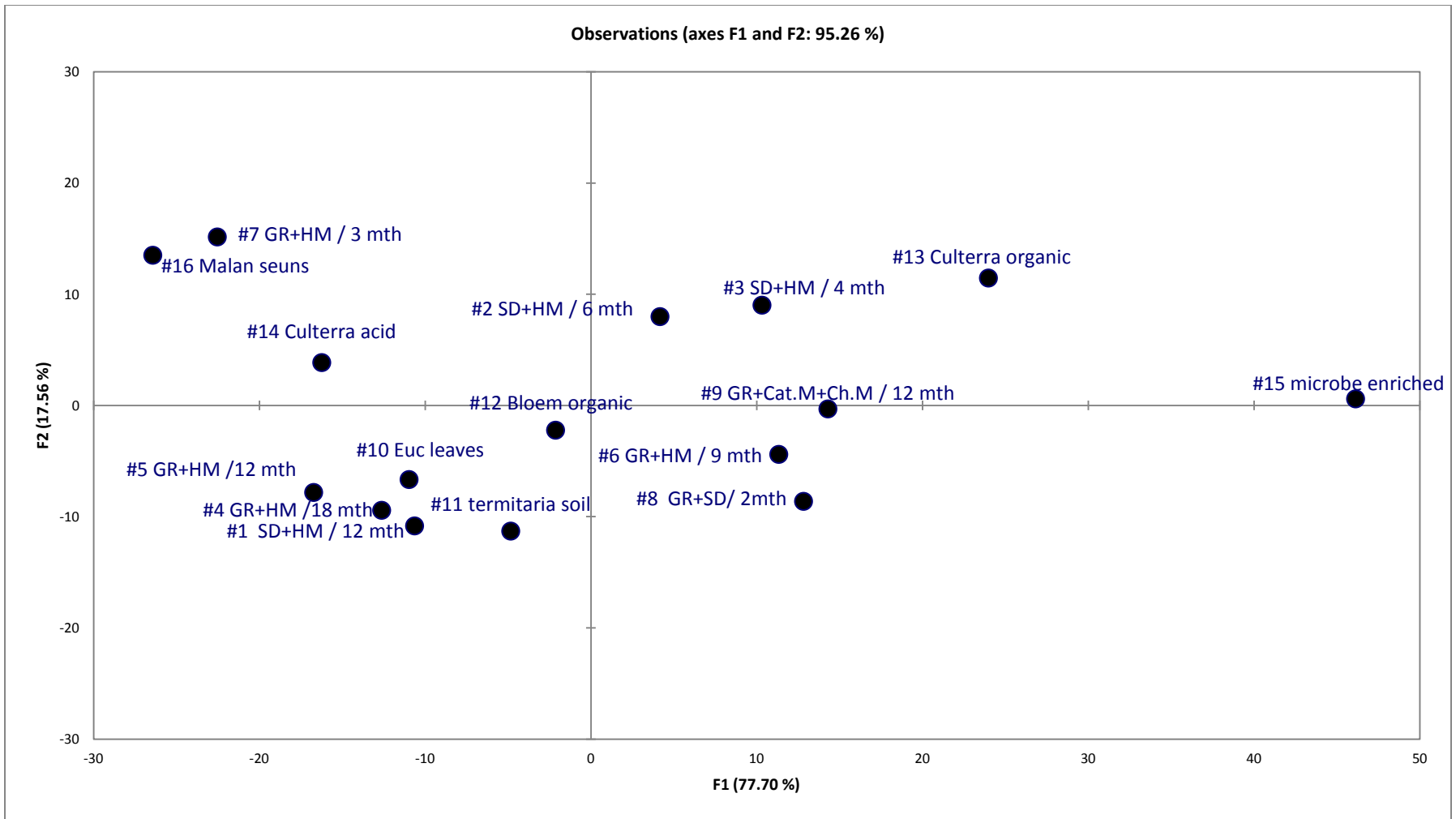


Figure 7: Principal Component Analysis showing the different clustering groups between the composts and termitaria soil based on MIR spectrophotometry.

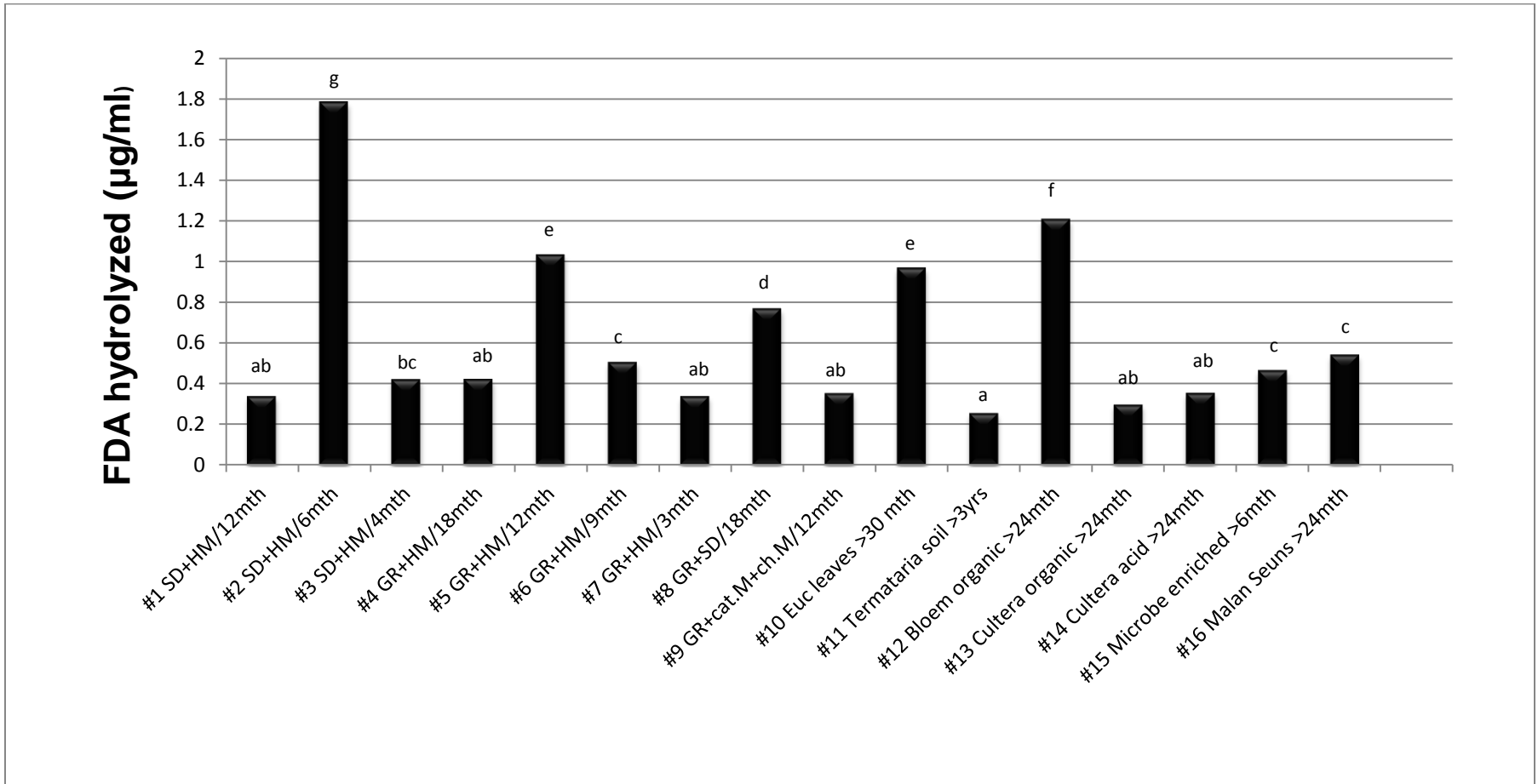


Figure 8: The Fluorescence diacetate hydrolyzed for the respective composts and termitaria soil. Bars not followed by the same letter are significantly different ($P < 0.05$).

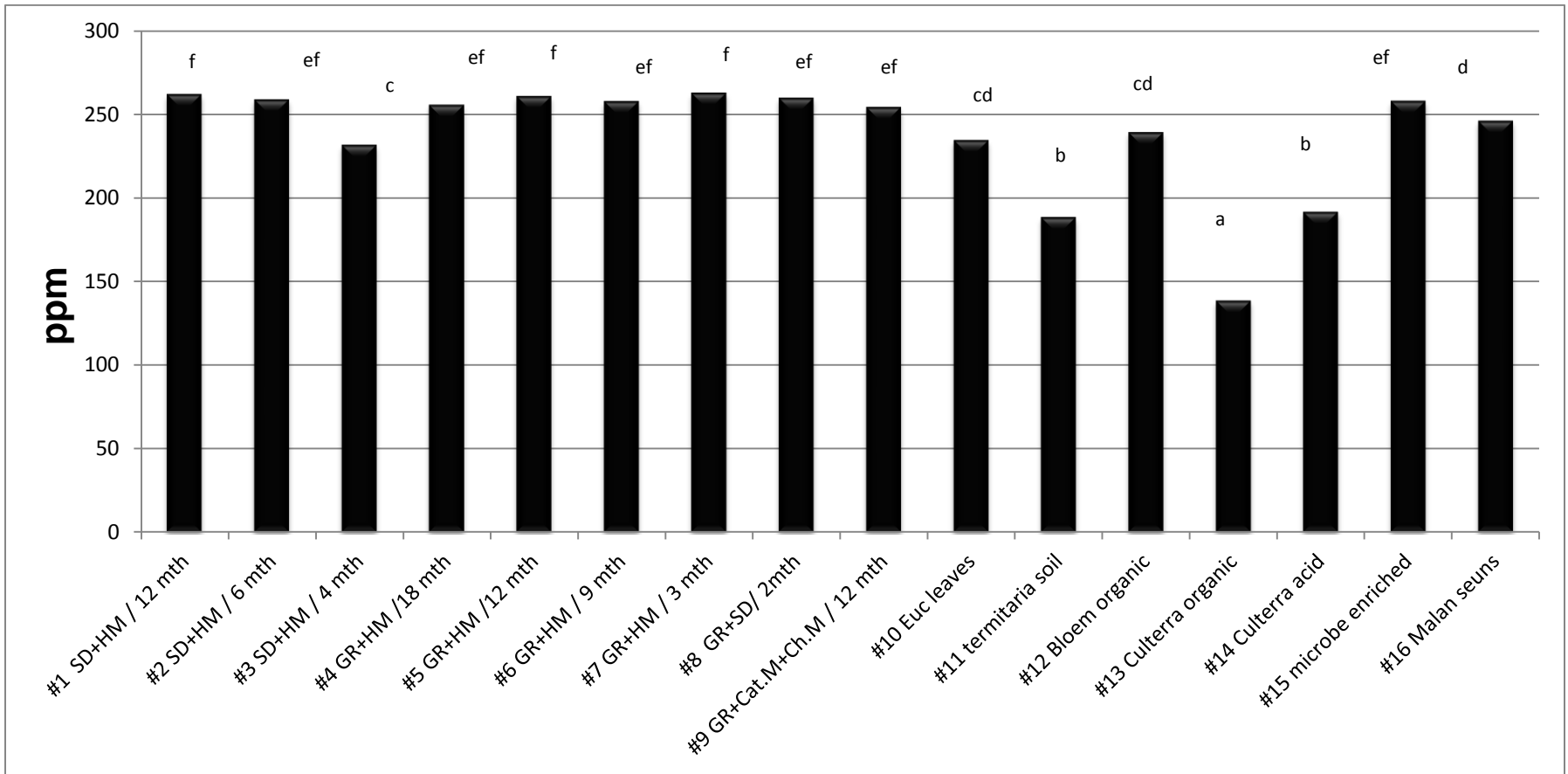


Figure 9: The active carbon (organic matter) of the respective composts and termitaria soil. Bars not followed by the same letter are significantly different ($P < 0.05$).

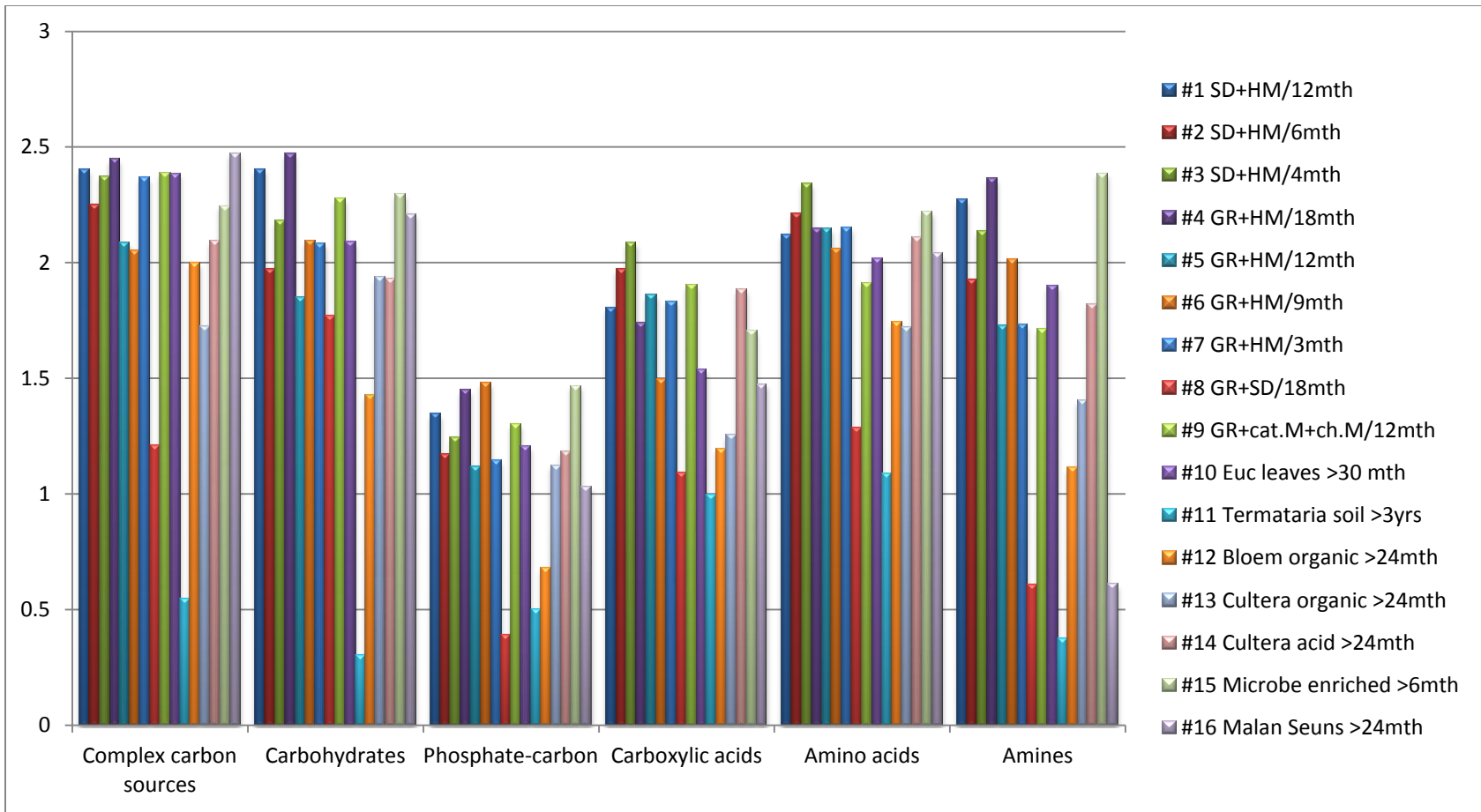


Figure 10: The Average Well Colour Development (AWCD) (590nm) for composts and termitaria soil over an incubation period of 144h.

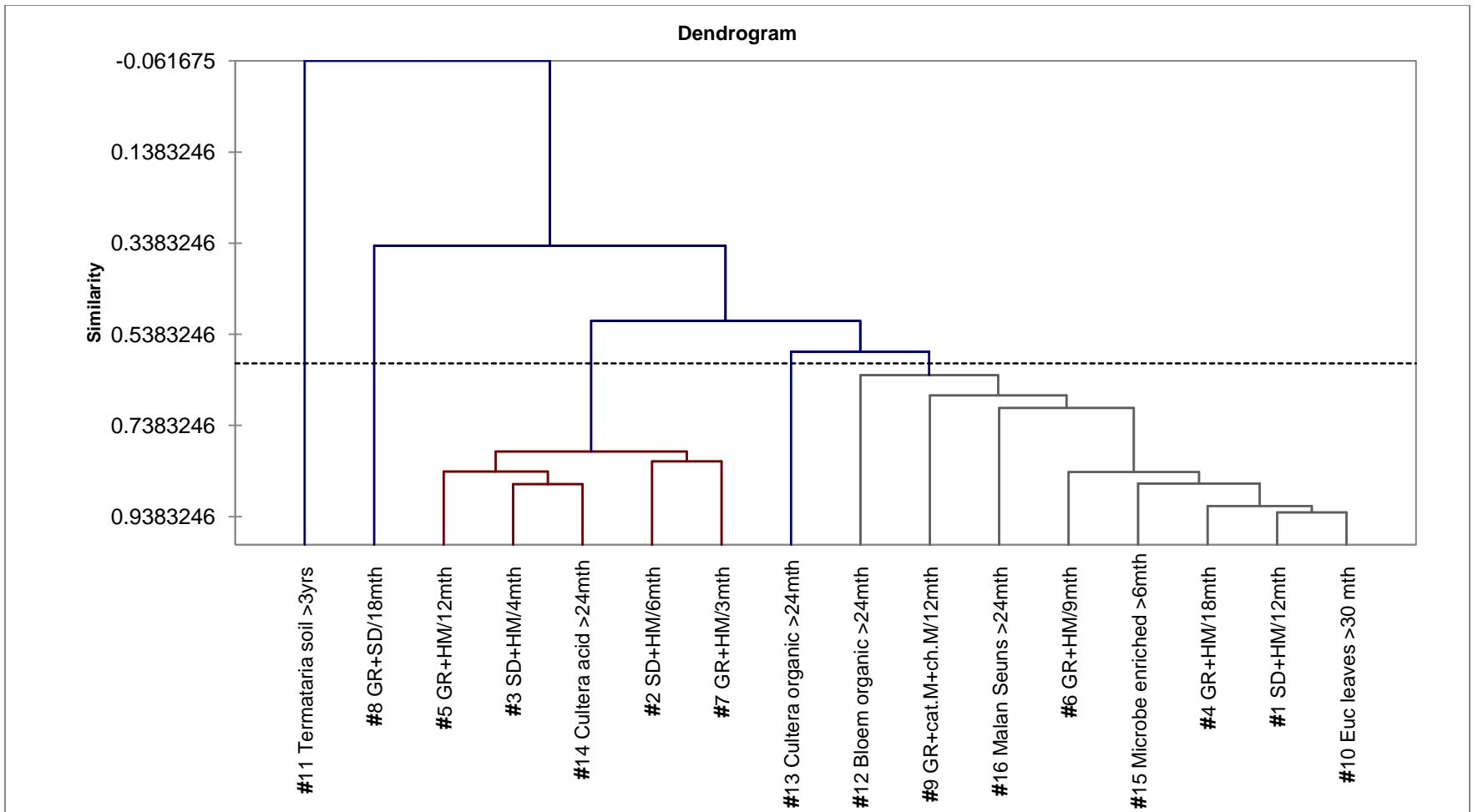


Figure 11: Dendrogram showing the similarities between the composts and termitaria soil based on Biolog Ecoplates®.

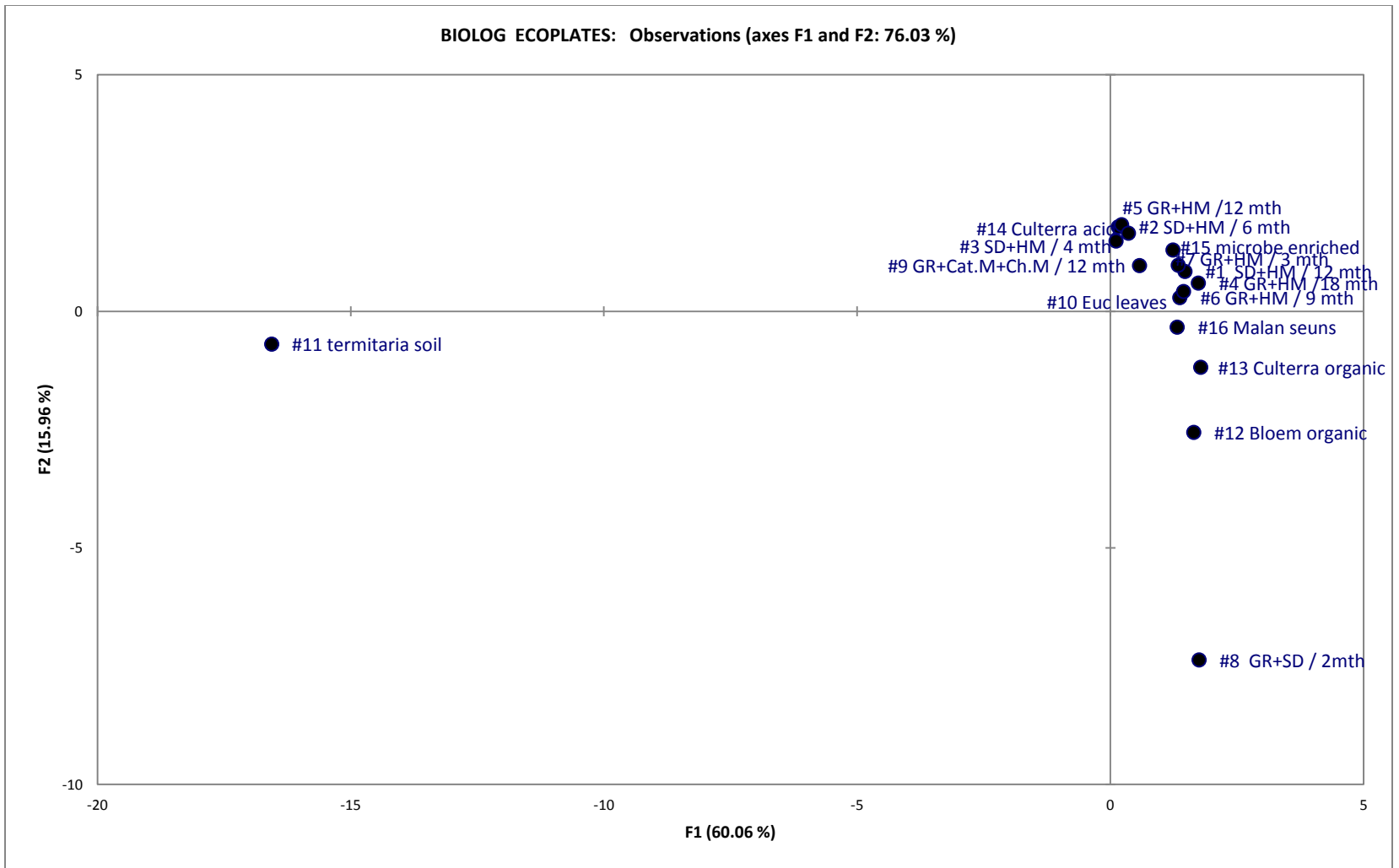


Figure 12: Principal Component Analysis showing the different clustering groups between the composts and termitaria soil based on Biolog Ecoplates®.

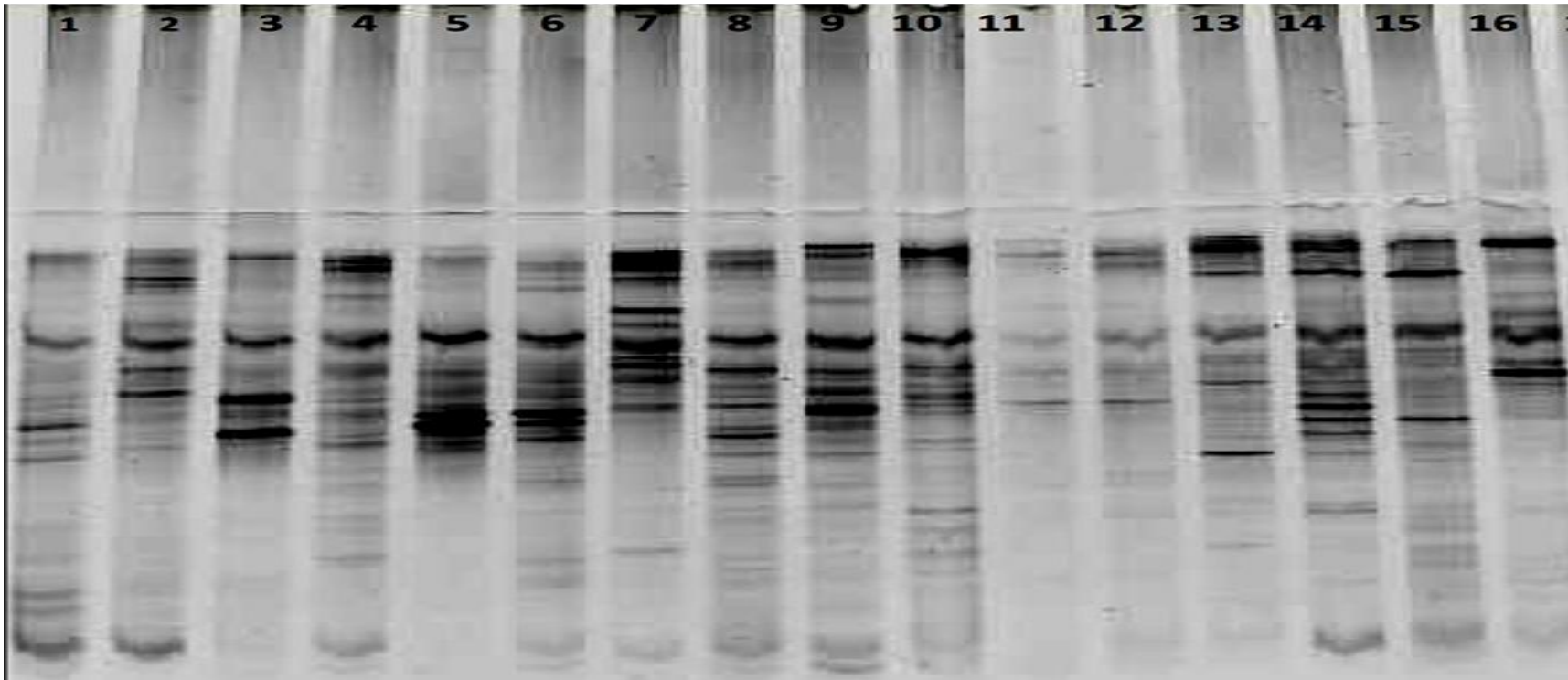


Figure 13: Denaturing gradient gel electrophoresis (DGGE) profile of 16S rDNA fragments. Each lane represents amplicons from genomic DNA extracts for each compost and termitaria soil.

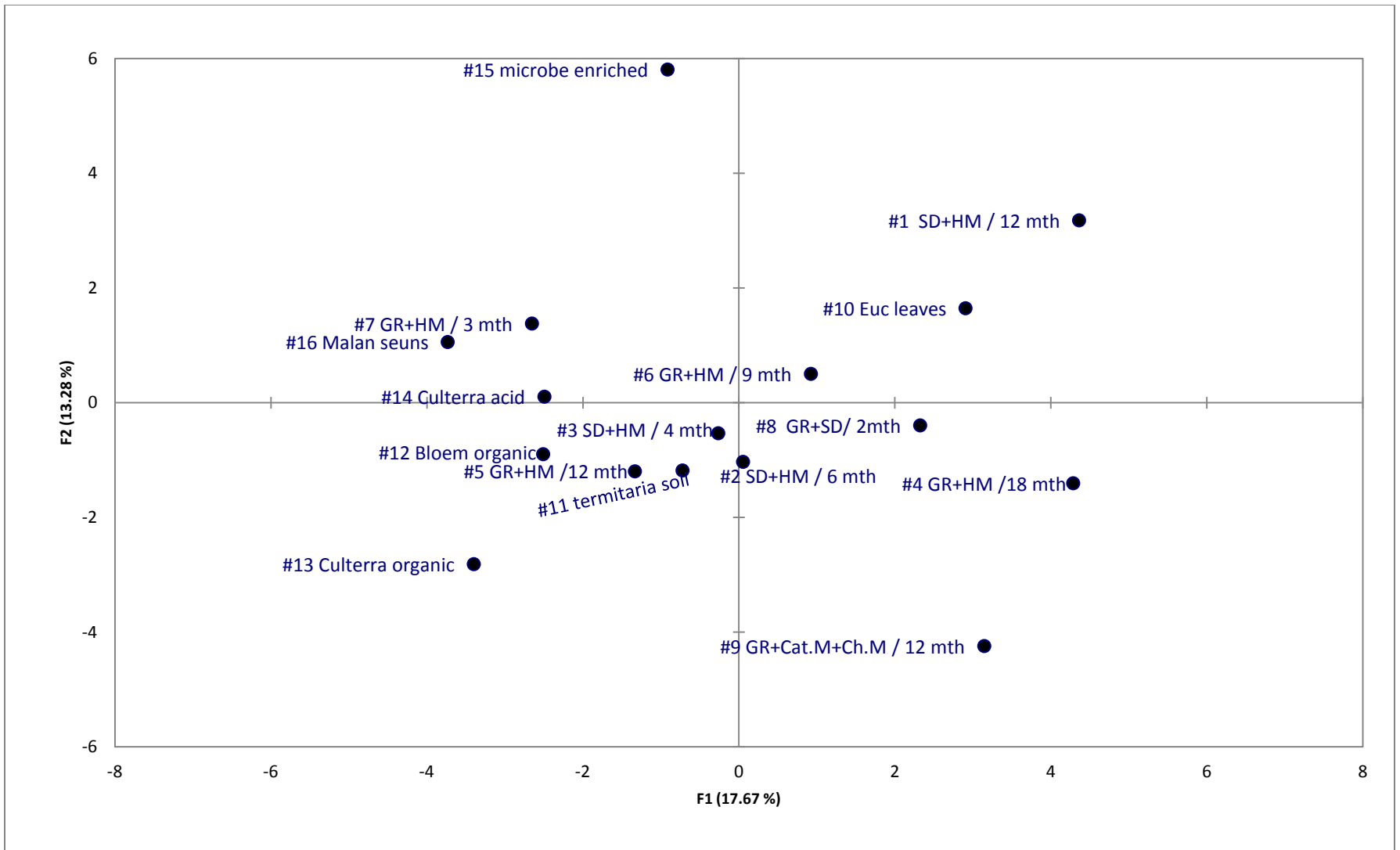


Figure 14: Principal Component Analysis showing the different clustering groups between the composts and termitaria soil based on DGGE.

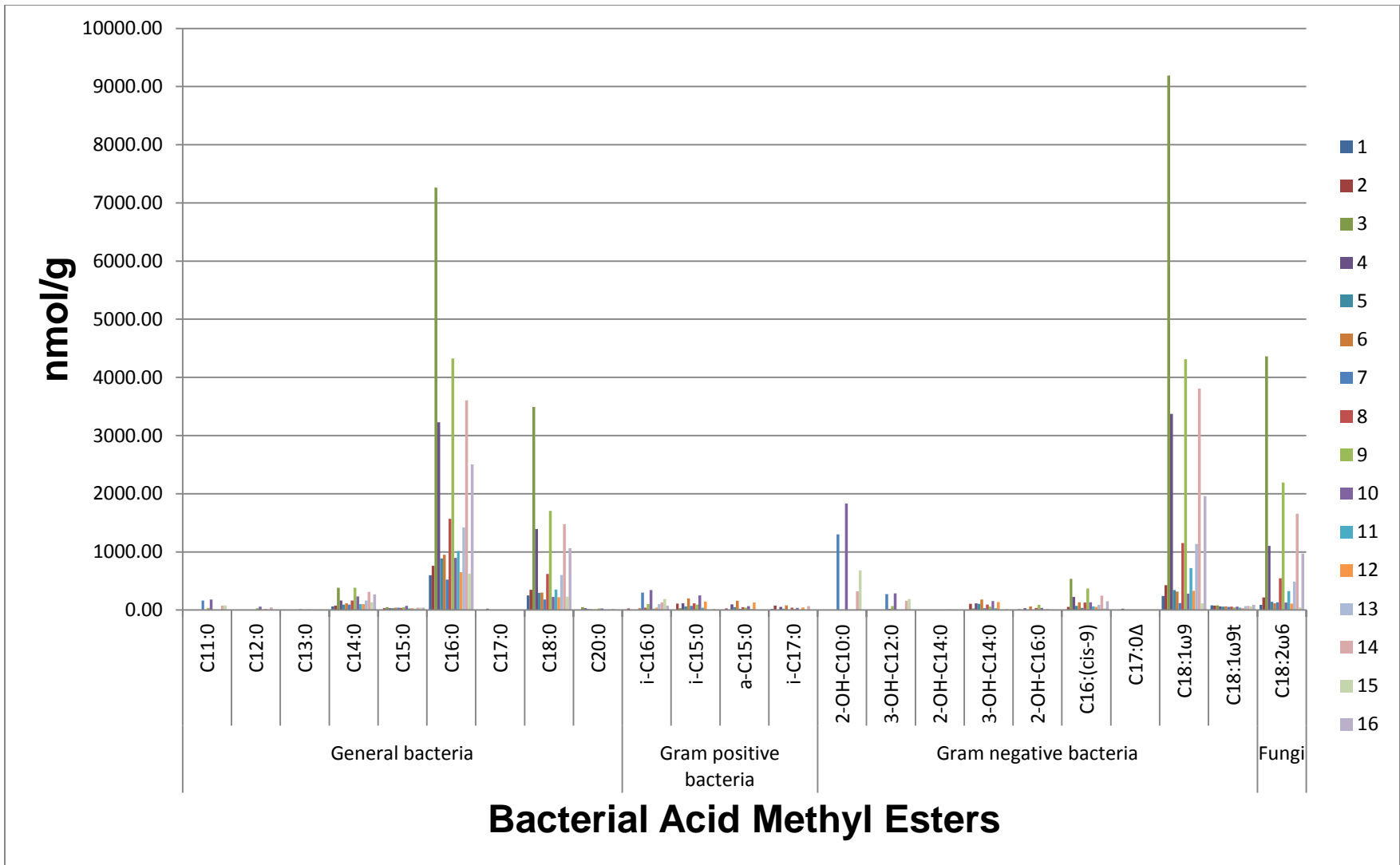


Figure 15: Bacterial Acid Methyl Esters analysis of the different composts and termitaria soil.

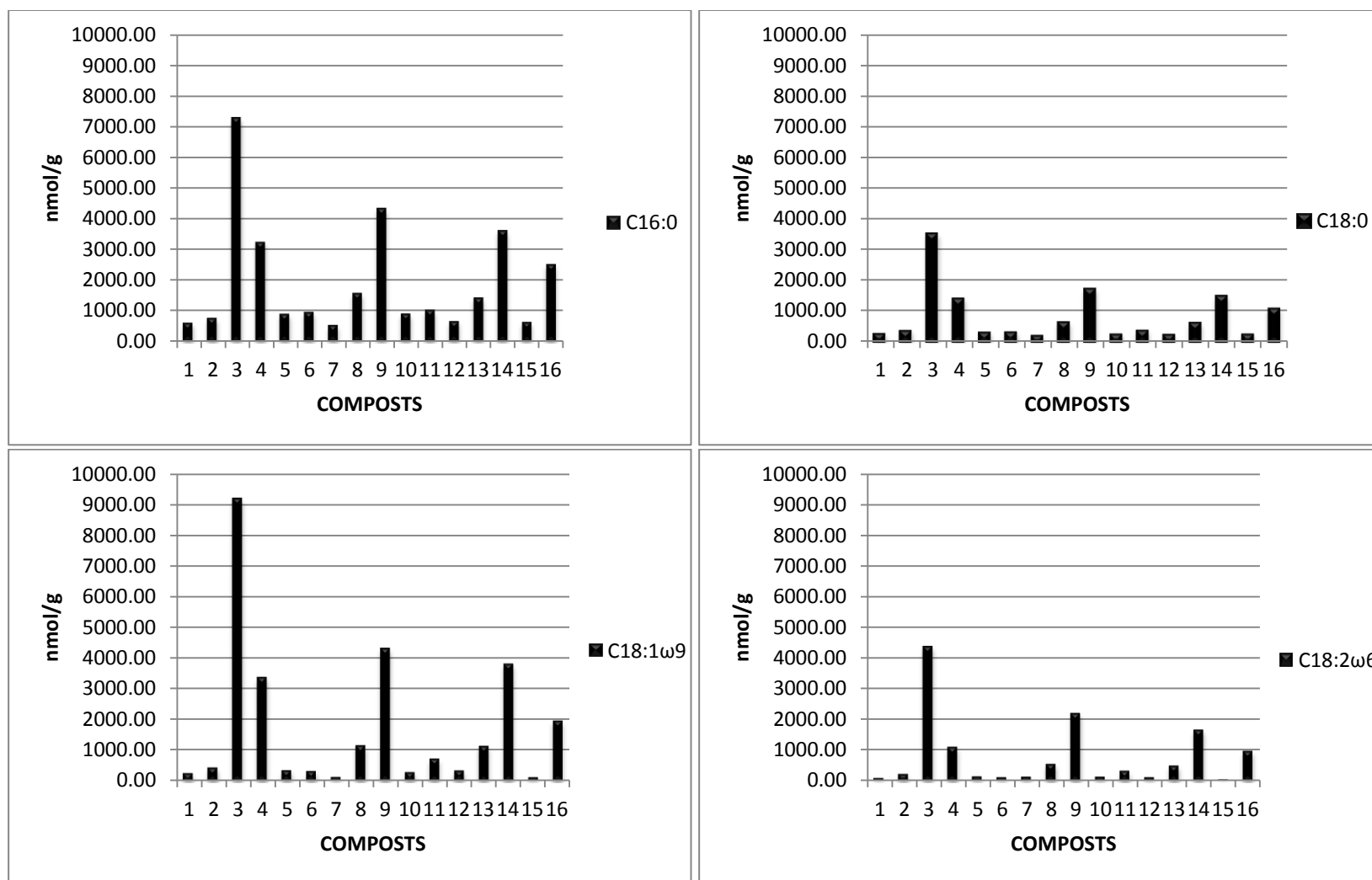


Figure 16: The most common BAMEs (C16:0, C18:0, C18:1 ω 9 and C18:2 ω 6) found in composts and termitaria soil. BAMEs C16:0 and C18:0 are representatives of general bacteria, while C18:1 ω 9 and C18:2 ω 6 are representatives of gram negative bacteria and fungi respectively.

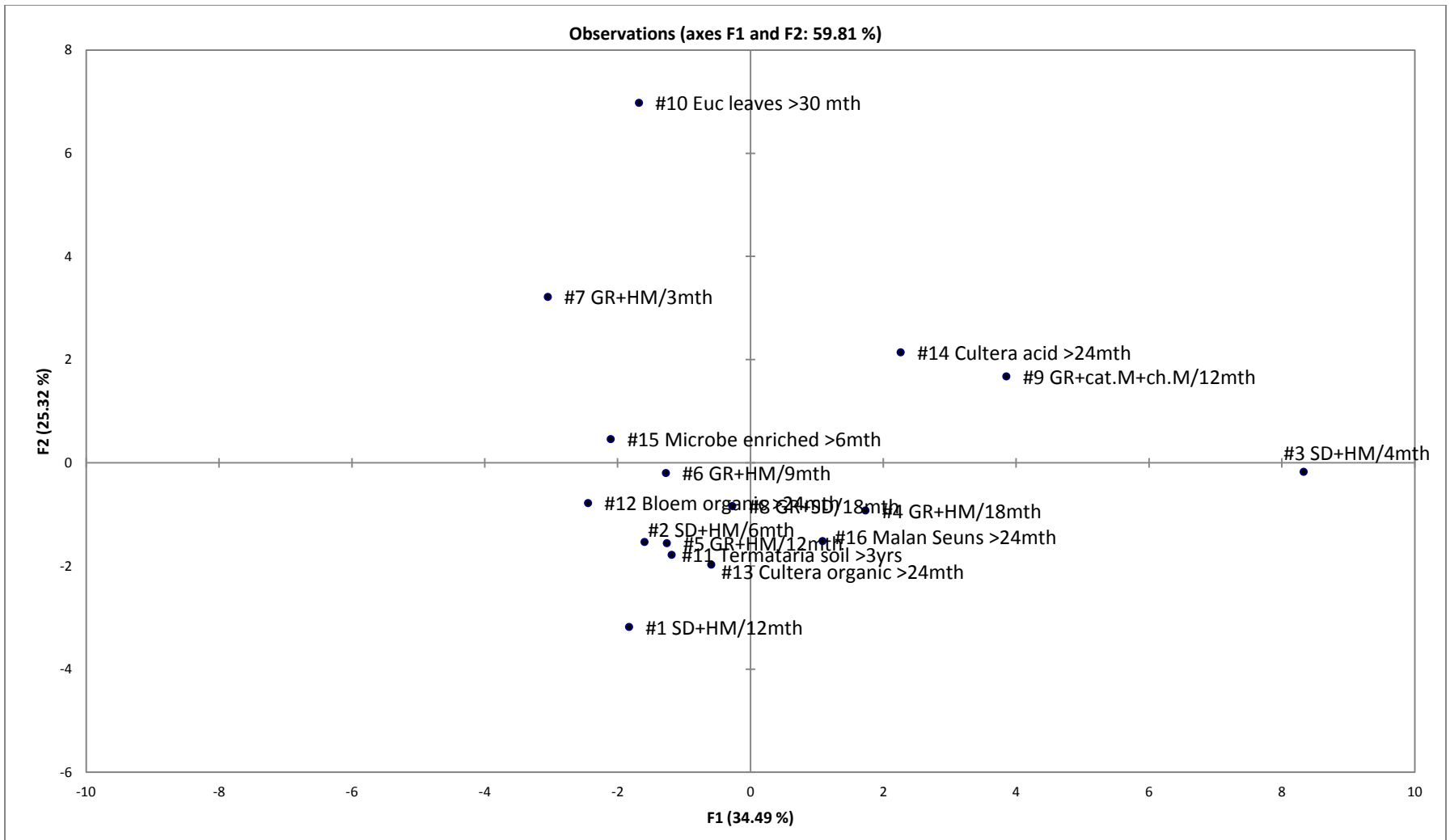


Figure 17: Principal Component Analysis showing the different clustering groups between the composts and termitaria soil based on PLFA.

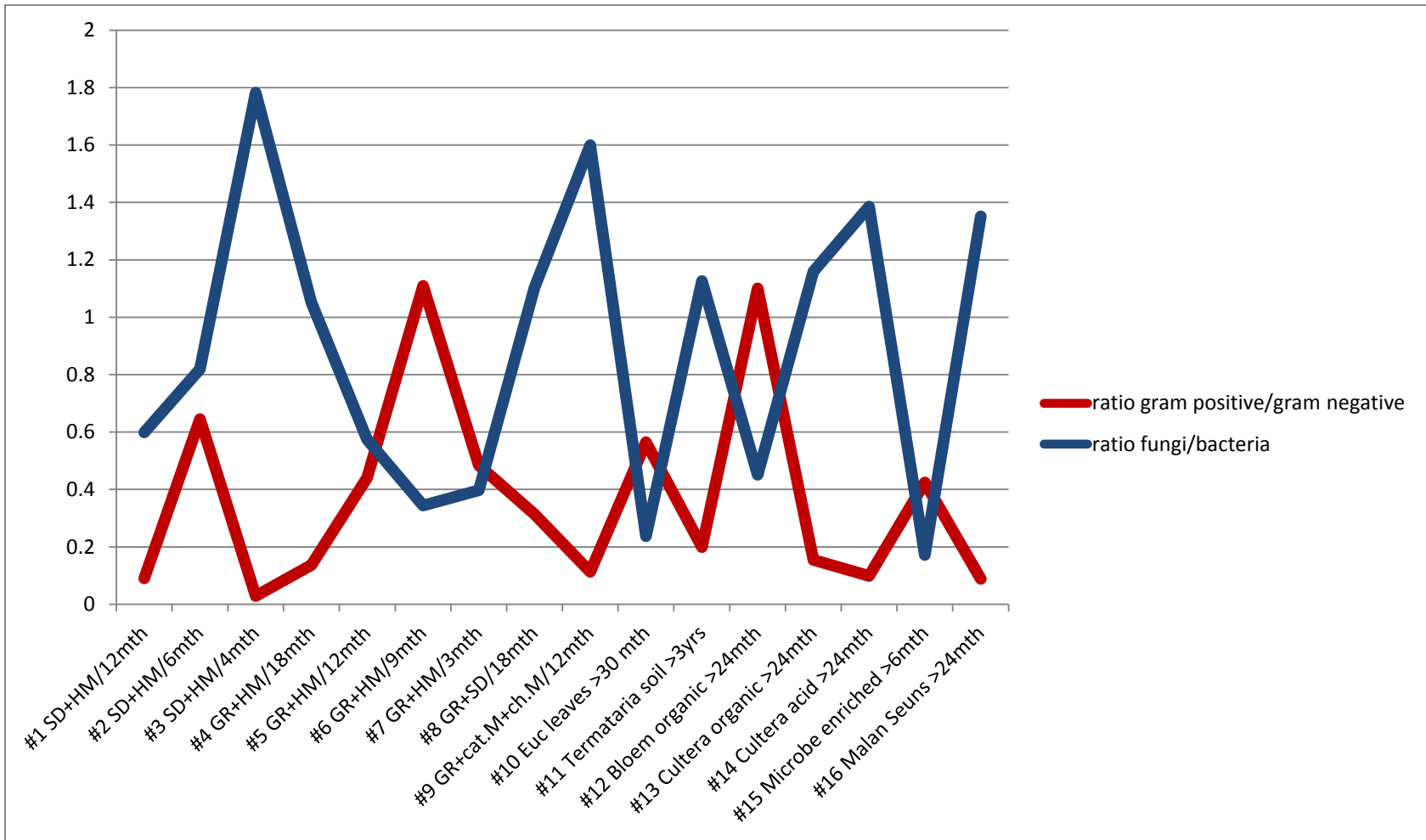


Figure 18: A reading analysis of the ratio between gram positive to gram negative bacteria and fungi to bacteria respectively for respective composts and termitaria soil.

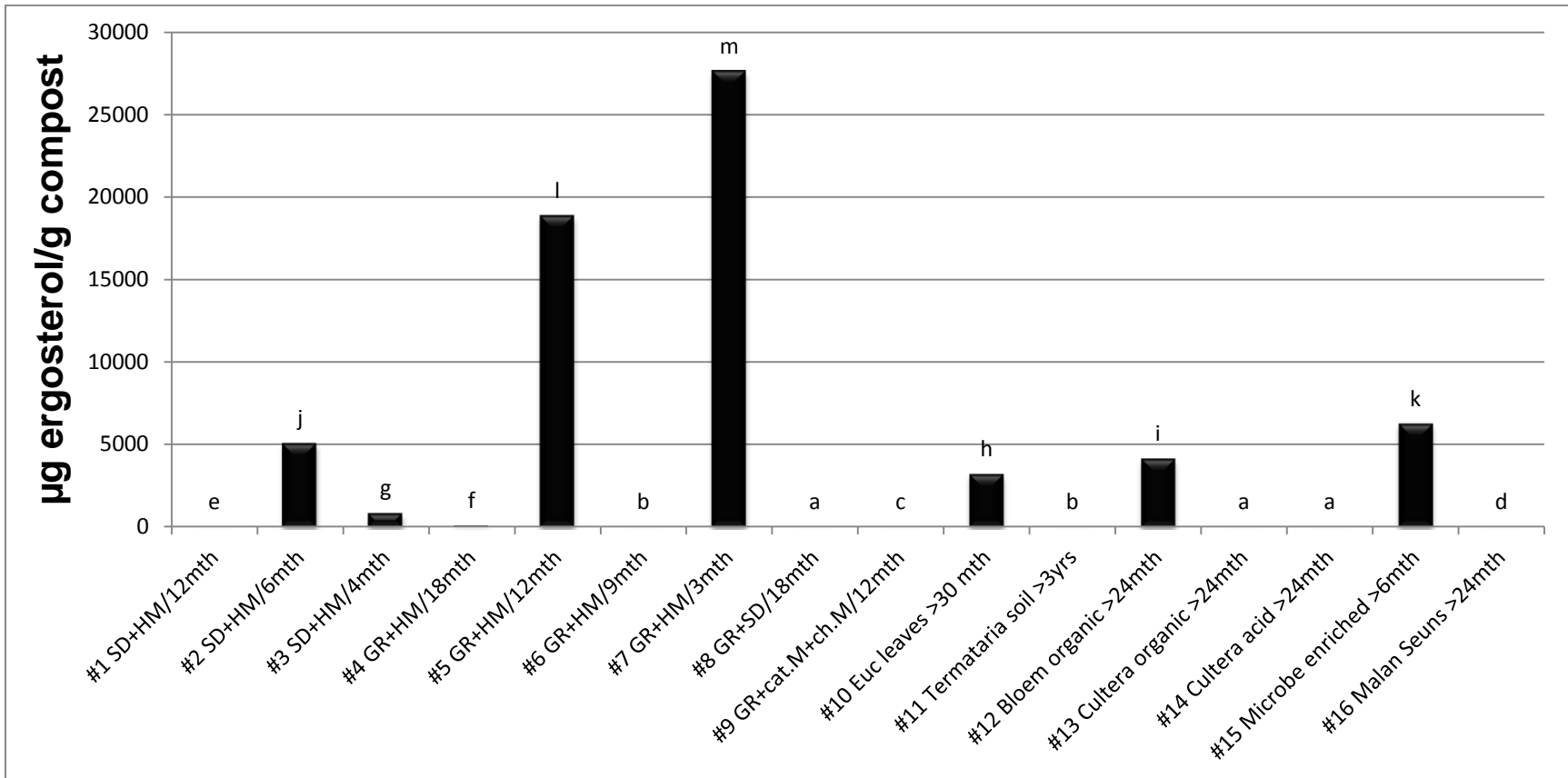


Figure 19: The ergosterol content of the respective composts and termitaria soil. Bars not followed by the same letter are significantly different ($P < 0.05$).

CHAPTER 3

THE INFLUENCE OF GENETIC VARIATION IN PATHOGENS ON THE DISEASE SUPPRESSIVE POTENTIAL OF COMPOST

ABSTRACT

The impact of genetic variation on the pathogenicity (ability to cause root rot) of *Rhizoctonia solani* isolates in various disease suppressive composts was investigated in the glasshouse. Techniques such as the FDA, DGGE, PLFA and ergosterol that examine the chemical and biological attributes of the composts were employed to evaluate the mechanisms of the isolate's response to the compost. The nutritional composition and fungal population density of composts were also studied. There was a highly significant difference between *R. solani* isolates in terms of their pathogenicity and interaction with compost amendments. The study could not however explain the specific mechanisms behind these interactions. Some isolates were suppressed by composts and caused no root rot while others were very pathogenic. The different responses of the isolates to compost amendments indicate that genetic variation of pathogens plays an important role in the disease suppressive ability of compost.

INTRODUCTION

Composts have been reported to provide natural biological control of root and foliage diseases (Lumsden *et al.*, 1981; Nelson, Kuter, & Hoitink, 1983; Tränkner, 1992; Ben-Yephet & Nelson., 1999; Boulter, Trevors & Boland, 2002; Cotxarrera *et al.*, 2002; Diab, Hu & Benson, 2003; Krause *et al.*, 2003; Hoitink & Changa, 2004; Ntougias *et al.*, 2008). Certain composts are known to display disease suppression to a broader range of pathogens than others (Scheuerell, Sullivan & Mahaffee, 2005; Termorshuizen *et al.*, 2006). It is believed that this is due to the composition of the compost and/or the presence of specific microorganisms in the composts (Scheuerell *et al.*, 2005). Hoitink & Fahy (1986) reported that *Aphanomyces* root rot of pea; *Rhizoctonia* root rot of bean, cotton and radish; *Fusarium* wilt of cucumber; *Pythium* damping-off of pea and *Phytophthora* crown rot of pepper were controlled by specific batches of compost. However, the same composts did not control *Fusarium* root rot of pea, *Fusarium* wilt of tomato and *Thielaviopsis* root rot of bean and cotton. This selectivity of disease suppression is because composts differ in substrate and microbial composition, and because the genetic makeup of the respective pathogens differs.

The composition of microbial communities differs between composts and has therefore been linked to the compost's inherent ability to suppress specific plant diseases (Tuitert, Szczech & Bollen, 1998; Diab *et al.*, 2003). Pugliese *et al.* (2008) found that the suppression of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Jarvis *et Shoem*), *Rhizoctonia solani* (Kühn) and *Phytophthora nicotianae* (Breda de Haan) were associated with *Fusarium* spp. (strain K5, K7, K9, and K11), bacteria (strain B17) and

Trichoderma spp. (strain E28 and E36), respectively, that were present in the composts. Thermophilic bacteria and fungi are reported to dominate in compost during the initial stage but, during the subsequent curing stage, mesophilic microorganisms recolonize the compost (Hoitink & Fahy, 1986). Depending on the stage of the compost and when it is applied to soil, will thus determine the suppressive effect it has on potential plant pathogens.

The application of composts increases the biomass and enzyme activity of resident beneficial microorganisms in soil (Schnürer & Rosswall, 1982). Beneficial microorganisms that control disease are known as antagonists, and disease control obtained through antagonists involves four basic mechanisms. Firstly, competition for nutrients where nutrient demand exceeds the immediate supply of nutrients (Craft & Nelson, 1996; Hoitink & Boehm, 1999). Secondly, production of antibiotics harmful to pathogens such as *Pythium* and *Rhizoctonia* damping-off (Craft & Nelson, 1996; Hoitink, Stone & Han, 1997; Bailey & Lazarovits, 2003; Raviv, 2008). For example, certain *Pseudomonas* spp. are known to produce antibiotics in the rhizosphere of plants that inhibit pathogens. The third mechanism is parasitism, where the pathogen is preyed upon by the suppressive agent (Diab *et al.*, 2003; Raviv, 2008). Diab *et al.* (2003) showed suppressiveness to *Rhizoctonia* damping-off in impatiens to be associated with the functional and population diversity of a stable compost-amended soil mix. Tuitert *et al.* (1998) also showed the suppression of *R. solani* in long matured compost to be associated with high populations of cellulolytic and oligotrophic actinomycetes. The fourth mechanism involves plant defence responses to the presence of antagonists, resulting in induced resistance (Zhang, Dick & Hoitink, 1996;

Zhang *et al.*, 1998). Zhang *et al.* (1996; 1998) found compost to induce resistance to *Pythium* root rot, cucumber anthracnose and bacterial speck of *Arabidopsis*. The addition of specific antagonistic fungi such as, *Verticillium biguttatum* (Gams), *Fusarium oxysporum* (non-pathogenic isolate), *Trichoderma asperellum* (Rifai) and *T. harmatum* to compost have also been implicated in the enhancement of disease suppressiveness (Postma, Montanari & van den Boogert, 2003; Fracchia *et al.*, 2006, Trillas *et al.*, 2006; Joshi *et al.*, 2009).

Most reports on compost disease suppressiveness are based on a single species of pathogen but in reality plants are in their lifetime exposed to numerous species of pathogens, or the same species comprising different genotypes. Each pathogen responds differently to varying compost amendments. For example, Termorshuizen *et al.* (2006) tested 18 composts against 7 fungal pathogens and the outcome was that each pathogen varied in response to compost amendment. This was attributed to competition-sensitivity in pathogens such as *F. oxysporum* and *R. solani* and the fact that there is some specificity or genetic variation in pathogens such as *R. solani*. Scheuerell *et al.* (2004) had previously reported that suppression of *Rhizoctonia* damping-off was not related to any specific compost factor but rather to genetic variation of the pathogen that made certain genotypes of it to be more resistant to certain composts than to others. It has been reported that some pathogen genotypes, for example anastomosis groups (AG) in *R. solani*, are more virulent than others and that their host specificity differs accordingly (Nelson *et al.*, 1996).

Determining the microbial composition of composts is important if we want to understand the mechanisms that are operating within them. Most studies to date make use of culture dependent methods such as the isolation and identification of microorganisms using culture media to identify microbial species present in the compost. However, culture media can detect only 1-15 % of microbes and are time consuming and unspecific (Boulter *et al.*, 2002; Novinscak *et al.*, 2008). Culture independent techniques offer the advantage of targeting a much wider range of microbes. Over the years, Real-Time Polymerase Reaction (qPCR) based methods have been the favoured approach for detection of fungal and bacterial pathogens in composts. Physiological profiling using techniques such as Biolog microplates and analysis of phospholipid fatty acids (PLFA), and DNA based techniques such as denaturing gradient gel electrophoresis (DGGE) may also lead to an improved understanding of the changes in microbial communities associated with disease control resulting from compost amendments (Pradhanang, Elphinstone & Fox, 2000; Parsons, Faggian & Lawrie, 2003; Noble & Coventry, 2005; Novinscak *et al.*, 2008).

The objective of this study was to examine the different responses of 7 isolates of *R. solani* to 10 commercial and homemade composts of different composition and age. Various microbiological and molecular methods were used to elucidate the inherent quantitative and qualitative microbial activities of the respective composts.

MATERIALS AND METHODS

Ten composts (commercial and homemade) were obtained from various nurseries (Table 1). The impact of genotype variation of the seven *R. solani* isolates

and their relationships with the chemical and biological attributes of the composts was tested in the greenhouse and laboratory as described below.

Pathogenicity bioassay

Fungal strains: Three isolates of *Rhizoctonia solani* were sourced from CBS Fungal Biodiversity Centre, viz: CBS 101779 *R. solani* AG 6 Japan, CBS 101780 AG 7 Japan and CBS 969, 96 AG 9, USA. Two isolates were sourced from ARC Plant Protection Research Institute viz: *R. solani* PPRI 10494, South Africa, AG unknown and PPRI 10041, South Africa, AG unknown. Two remaining isolates were obtained from the Department of Plant Science, University of the Free State culture collection, viz: CCP 160 AG 1 and CCP 159 AG group unknown. The fungi were grown on potato dextrose agar (PDA) containing streptomycin (0.5 mg/l) and incubated for 7 days at 24 °C. Mycelial disks 5 mm in diameter were taken from the colony margin of a 7-day-old culture and used to prepare inoculum, as described below.

Inoculum preparation: Unrolled oats was soaked in 100 ml distilled water in 1l Ehrlenmeyer flasks for 20 hours. Excess water was removed and the seed mixture autoclaved for 1 hour at 121 °C on two consecutive days, and then allowed to cool down to 25 °C. Agar discs containing mycelium from each of the seven *R. solani* isolates were added to respective flasks and incubated at 25 °C for 2 weeks with intermittent shaking. The colonized seed was then removed from flasks, air dried in a sterile environment at 30 °C for 72 hours, ground into a fine powder using a coffee grinder and stored in sterile containers at room temperature.

Greenhouse bioassay: Pathogenicity trials were conducted according to Krause, Madden & Hoitink (2001) on radish seedlings with the seven isolates and ten composts in the greenhouse in order to evaluate isolate virulence as affected by the different composts. Disease suppressiveness of each compost was evaluated using inoculum prepared as described above. Compost was mixed with soil in a ratio of 2:3 (40%; 60%), and then placed in 10 x 10 cm plastic pots. The mixture was allowed to stabilize for 7 days, and watered daily. Inoculum (25 g) of each *R. solani* isolate was added to each pot and 10 radish seeds were then distributed evenly in each pot. Soil without compost or inoculum was used as negative control treatment. The positive control included soil without compost inoculated with each of the isolates.

A randomized complete block design was used for the experiment and each treatment was replicated four times. Pots were watered daily and incubated for 3 weeks. The numbers of germinated seedlings in each pot were counted after three weeks and the wet biomass of each seedling was determined. The experiment was conducted twice and data collected were subjected to analyses of variance (ANOVA) using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Data and means were separated using Fischer's least significant difference ($LSD_{0.05}$) test.

Compost evaluation

Chemical composition of the composts: A chemical analysis of composts was conducted using the method by Hesse (1971) to evaluate the nutrient composition of

composts and their pH values. The procedure was carried out at Department of Soil, Crop and Climate Sciences of the University of the Free State, Bloemfontein.

Fungal diversity of composts: A sample of each compost (1 g) was placed in 10 ml of sterile distilled water in a water flask and shaken mechanically for 10 min. The suspension was diluted (10^{-1} to 10^{-6}) and 0.1 ml of each dilution aliquoted onto separate petri-dishes (50 % malt extract agar) in 4 replications. The plates were incubated for 48 hours at 22 °C and the total number of fungal colonies growing in each plate was recorded. Dilution plates that had colonies between 5 and 200 were selected and the total number of fungal colonies/or number of colony forming units (CFUs) was estimated (Kena, 2002; Pugliese *et al.*, 2008; Sharma, 2010; Sharma, 2011). Hyphal tips were removed from each colony with a sterile dissecting needle and placed onto malt extract agar supplemented with 0.5 mg of streptomycin sulphate. After incubation (4-10 d) at 25 °C, the fungi were classified into genera using standard mycological procedures and available literature. Isolates from certain genera that could not be classified into species were transferred onto specific agar media and/or sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, for sequencing to identify to species level.

Fluorescein diacetate (FDA): The procedure was adapted from Adam & Duncan (2001), and as modified by Zabaloy, Garland & Gómez (2008) to measure microbial activity in the composts by the rate of FDA hydrolysis. Two solutions were prepared, namely, 1) a substrate solution containing 0.1 g of FDA which was dissolved in 100 ml of acetone and stored at -20 °C, and 2) a buffer solution containing 8.7 g of K_2HPO_4 and 1.3 g of KH_2PO_4 , dissolved in 1l of deionized water (pH 7.6) and stored at 4 °C.

A 2 g sample of compost was placed in a 50 ml test tube and 20 ml of 60 mM potassium phosphate buffer (pH 7.6) was added followed by 0.20 ml of FDA substrate. The tubes were incubated at 28 °C for 20 min and shaken three times by hand. The tubes were then placed on an orbital shaker at 300 rpm for 10 min at room temperature before 15 ml of 2:1 chloroform: methanol solution was added and the suspension briefly swirled to terminate FDA hydrolysis. After that the supernatant was placed in 1.5 ml eppendorf tubes and centrifuged for 3 min at 300 rpm to remove excess debris, whereafter the content (1.2 ml) was pipette and placed in separate cuvettes. The spectral absorbance was measured on a spectrophotometer (Varian 8x6 Multicell Block Water Thermostatted Series II, Australia) set at a wavelength of 490 nm. The amount of FDA hydrolyzed by the respective composts was determined from the standard curve and expressed as µg/ml FDA hydrolyzed. A standard curve was prepared by adding 0 µl, 10 µl, 20 µl, 40 µl, 60 µl, 80 µl, and 100 µl of FDA to 5 ml of the potassium phosphate buffer in test tubes. The test tubes were placed in water bath for 60 min and then 15 ml of 2:1 chloroform: methanol solution was added and the suspension swirled to terminate FDA hydrolysis. The absorbance was measured on a spectrophotometer set at a wavelength of 490 nm and plotted on a graph. Analyses of variance (ANOVA) using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) was then performed on the data and means were separated using Fischer's least significant difference ($LSD_{0.05}$) test.

Denaturing Gradient Gel Electrophoresis (DGGE): DGGE is one of the fingerprinting methods used to describe and compare microbial communities in a given environment

(Muyzer *et al.*, 1997; Green, Leigh & Neufeld, 2009). The technique involves the following steps:

DNA extractions: PowerSoil[®] DNA isolation kit (MO BIO Laboratories, Inc.) was utilized for DNA extractions on the 10 composts. The procedure was conducted according to the manufacturer's instructions.

DNA concentrations and purity: The concentrations and purity of genomic was determined using a Nano Drop spectrophotometer 3300 fluorospectrometer (Thermo Scientific) prior to 16S rDNA Polymerase Chain Reaction (PCR) amplification. The community rDNA's were PCR amplified from 1-50 ng of bulk DNA in reaction mixtures using a standard procedure (SOP_29) developed in the Metagenomics Platform laboratory of the University of the Free State (UFS), Bloemfontein. The PCR amplified fragments were verified on a 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination. A nested PCR approach was applied where a fragment of the same 16S rDNA region was obtained for all specific bacterial groups using the target 16S rDNA PCR products to amplify DNA fragments suitable for DGGE analysis. The DNA fragments were verified on a 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination and the amplified DNA fragments were exposed to a gradient of denaturant (urea / formamide) at an elevated temperature (60 °C) within the polyacrylamide gel (Tzeneva *et al.*, 2008). The analysis was carried out according to the standard procedure (SOP_19b) developed in the Metagenomics Platform laboratory using a DCode[™] Universal Mutation Detection System (BioRad).

PCR amplification of bacterial 16S rRNA gene: Bacterial-specific primers 27F and 1492R (Weisburg *et al.*, 1991) were used in the PCR amplification and the PCR reaction mixture contained 10 µM of each primer set, 10 mM of dNTPs, 1 – 50 ng of genomic DNA, 1.25U of Taq DNA polymerase (SuperTherm) and reaction buffer in a final volume of 50 µl. The reaction mixture was incubated at 95 °C for 2 min and this was followed by 30 cycles of amplification. Each cycle consisted of denaturation at 95 °C for 30 sec, annealing at 52 °C for 45 sec and extension at 72 °C for 1 min. While the final extension was at 72 °C for 10 min, this was followed by visualization on a 1% agarose gel electrophoresis stained with ethidium bromide under UV illumination. Nested PCR was performed using gel stabbed 16S rDNA products as templates and the same PCR program was used for PCR amplification with primers set 341F-GC and 517R (Muyzer *et al.*, 1997).

DGGE and analysis: DGGE analysis was carried out according to the standard procedure (SOP_19b) developed in the Metagenomics Platform laboratory using a DCode™ Universal Mutation Detection System (BioRad), followed by staining and visualization under UV transillumination. The DGGE banding profile of each sample was quantified and compared with Quantity One® 1- D Analysis imaging software available from the BioRad Molecular Imager Gel Doc™ XR System to determine relatedness (SOP_66) (Green *et al.*, 2009) and principal component analysis (PCA) was performed on the data.

Phospholipid Fatty Acid (PLFA) analysis: PLFA analysis evaluates total microbial composition in an environment. The procedure was conducted according to the method modified by White *et al.* (1979) as described by Marschner (2007) as follows:

Lipid Extraction: Compost samples (2 g) were weighed in 25 ml glass centrifuge tubes, whereafter 1.5 ml citrate buffer, 1.9 ml chloroform, 3.8 ml methanol and 2 ml Blight and Dyer reagent were added to the tubes, and vortexed for 1 min each. The tubes were shaken for 2 h, followed by centrifuging at 3500 g for 10 min. The supernatant was transferred into a clean 25 ml glass centrifuge tube. The substrate pellet was again washed with 2.5 ml Blight and Dyer reagent (vortexed and centrifuged as previously described) and the supernatant combined with the previously obtained supernatant.

Phase Separation: The supernatant was then diluted with 3.1 ml chloroform and 3.1 ml citrate buffer, vortexed and centrifuged at 3500 g for 10 min. Following this procedure, 1–3 ml from the lower (organic) phase was transferred into a new 10-ml glass tube, and dried at 40 °C under a stream of N₂.

Lipid Fractionation: Dried samples (organic phase) were dissolved in 300 µl chloroform and then pipetted to the conditioned (2x 1 ml chloroform) silica-bonded column. The pipettes were rinsed with 300 µl chloroform between samples. Neutral lipids were eluted with 5 ml chloroform followed by elution of glycolipids with 10 ml acetone and both were discarded. Phospholipids were then eluted with 5 ml methanol and transferred to a 10 ml centrifuge tube, dried at 40 °C under a stream of N₂.

Alkaline Methanolysis: A 30 µl internal standard (C19:0) was added to dried samples and then suspended in 1 ml methanol toluol solution (1:1, v/v). Thereafter, 1 ml 0.2 M

KOH-MeOH was added to the suspensions, vortexed briefly and incubated for 15 min at 37 °C. A 2 ml hexane–chloroform solution, followed by 0.3 ml of 1 M acetic acid and 2 ml deionized water were added, vortexed for 1 min, and centrifuged for 5 min at 3500 g. The supernatant was transferred into 10 ml glass tubes and 2 ml of a hexane–chloroform solution added, vortexed and the supernatant combined with the previously obtained one. The suspensions were dried at 40 °C under a stream of N₂.

Fatty Acid Determination and data analysis: Dried extract was dissolved in 130 µl isooctane. Fatty acid methyl esters (FAME) were quantified using a Varian 430 flame ionization gas chromatograph (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 µm ID, 0.2 µm film thickness). Column temperature was 40–230 °C (hold 2 minutes; 4 °C/min; hold 10 min). Fatty acid methyl esters in isooctane (1 µl) were injected into the column using a Varian CP 8400 autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19).

Peak identification of the fatty acids and the fatty acid profile were done with an external standard, Supelco 37 component FAME mix and the Sigma bacterial acid methyl ester (BAME) mix. Calculations were performed according to the equation listed by Marschner (2007), and data classified into different Bacterial Acid Methyl Esters

(BAMEs) according to Whalen & Sampredo (2009). The BAMEs were then grouped in to their respective microbial groups and PCA was performed.

Ergosterol analysis: The procedure estimates fungal biomass in a substrate, and was conducted according to the method of Ruzicka *et al.* (2000) and Klamer & Bååth (2004). Compost samples (20 g) were added to 50 ml of methanol and mixed in a 100 ml beaker for 90 min, using a magnetic stirrer. The mixture was allowed to settle and 25 ml of the clean extract was added to a test tube with a screw cap, containing 3 g of KOH pellets. The mixture was stirred in a vortex mixer to dissolve the KOH crystals, whereafter 10 ml of n-hexane was added and the mixture was incubated for 30 min in a 75°C water bath. Distilled water (5 ml) was added, mixed well and cooled to room temperature. The hexane supernatant was transferred to a 50 ml beaker, and n-hexane (10 ml) was added to the rest of the aliquot in the test tube and mixed well. The hexane layer was removed and added to the earlier aliquot. The last two steps were repeated. The hexane extract was evaporated in the beaker, until dry in a hot water bath. The residue was dissolved in 5 ml methanol and filtered through Acrodisc GHP membrane filter. The filtrate was analyzed on a HPLC (Flexar HPLC Detector, Perkin Elmer) machine. The amount of ergosterol content was determined with reference to a standard curve prepared according to Jambunathan, Kherdekar & Vaidya (1991), and expressed as µg/g ergosterol. The standard curve was prepared using the following ergosterol standards concentrations: 0, 1, 5, 10, 15, 20 and 25 µg ergosterol /ml methanol. The results were subjected to an ANOVA using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA), and data and means separated using Fischer's least significant difference ($LSD_{0.05}$) test.

RESULTS

Pathogenicity bioassay

Greenhouse bioassay. The mean germination % and mean biomass (g) of radish seedlings for the seven *R. solani* isolates were significantly different from each other ($P < 0.05$) (Figure 1, 2 and 3). The responses of the respective isolates to the composts were variable (Table 2 and Figure 4). Isolates CCP160 and PPRI10041 were the most pathogenic isolates with an average mean germination of 15.4 and 15.6 % respectively. These two isolates were closely (>74 %) related to each other (Figure 4). The highest mean germination was recorded on compost C and B respectively, for isolate CCP160 and PPRI10041 both at 23.8 %. They were however significantly lower ($P < 0.05$) than the negative control treatment but not significantly different from the positive control treatment and any of the other composts.

Isolates PPRI10494, CBS101780 and CCP159 respectively, ranked 3rd, 4th and 5th in terms of mean germination % and biomass (g) of radish seedlings. The highest germination was for isolate PPRI10494 in compost I (40 %) and although significantly lower than the negative control, it was higher than the positive control treatment. However, isolate PPRI10494 did not closely group with isolates CCP159 and CBS101780 (Figure 4). Other composts (A-H, and J) for this isolate were not significantly different in terms of germination, and ranged between 0-27 %. Isolate CBS101780 recorded a higher germination in composts A, B and I (32.5, 37.5 and 28.8 %) than in other composts (range 2.5 to 21.3 %) and positive control (3.8 %), but lower than the negative control (51.3 %). Isolate CCP159 recorded a mean germination of

53.8 % in compost B, which was not significantly different to soil negative control (63.8 %), but significantly higher than the rest (Compost A, C-J and positive control) which ranged between 0 and 32.5 %.

Isolates CBS969.96 and CBS101779 were the least pathogenic, however, resulting in an average germination of 25.1 and 37.3 % respectively. The two isolates were distant from each other based on Principal component analysis (PCA) (Figure 4.) Isolate CBS969.96 recorded a germination of 35 % in composts H and I followed by compost G with 33.8 %. Other composts (A-F, and J) were in the range 7-23 %. Isolate CBS101779 recorded high germination of 72.5 % in compost C, followed by 60 % in compost A, 55 % in compost I and 43.8 % in compost B. Other composts (D-H, and J) were in the range of 1.25-33.8 %. Correlations (Table 3) between the isolates were positive but few were highly significant ($P < 0.01$). The relationship between mean germination % of each isolate and data obtained from using the biochemical and molecular techniques varied considerably and no trends could be discerned.

Compost evaluation

Chemical composition of composts: The chemical composition (Table 4) showed high nutrient levels (Ca, K, Mg, Na, P, N, and C) in all composts with the exception of composts D and H. The pH values of composts were also measured and were in an optimum range (6.2-8.5) except compost H which had low pH (4.8). Overall, the seven *R. solani* isolates were negatively affected by highly nutrient levels in the composts since high nutritious composts, A-C and I, showed a broader range of suppression to the seven isolates compared to less nutritious composts D and H. The observation was

not always consistent, since none of the seven isolates were suppressed by other high nutritious composts (E-G and J). It appears that pH values did not affect growth of the seven isolates as no clear relationships were discernible. The correlation (Table 3) between germination % of the seven isolates with nitrogen and carbon content was not significant ($P < 0.01$).

Fungal diversity: Fungal density differed markedly amongst composts (Table 5). Twelve genera of fungi were isolated in the composts of which *Aspergillus fumigatus* (Fresen) was the most common species occurring in seven composts (Table 6). *A. niger* was the second common species occurring in five composts, while *Fusarium solani* (Mart.) Sacc. and *Paecilomyces inflatus* (Burnside Carmichael) occurred in four composts. It is not clear if any of these species played a role in inhibiting growth of the seven *R. solani* isolates; however, *F. culmorum* was unique to compost B which showed some significant suppression levels to isolates CCP159 and CBS101780 while *Penicillium simplicissimum* (Oudem.) Thom was unique to compost A which effectively suppressed isolate CBS101779. In addition, *Trichoderma harzianum* (Rifai) and *T. viridae* which are reported to be antagonistic microorganisms were present in composts D and I. None of the *R. solani* isolates were however consistently suppressed by the *Trichoderma* spp. Only isolates CBS101779 and PPRI10494 showed some level of suppression, presumably due to *Trichoderma* spp. present in compost I. Despite the presence of *T. viridae* in Compost D, none of the isolates were suppressed. Overall, the presence of fungi in composts did not consistently correlate with the suppression of any of the isolates. For example, the high number of fungal species present in compost D consistently failed to suppress disease by any of the isolates, while the low number of

fungal species present in compost C effectively suppressed isolate CBS101779. In contrast, the high number of fungal species in compost I was associated with some level of suppression to isolates CBS101779 and PPRI10494, while the significant lack of fungal species in compost J was consistent with a lack of disease suppression for all isolates.

Fluorescein diacetate (FDA): The FDA hydrolyzed in the respective composts were not significantly different ($P < 0.05$) (Figure 5) and thus no relationships with the seven *R. solani* isolates could be derived. For instance, Isolates CBS101779, PPRI10494 and CCP159 were suppressed by at least one compost, each of which recorded microbial activity not significantly different from composts that consistently failed to suppress disease. The correlation (Table 3) between germination % of the seven isolates with the rate of FDA hydrolyzed was not significant ($P < 0.05$).

Denaturing Gradient Electrophoresis (DGGE): The 16S rDNA fragments confirmed that there were bacteria in the composts (Figure 6). Certain band patterns were present in all composts while other bands were strictly associated with certain composts. However, no consistent relationships could be derived between germination % or biomass (g) of *R. solani* isolates with the DGGE banding patterns. Isolates CBS101779 and PPRI10494 were suppressed by compost I which was similar to compost J that did not suppress any of the isolates. Isolates CCP159 and CBS101779 were suppressed by composts B and C which were more similar to each other while isolate CBS101779 was also suppressed by compost A which did not resemble close similarities to the other composts. However, compost A clustered more closely with composts D, F and G

which failed to suppress the isolates (Figure 7). Composts B, C, E and I scattered outside with composts C and I being the highest in terms of overall germination % (>30 %) of the isolates, and each suppressed at least one isolate. Compost B and E varied in terms of average germination of the isolates, at 28 and 11 % respectively, with only compost B suppressing isolate CCP159 and slightly less, isolate CBS101779.

Phospholipid fatty acid analysis: The phospholipid fatty acids (PLFA) analyses detected the presence of general bacteria (5-13 % mole), gram positive bacteria (0.2-4.5 %), gram negative bacteria (2-8.5 %) and fungi (2-30.3 %) (Table 7 and Figure 8 and 9). Only isolate CBS 101779 showed suppression from compost A displaying a high ratio of gram positive to gram negative bacteria (1.04). However, no suppression of isolate CBS101779 was shown by the other composts having a high ratio of gram positive to gram negative bacteria, viz. composts H, G, E and D. Isolate CBS 101779 was also suppressed by compost C with a lower ratio of gram positive to gram negative bacteria (0.04). However, isolate CBS 101779 was suppressed by compost A which exhibited a high ratio of fungi to bacteria but the suppression of this isolate was not consistent with the high fungal ratio of other composts such as B, E and F. Inconsistencies were however observed with composts C, H and I that exhibited low ratios of fungi to bacteria. Compost C and I could suppress CBS 101779 but compost H could not. No other relationships between bacteria-fungi ratios and germination % of the isolates could be discerned. There was a positive correlation (Table 3) between total PLFA content of each isolate and pathogenicity, the highest being for isolates CCP 160 ($r = 0.72$), CBS 11779 ($r = 0.66$) and PPRI 10494 ($r = 0.52$).

Ergosterol analysis: The fungal biomass present in respective compost samples differed significantly ($P < 0.05$) (Figure 10). High ergosterol content (e.g. compost D and I) was however inconsistent in terms of disease suppression. The high ergosterol content of compost I suppressed isolates CBS101779 and PPRI10494 but high ergosterol content in compost D did not suppress any of the isolates. Similar inconsistencies were observed in composts with low ergosterol readings (e.g. compost C and G). Compost C suppressed isolate CBS101779 while compost G did not suppress any of the isolates. Ergosterol contents also did not correlate significantly ($P < 0.01$) with any of the isolates in terms of reducing germination % (Table 3).

DISCUSSION

Disease suppressiveness of composts on multiple pathogens was studied by Termorshuizen *et al.* (2006) on 7 pathosystems and the authors found a highly significant variation in terms of pathogen-compost interactions. Predictive variables for pathogen response to the physico-chemical and biological characteristics of compost remain unknown. This is because the sensitivity of pathogen genotypes towards composts differs and the mechanisms of disease suppression by composts also vary (Scheuerell *et al.*, 2004; Termorshuizen *et al.*, 2006). The suppression of *R. solani* is reported to be related to genetic variation in the pathogen with some genotypes being more sensitive to certain composts and less to others (Scheuerell *et al.*, 2004).

In the present study, the response of the seven isolates of *R. solani* to suppression by various composts varied. With the exception of isolate CBS101779 no other isolates recorded a germination % higher than that of the negative control in soil

amended with compost. However, this isolate was suppressed by composts A, B and I, although in these instances germination % was lower than the negative control. Isolates PPRI10041, CCP160, CBS969.96, CBS101780 and PPRI10494 proved to be resistant to compost; with the highest germination being 40 % for isolate PPRI10494. Isolate CCP159 was only susceptible to compost B, although, germination % was lower than the negative control.

The mechanisms of *R. solani* suppression by composts can include competition, antibiosis and parasitism (singularly or in combination) by specific antagonistic microorganisms (Hoitink & Fahy, 1986; Chen, Hoitink & Madden, 1988; Nelson, 1992; Hoitink *et al.*, 1997; Lucas, 1998). However, suppression of plant diseases by composts is not consistently related to its inherent microbial activity. For example, suppression of *Pythium* damping-off of cucumber was found to be related to microbial activity and ammonium volatilization of composts, while the suppression of *Rhizoctonia* damping-off was not related to any discernible compost factor (Bonanomi *et al.*, 2010).

Most mechanisms involved in disease suppression are related to characteristics of the compost/or soil medium and scant attention has been devoted to the influence of pathogen genotype. The seven *R. solani* isolates used in the present study differed in terms of their virulence to radish seedlings in the presence of different composts. A possible explanation is related to differences between genotypes (or anastomosis groups in case of *R. solani*). Isolates within one anastomosis group can also behave differently (Engelkes & Windels, 1996; Reeleder *et al.*, 1996; Villaguan, Kageyama & Hyakumachi, 1996; Schneider, Kocks & Schilder, 2001; Termorshuizen *et al.*, 2006).

Isolates, PPRI 10041 (AG unknown), PPRI 10494 (AG unknown) and CCP 160 (AG 1) were the most virulent while CB101779 (AG 6) was the least virulent. Isolates, CBS 101780 (AG 7), CBS 969, 96 (AG 9) and CCP 159 (AG unknown) ranked in the mid range in terms of pathogenicity. The different responses of the seven isolates in respective composts indicate that the sensitivity of pathogens to biotic and abiotic factors in compost amendments can differ significantly.

The genetic diversity of *R. solani* in natural populations has always been a challenging study due to complexes of genetically distinct groups that have diverse life histories (Cubeta & Vilgalys, 1997). Nonetheless, anastomosis grouping remains the most important factor in understanding the genetic diversity within *Rhizoctonia* spp. There are about 12 anastomosis groups (Cubeta & Vilgalys, 1997) and the response of these groups to different plant species or fungicides is well documented. For example, Windels & Nabben (1988) tested 361 cultures of *R. solani* from sugar beet seedlings and their findings were that the AG 4 group was the most virulent followed by AG 5, AG 2, AG 1 and AG 3. In another study, the anastomosis groups of *R. solani* were variable in response to treatments by fungicides carboxin, PCNB, chlorothalonil and triadimefon (Martin, Lucas & Campbell, 1984). The variability responses of anastomosis groups of *R. solani* are possibly due to somatic interactions between genetically distinct or similar individuals of this species (Sneh, Burpee & Ogoshi, 1991).

The evaluation of microbial activity and community structure of composts measured by FDA, PLFA, ergosterol and DGGE, to qualitatively and quantitatively evaluate the effect of genetic variation of the 7 *R. solani* isolates in differing compost

environments, proved to be an elusive task. The composts all exhibited high microbial counts, which is considered crucial in disease suppression. The 16S rDNA fragments confirmed that there were bacteria in the samples, and based on PLFA analyses, gram negative bacteria dominated the bacteria group. According to Tunlid *et al.* (1989) and Atkinson, Jones & Gauthier (1997), gram negative bacteria are the dominant biological control agents in suppressive bark compost, and straight chain monoenoic and cyclopropane fatty acids dominate in disease suppressive composts (Tunlid *et al.*, 1989). These findings were consistent for the composts evaluated in the present study. However, none of these techniques could explicitly explain variation in the pathogenic response of different isolates in different composts.

It is not clear if any of the fungal species isolated played any role in disease suppressiveness of composts. According to the literature listed above, various *Trichoderma* spp. are antagonistic to *R. solani*. However, the genus was not consistently involved in the suppression of *R. solani* isolates used in the present study. The most dominant fungal species including *T. viridae* found in compost D failed to suppress a single isolate of the pathogen. Isolates CBS101779 and PPRI10494 were however suppressed by compost I which exhibited a high number of fungal species including *T. viridae* and *T. harzianum*. The observation was not always consistent as isolate CBS101779 proved to be ineffective in compost C which exhibited the least number of fungal species.

Total N, C and pH have been shown to play an important role in the development of the microbial community structure (Marschner, Crowley & Yang, 2004). High nutrient

values in composts enhance plant diseases suppressiveness (Hoitink & Boehm, 1999). Overall, the isolates were suppressed by composts with high nutrient levels although not all composts with high nutrient levels could suppress disease. The pH values of composts did not show any discernible relationships with the pathogenicity of the seven isolates. This is consistent with Grosch & Kofot (2003) whom reported that pH levels of soil do not affect *Rhizoctonia* pathogenicity on lettuce.

The pathogenicity of the seven *R. solani* isolates on radish seedlings in different composts varied significantly. Since some isolates were suppressed by certain composts and others not, the mechanisms of these interactions are not clear. No isolate was suppressed by all the composts and no single compost could suppress all the isolates. In general, most isolates were not suppressed by compost and there were isolates that exhibited no suppression in any of the compost used. This suggests that the genotypes of a specific pathogen in response to compost suppression are highly variable. Nevertheless, infested radish grown on soil amended composts exhibited a higher germination % and biomass (g) than on infested soil only. This illustrates that despite inconsistencies in the response of the seven isolates to different composts, the usefulness of compost as a soil amendment is justified.

No relationship could be found between microbial activity and structural diversity in composts and genetic variation of the pathogen. Each technique simply provided a profile of the compost or substrate used. Variation in disease suppression has been reported to depend on the type of compost and substrate used. This is because microbial communities are likely to vary with the substrate used to make the compost,

and the nutrient composition of the compost differs. Compost may be highly suppressive to one pathogen but less or not suppressive to another (Pérez-Piqueres *et al.*, 2006; Termorshuizen *et al.*, 2006). However, understanding the genetic variation of a specific pathogen such as *R. solani* in response to compost remains a challenge. The fact that different genotypes of a pathogen can behave differently in different composts needs to be addressed. Optimization of the preparation and use of composts in different soils for the suppression of a broad range of plant pathogens represents a very interesting topic for further research.

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Table 1: Characteristics of composts used in the study.

NO.	Composition/Brand	Abbreviation	Age (month)
A	Garden refuse + leaves	GR+LEV /18	12
B	Garden refuse + leaves + horse manure	GR+LEV + HM/12+	12
C	Sawdust from <i>Eucalyptus grandis</i> + horse manure	SD+HM /22 mth	22
D	<i>E. grandis</i> leaves	Euc leaves/24 mth	24
E	Garden refuse + <i>E. grandis</i> Leaves + cattle manure	GR+ LEV +HM /10	10
F	Bloem organic compost	Bloem organic	>24
G	Culterra organic compost	Culterra organic	>24
H	Culterra acid compost	Culterra acid	>24
I	Terra microbe enriched compost	Microbe enriched	>6
J	Malan Seuns nursery	Malan seuns	>24

Table 2: Interactions between the seven isolates of *R. solani* and the ten different composts. Each value is a mean percentage of four replicates of 10 seeds. Means within a column not followed by the same letters are significantly different (P<0.05).

Composts	CBS 969.96	CBS 101780	CBS 101779	PPRI 10041	PPRI 10494	CCP 159	CCP 160	Average
#A GR+Lev/18	11.3a	32.5c	60.0ef	18.8b	6.25ab	32.5c	10.0ab	24.5
#B GR+Lev+HM/12+	16.3a	37.5c	43.8de	23.8b	16.3bc	53.8d	3.75ab	27.9
#C SD+HM/22mt	25.0bc	18.8bc	72.5f	21.3b	26.3c	21.3bc	23.8b	29.8
#D Euc lev/24mt	10.0a	15.0ab	23.8bc	12.5ab	1.56ab	0a	12.5ab	10.8
#E GR+Lev+HM/10mt	18.8a	12.5ab	32.5cd	3.75ab	1.25ab	3.75ab	7.50ab	11.4
#F Bloem org	7.50a	2.50a	1.25a	0a	0a	2.50ab	0a	1.96
#G Cultera org	33.8c	21.3bc	17.5bc	10.0ab	18.8bc	3.75ab	13.8b	17.0
#H Cultera acid	35.0c	20.0bc	33.8cd	16.3b	13.8bc	16.3bc	8.75ab	20.5
#I Microbe enriched	35.0c	28.8c	55.0ef	13.8b	40.0d	30c	11.3	30.5
#J Malan Seuns	22.5b	17.5bc	30.0cd	11.3ab	18.8bc	21.3bc	15.0b	19.5
#C1 Control -ve	80.0d	51.3d	71.3f	53.8c	67.5e	63.8d	75.0c	66.1
#C2 Control +ve	6.25a	3.75ab	6.25ab	2.50ab	3.75ab	23.8bc	3.75ab	7.14
Average	25.1	21.8	37.3	15.6	17.8	22.7	15.4	

Table 3: Pearson correlation (r) between germination % of 7 *R. solani* isolates, phytotoxicity, N and C contents, pH, fungal populations, FDA, PLFA and ergosterol. $r > 75\%$ are highly significant ($P < 0.01$).

	Germ% CBS 969.96	Germ% CBS 101780	Germ% CBS 101779	Germ% PPRI 10041	Germ% PPRI 10494	Germ% CCP 159	Germ% CCP 160
Germ% CBS 969.96	1.00						
Germ% CBS 101780	0.24	1.00					
Germ% CBS 101779	0.24	0.64	1.00				
Germ% PPRI 10041	0.20	0.82*	0.76*	1.00			
Germ% PPRI 10494	0.75*	0.47	0.55	0.45	1.00		
Germ% CCP 159	0.06	0.86*	0.62	0.77*	0.46	1.00	
Germ% CCP 160	0.41	0.09	0.56	0.40	0.50	0.06	1.00
Germ% radish	0.26	0.73	0.71	0.43	0.55	0.62	0.17
radish biomass	0.22	0.72	0.74	0.45	0.59	0.66	0.12
N%	0.21	0.24	0.40	0.14	0.67	0.31	0.14
C%	0.47	0.19	0.14	0.03	0.25	0.06	0.18
pH	0.11	0.08	0.26	0.48	0.15	0.04	0.17
Fungal populations	0.13	0.38	0.19	0.21	0.01	0.19	0.19
FDA	0.22	0.02	0.04	0.11	0.28	0.02	0.03
PLFA	0.18	0.02	0.66	0.40	0.52	0.16	0.72
Ergosterol	0.23	0.35	0.10	0.20	0.15	0.28	0.16

Table 4: Chemical composition of the respective composts

Compost	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	N %	N mg/kg	C %	C mg/kg	pH
A	6255.0	3850.0	4600.0	420.0	3938.5	0.768	7682.0	6.96	69600.0	7.23
B	4080.0	2600.0	1440.0	138.0	2868.5	0.796	7955.0	7.64	76400.0	7.05
C	6490.0	2650.0	1880.0	272.0	3468.5	1.070	10695.0	13.95	139500.0	6.20
D	345.0	1850.0	620.0	98.0	1413.0	0.529	5289.7	9.15	91500.0	6.49
E	5645.0	5150.0	1940.0	254.0	4117.5	0.520	5202.0	3.97	39700.0	7.61
F	3665.0	7300.0	920.0	292.0	2685.0	0.923	9234.3	14.10	141000.0	8.15
G	3495.0	6000.0	800.0	228.0	2333.5	0.807	8074.5	12.30	123000.0	8.13
H	1200.0	1515.0	460.0	82.0	891.5	0.416	4159.9	30.70	307000.0	4.83
I	7825.0	6450.0	1980.0	168.0	5443.0	1.396	13957.0	12.80	128000.0	8.45
J	3895.0	485.0	1100.0	56.0	1705.0	0.521	5206.9	27.60	276000.0	8.37

Table 5: Fungal population density at 10^{-1} dilution

Composts	Colony forming units (CFU)/ g compost
A	6.70×10^4
B	7.20×10^4
C	0.40×10^4
D	12.2×10^4
E	6.10×10^4
F	0.75×10^4
G	0.68×10^4
H	4.70×10^4
I	10.8×10^4
J	1.10×10^4

Table 6: Fungal species isolated in the respective composts

Fungal species	Composts									
	A	B	C	D	E	F	G	H	I	J
<i>Aspergillus flavus</i> Link							X		X	
<i>Aspergillus fumigatus</i> Fresen	X				X	X	X	X	X	X
<i>Aspergillus niger</i> van Tieghem	X	X		X	X				X	
<i>Aspergillus sp</i>									X	
<i>Beauveria bassiana</i> (Bals.-Criv.) Vuill.						X				
<i>Cladosporium cladosporioides</i> (Fresen) de Vries					X					
<i>Clonostachys rosea</i> (Link: Fries) Schoers, Samuels, Seifert & Gams				X						
<i>Colletotrichum gloeosporioides</i> Dickman				X		X				
<i>Emericella nidulans</i> Winter							X		X	
<i>Fusarium culmorum</i> Sacc.		X								
<i>Fusarium dimerum</i> Penzig					X					
<i>Fusarium equiseti</i> (Corda.) Sacc.		X			X					
<i>Fusarium lateritium</i> Nees				X						
<i>Fusarium nelsonii</i> Marasa & Logrieco		X							X	
<i>Fusarium oxysporum</i> Schlecht.	X			X		X				
<i>Fusarium solani</i> (Mart.) Sacc.	X	X							X	X
<i>Fusarium sporotrichioides</i> Sherb									X	
<i>Fusarium sp.</i>				X	X	X			X	
<i>Fusarium verticillioides</i> Sacc.						X				
<i>Fusarium virguliforme</i> O'Donnell & Aoki				X						
<i>Geotrichum sp.</i>								X		
<i>Paecilomyces inflatus</i> Burnside Carmichael				X		X		X	X	
<i>Penicillium chrysogenum</i> Thom.				X					X	
<i>Penicillium decumbens</i> Thom.			X	X				X		
<i>Penicillium glabrum</i> Wehmer Westling		X			X					
<i>Penicillium janthinellum</i> Biourge				X				X		
<i>Penicillium lividum</i> Westling				X		X				
<i>Penicillium raistricki</i> Raistricki			X	X						
<i>Penicillium simplicissimum</i> (Oudem.) Thom	X									
<i>Rhizopus oryzae</i> Went & Prins. Geerl		X				X				
<i>Trichoderma harzianum</i> Rifai									X	
<i>Trichoderma viridae</i> Pers.				X					X	

Table 7: The average phospho-lipid fatty acids (PLFAs) % of different microbial groups in the tested composts

Microbial groups	Total PLFA (%) Composts									
	A	B	C	D	E	F	G	H	I	J
General bacteria	5.62	6.39	9.10	7.79	6.28	5.48	7.70	12.6	8.06	5.23
Gram positive bacteria	4.11	3.20	0.25	4.01	4.14	3.32	4.31	2.82	3.09	0.60
Gram negative bacteria	3.97	4.26	6.00	4.24	4.07	4.50	3.93	2.01	5.87	8.22
Fungi	30.3	24.3	2.46	14.1	24.7	28.9.	17.7	4.80	3.72	9.88
Ratio Gram positive/Gram negative	1.04	0.73	0.04	0.95	1.02	0.74	1.10	1.40	0.53	0.07
Ratio fungi/bacteria	2.21	1.77	0.16	0.88	1.71	2.18	1.11	0.28	0.22	0.70
Ratio cyclo/monounsaturated precursor	0.14	0.19	0.00	0.00	0.11	0.09	0.16	0.00	0.00	10.2

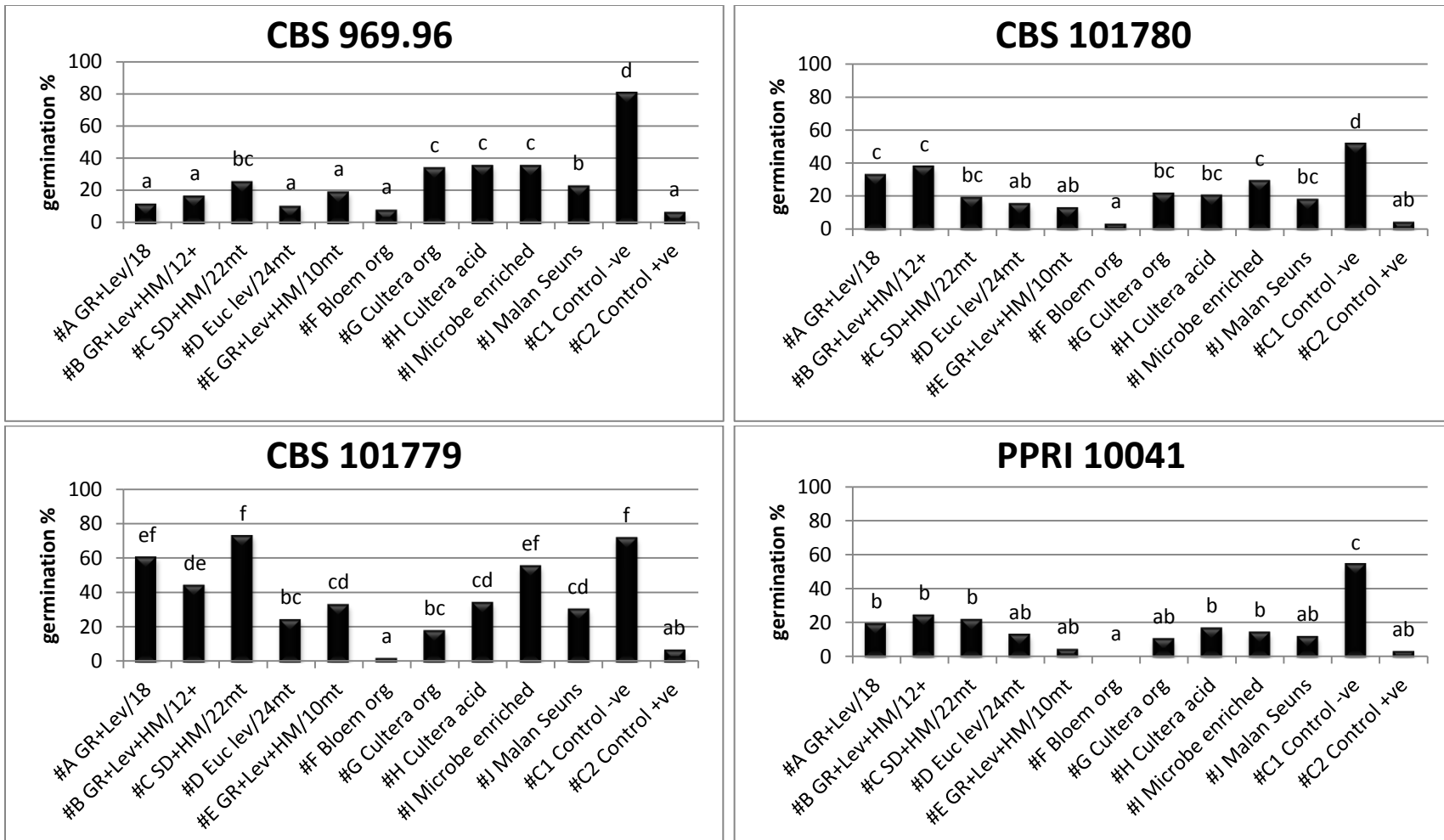


Figure 1: Mean germination percent of radish (n=10, rep= 4) for different composts in response to 7 isolates respectively. The experiment included two controls; negative control (soil) and positive control (soil infested with each of the 7 isolates respectively). Bars not followed by the same letter are significantly different (LSD 0.05=12.613).

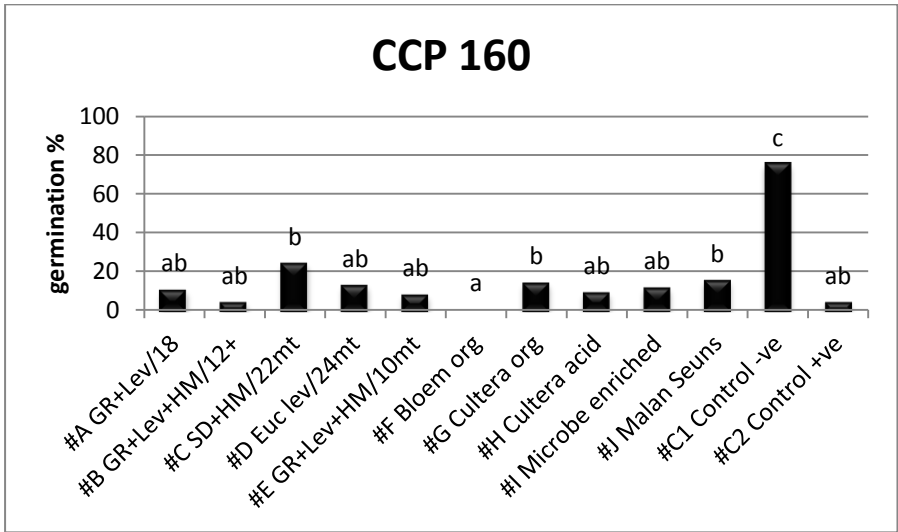
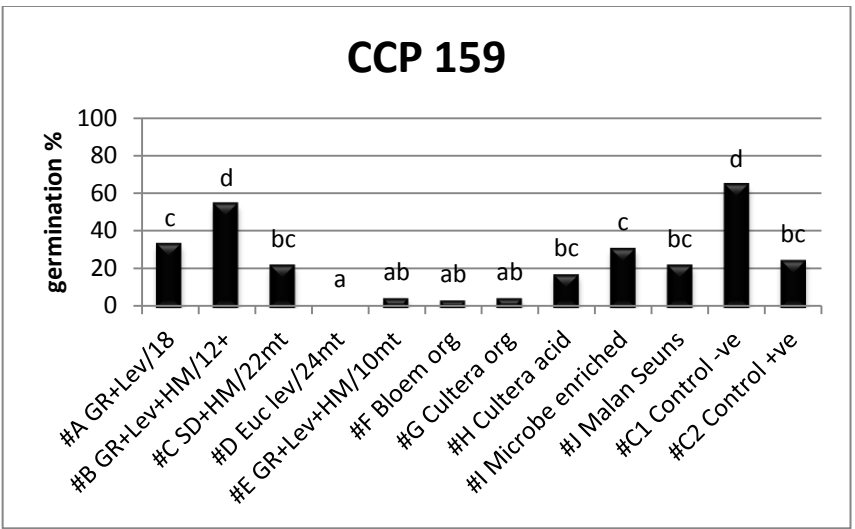
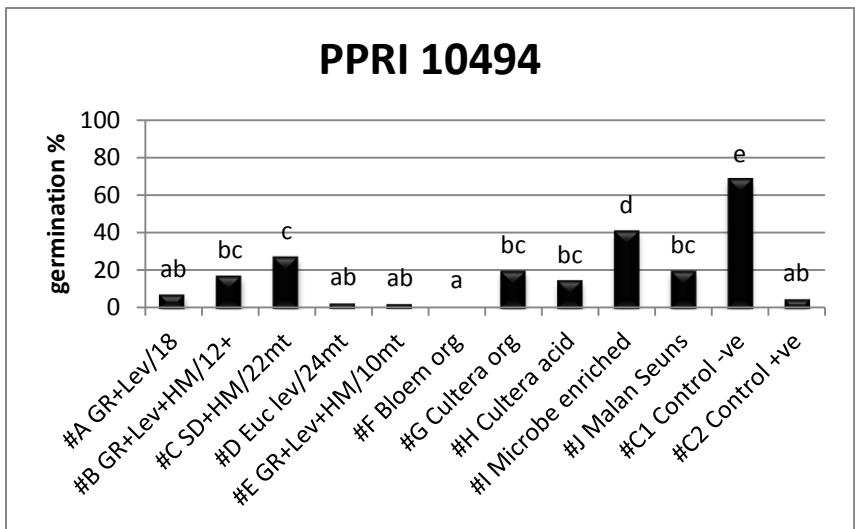


Figure 1: (Continued)

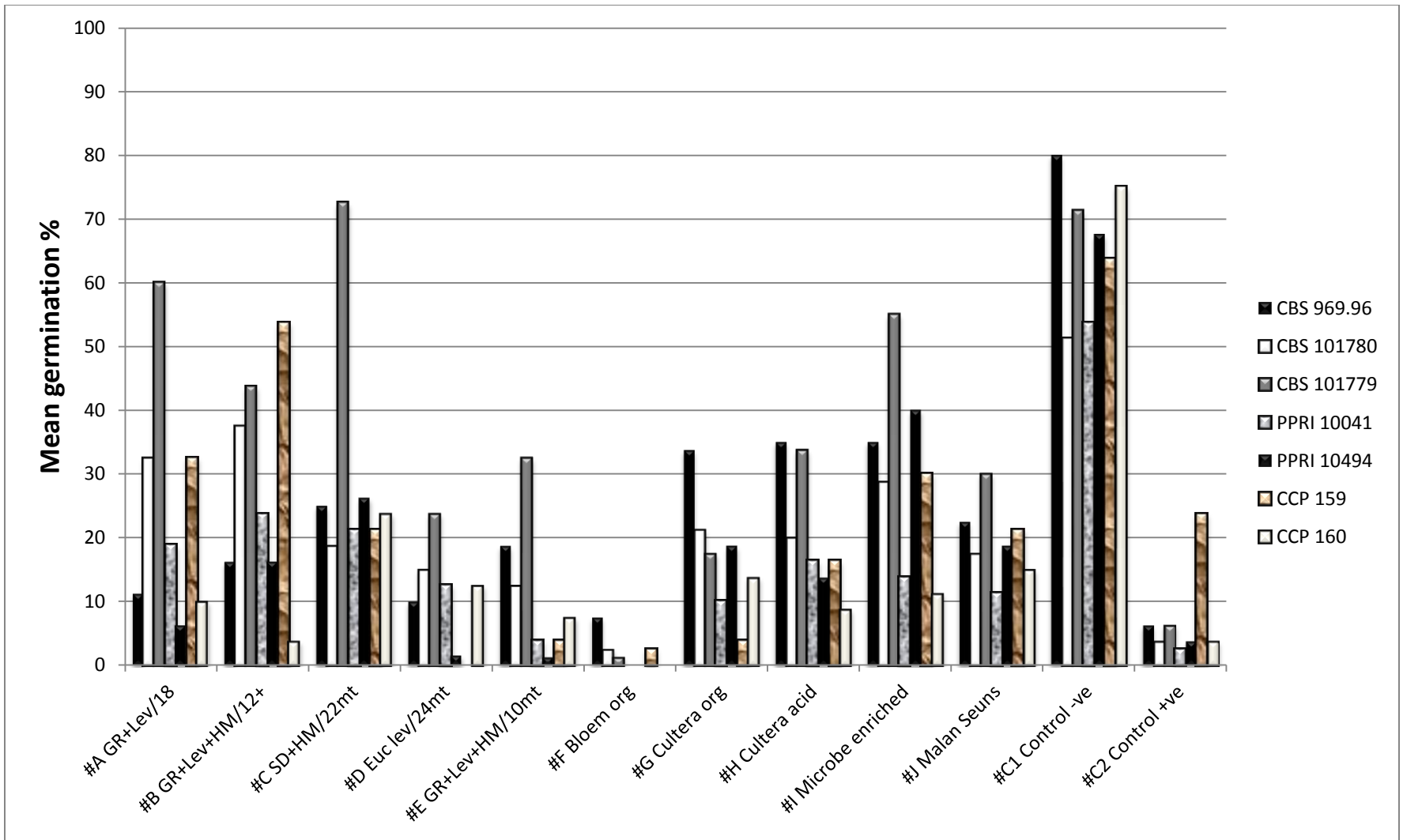


Figure 2: Mean germination percent of radish (n=10, rep= 4) seedlings for different composts combined in response to 7 isolates.

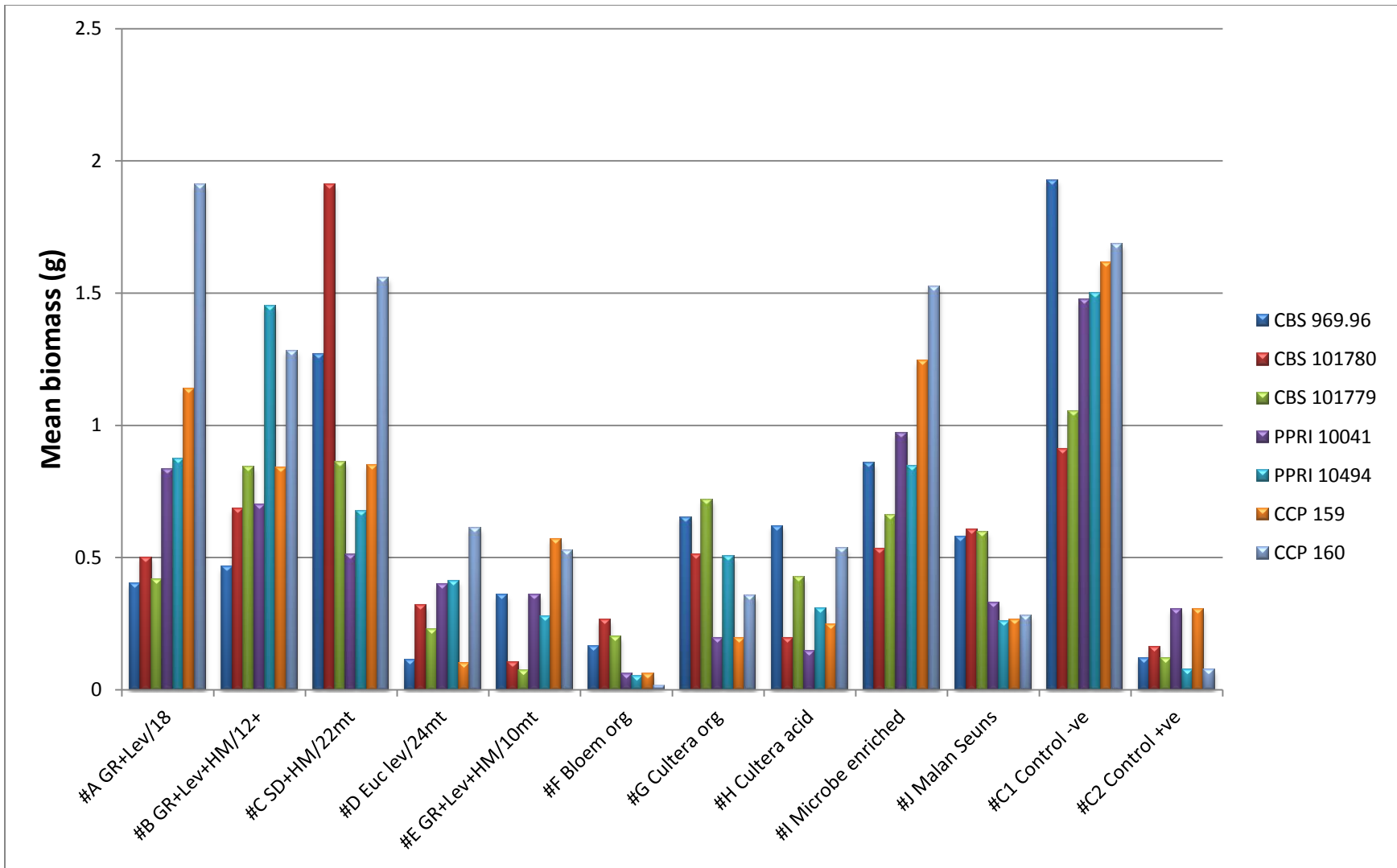


Figure 3: Mean biomass of radish (n=10, rep= 4) seedlings for different composts in response to 7 isolates of *R. solani*.

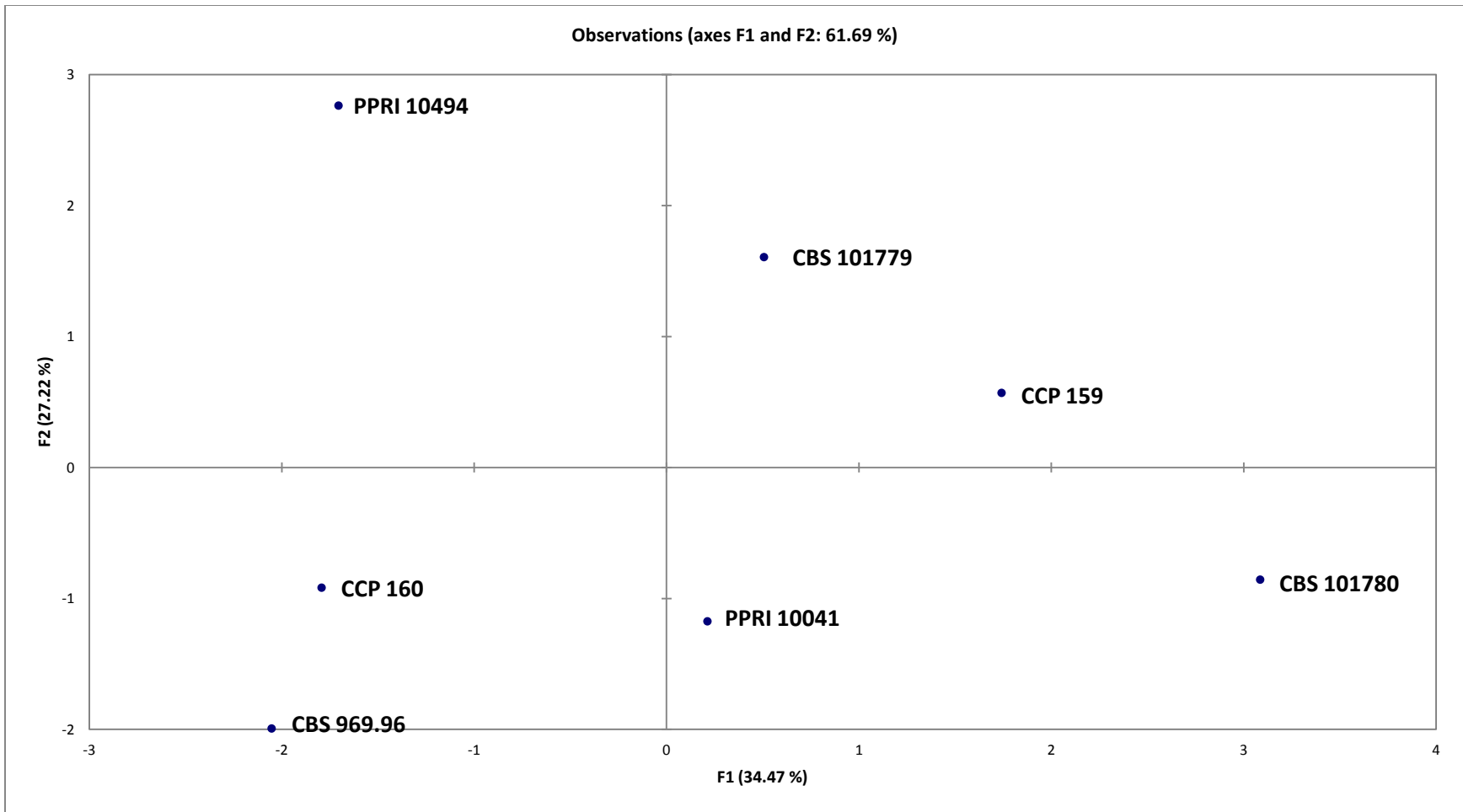


Figure 4: Principal Component Analysis (PCA) showing the different clustering groups of 7 isolates of *R. solani* based on germination percent of radish (n=10, rep= 4).

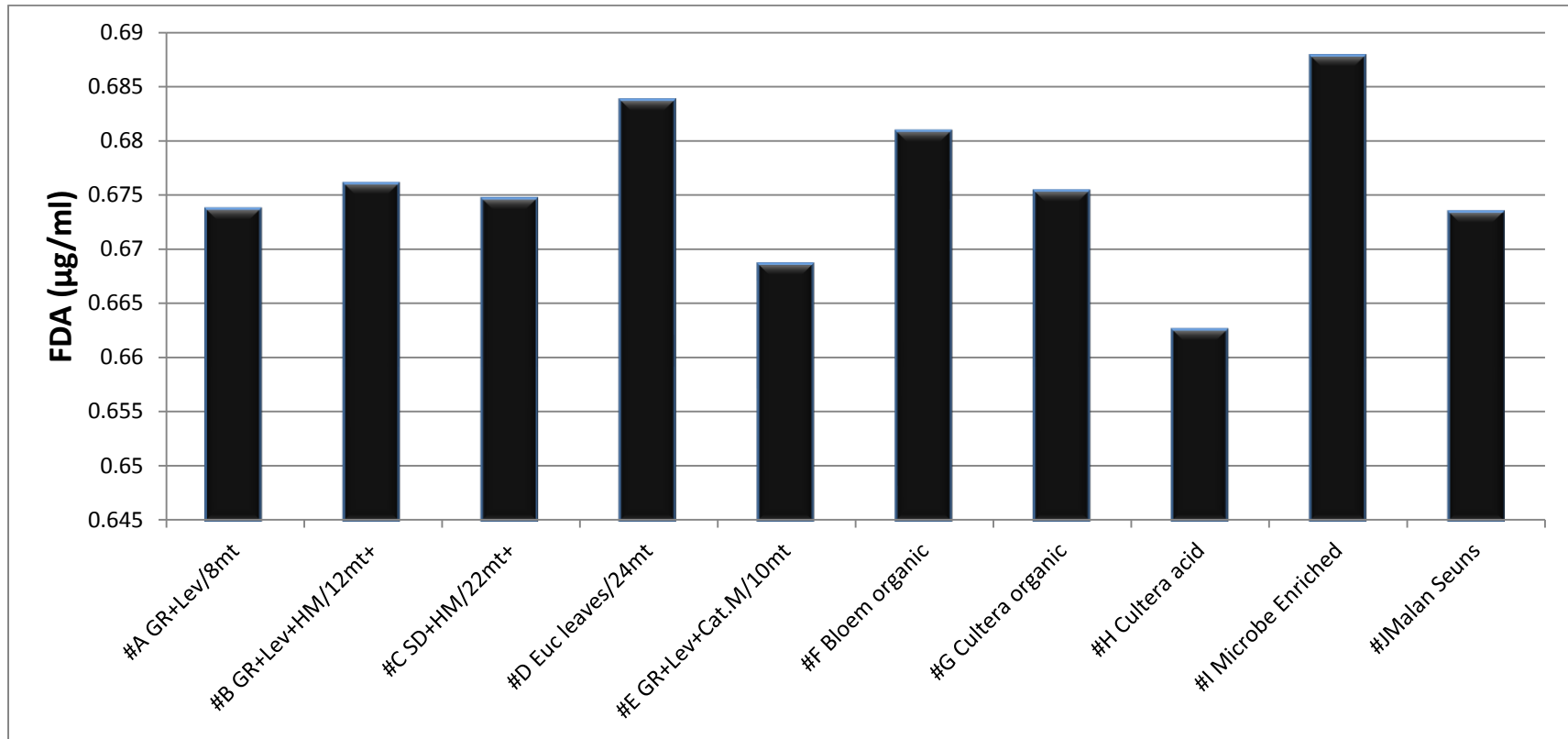


Figure 5: Microbial activity of composts in terms of fluorescence diacetate (FDA) hydrolysis. The FDA values were not significantly different ($P < 0.05$).

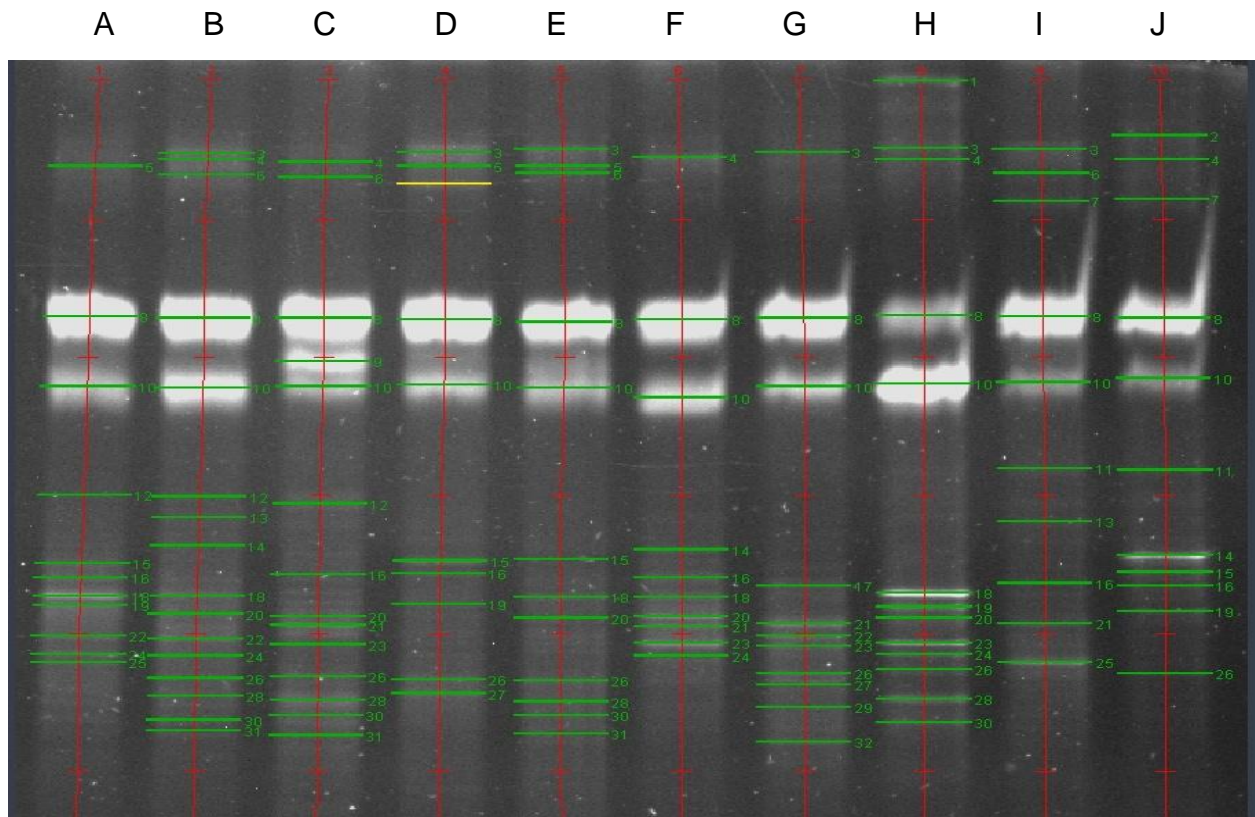


Figure 6: Denaturing gradient gel electrophoresis (DGGE) profile of 16S rDNA fragments of the 10 composts. Each sample represents a column and each prominent band represents bacterial phylotype.

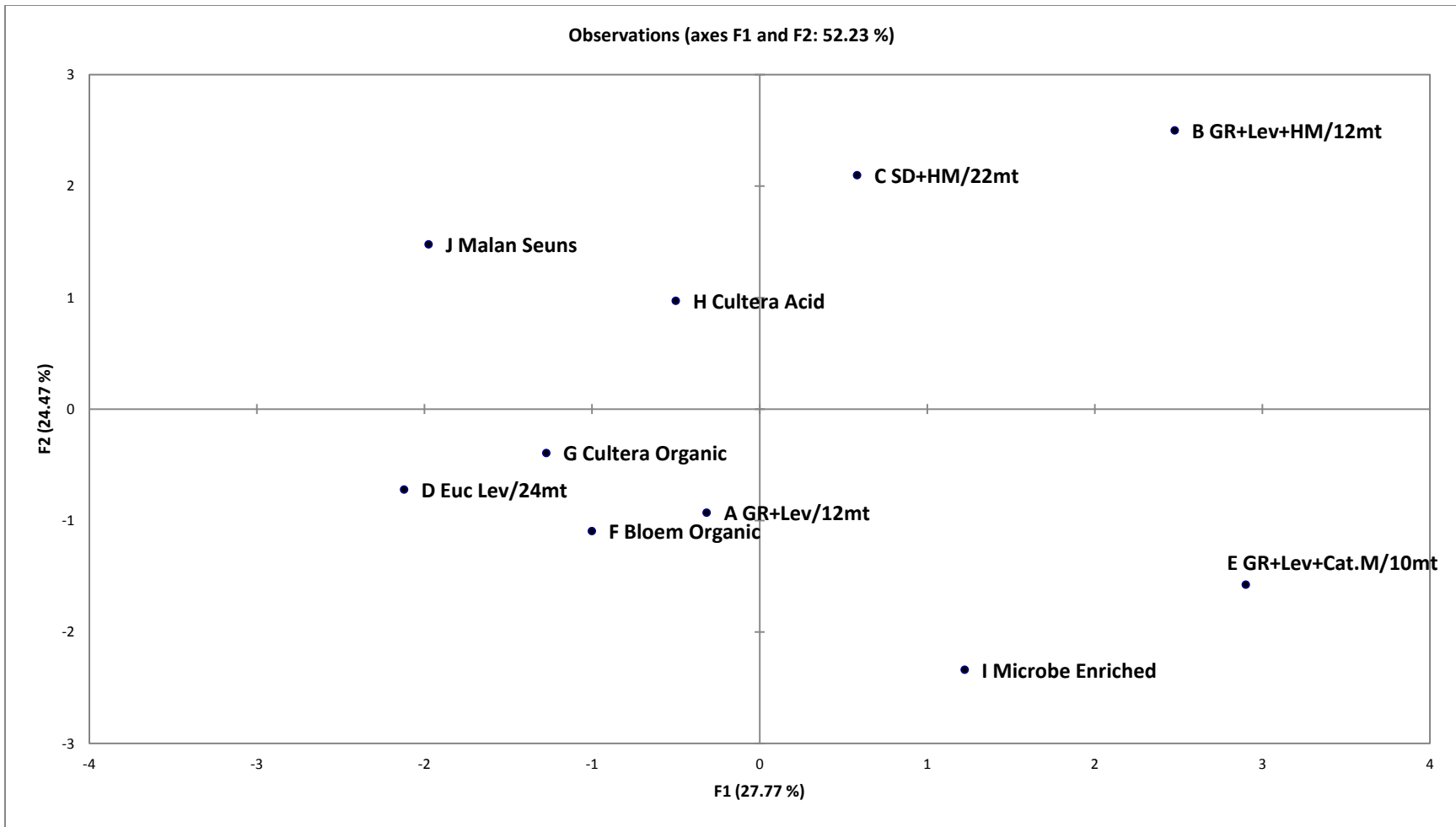


Figure 7: Principal Component Analysis (PCA) showing the different clustering groups of respective composts based on DGGE analyses.

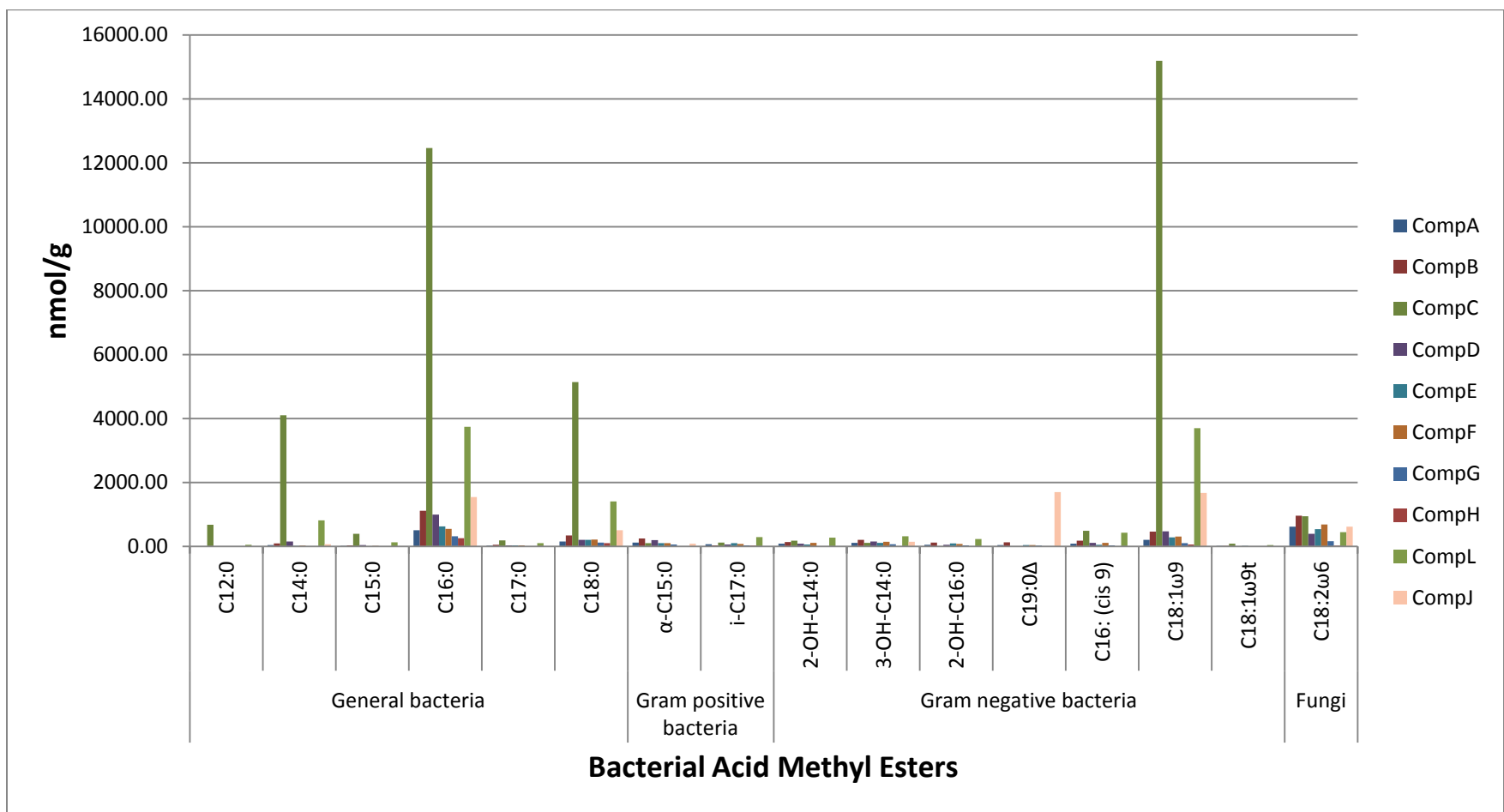


Figure 8: Bacterial Acid Methyl Esters analysis of the different composts

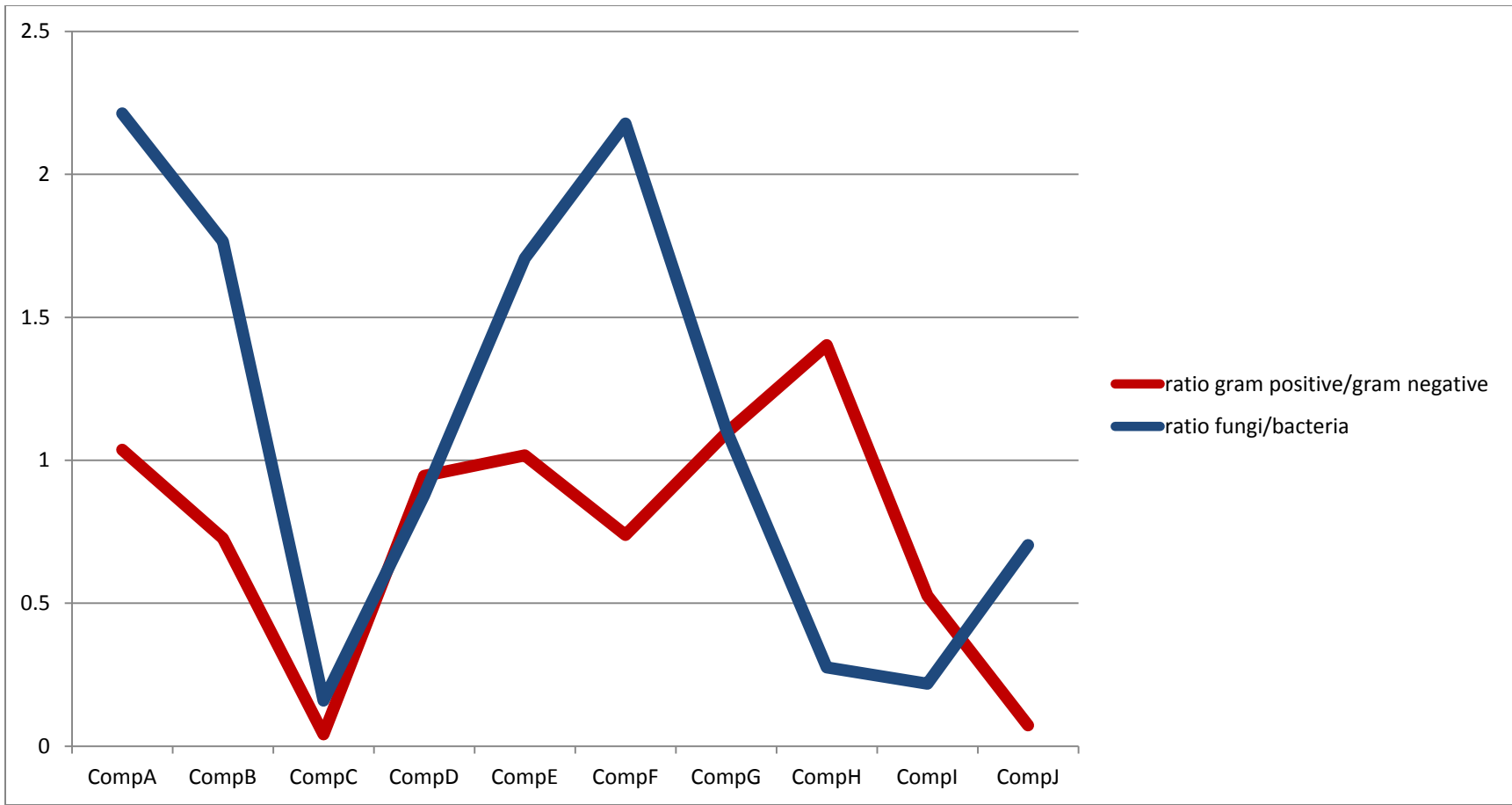


Figure 9: A reading analysis of the ratio between gram positive to gram negative bacteria and fungi to bacteria respectively for respective composts.

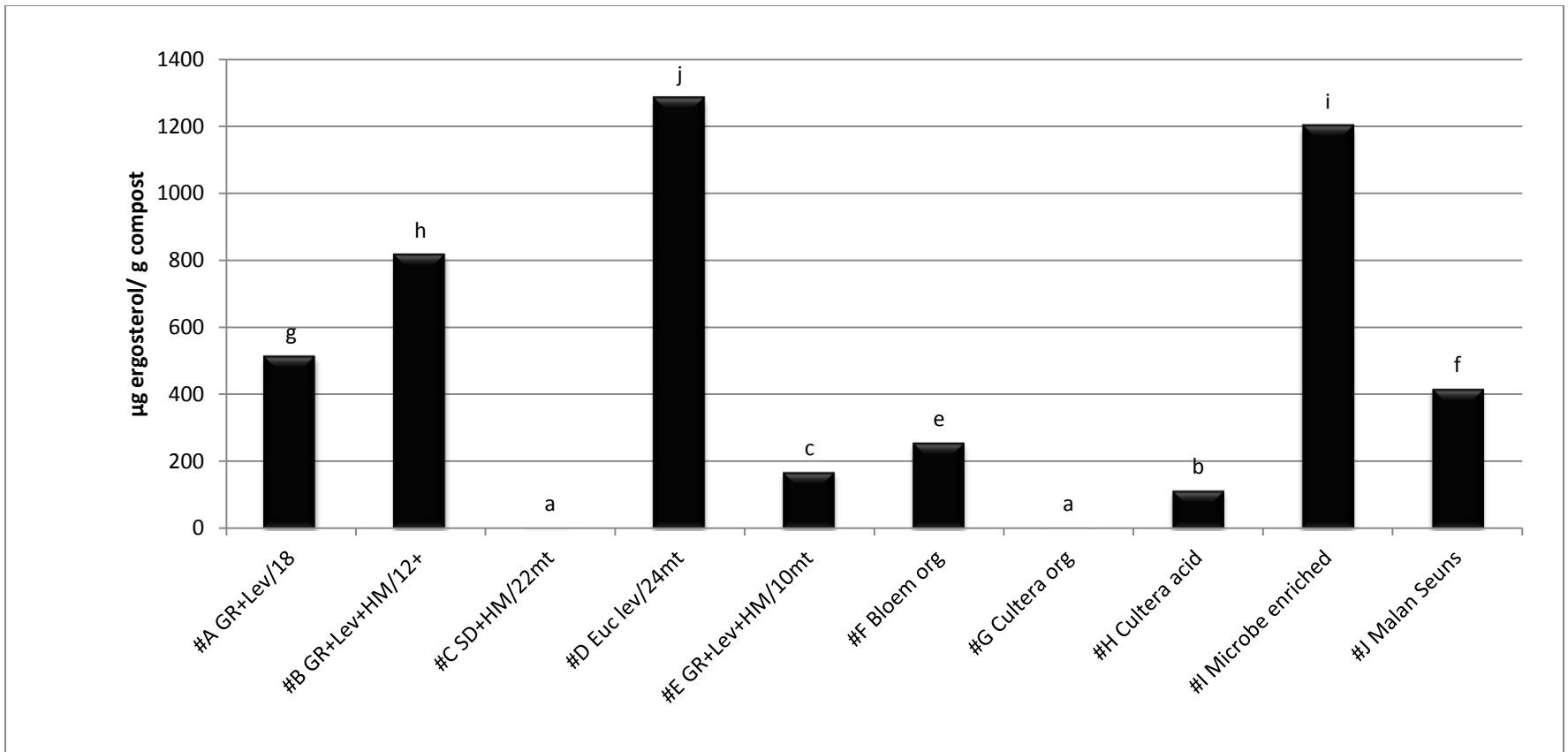


Figure 10: Ergosterol content (n=2) of composts. Bars not followed by the same letter are significantly different (P<0.05), LSD= 17.2959.

CHAPTER 4

INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN RADISH PLANTS BY COMPOST

ABSTRACT

Six composts of different ages and maturity were examined in the glasshouse for their inhibitory effect on *Alternaria raphani* leaf-blight of radish (*Raphanus sativus*) seedlings. In a comparative study, *A. raphani* development on radish seedlings grown in soil, with or without salicylic acid treatment of leaves, was examined. The levels of two pathogenesis-related (PR) proteins, β -1,3-glucanase and peroxidase in radish seedlings were measured using biochemical techniques. The nutritional composition and fungal population densities of composts to evaluate mechanisms of compost's induction of SAR were also studied. Radish plants grown on soil amended with two specific batches of compost displayed reduced blight symptoms and exhibited higher levels of PR proteins than plants with severe blight symptoms. Composts thus reduced disease incidence by priming plants thus enabling them to respond quicker to pathogen challenge. Salicylic acid sprayed onto leaves also induced increased defence responses which however did not differ significantly upon pathogen challenge. Composts that induced higher activity of PR proteins exhibited high nutritional composition but were inconsistent in terms of fungal population densities. The results of this study indicate that composts suppressed *Alternaria* blight of radish by inducing plant defence responses.

INTRODUCTION

Composts have been reported to suppress numerous soilborne diseases such as damping-off, root rots, wilts, and several diseases of turf grass (Lumsden *et al.*, 1981; Nelson, Kuter, & Hoitink, 1983; Ben-Yephet & Nelson., 1999; Boulter, Boland & Trevors, 2000; Cotxarrera *et al.*, 2002; Diab, Hu & Benson, 2003; Krause *et al.*, 2003). According to available literature, only soilborne and not foliar diseases, are typically suppressed by compost. There is however mounting evidence that compost amendments can also suppress foliar diseases (Tränkner, 1992; Abbassi *et al.*, 2002; Ntougias *et al.*, 2008). The suppressive mechanism present in compost for suppression of foliar diseases is via the induction of systemic acquired resistance (SAR) which is a state of plant resistance in response to pathogen attack or certain chemical factors (Zhang, Dick & Hoitink, 1996; Nelson & Boehm, 2002; Pharand, Carisse & Benhamou, 2002; Krause *et al.*, 2003; Vallad, Cooperband & Goodman, 2003; Kavroulakis *et al.*, 2005).

SAR is triggered when plants are exposed to foreign invaders such as virulent, avirulent and non-pathogenic microbes, or chemical agents such as salicylic acid, 2,6-dichloro-isonicotinic ester, benzo-thiadiazole-7-carbothioic acid, methyl ester, siderophores, and lipopolysaccharides (Hoitink & Boehm, 1999; Abbasi *et al.*, 2002; Vallad *et al.*, 2003). Various factors such as pH and nutritional components including calcium and nitrogen in composts have also been reported to induce SAR (Zhang *et al.*, 1996; Hoitink & Boehm, 1999). These nutrients provide energy important for plant defenses against pathogens (Heil, 2002). Certain microorganisms present in composts

have also been reported to induce SAR in plants such as radish and cucumber (Trillas-Gay, Hoitink & Madden, 1986; Zhang *et al.*, 1996). These microorganisms include *Trichoderma hamatum* (Bonord.) Bainier and *Pantoea agglomerans* (Ewing & Fife) which were found to induce SAR to bacterial foliar spot of radish caused by *Xanthomonas campestris* (Pammel & Dowson) (Abbasi *et al.*, 2002). Determining the microbial composition of composts is therefore important if we want to understand the mechanisms of SAR induction that are operating within them. Culture dependent methods such as the isolation and identification of microorganisms present in compost using culture media are to date the most widely used procedures (Boulter, Trevors & Boland, 2002; Novinscak *et al.*, 2009). The microorganisms present in some composts are said to colonize the roots and remain at certain threshold levels that induce systemic resistance in plants (Zhang *et al.*, 1998).

SAR development is accompanied by the expression of a set of genes in the plant coding for pathogenesis-related (PR) proteins including chitinases, glucanases and thymidine-like proteins with antifungal activities, as well as phytoalexins (Linthorst, 1991; Van Peer, Nieman & Schippers, 1992; Ryals *et al.*, 1996; Sticher, Mauch-Mani & Métraux, 1997; Boulter *et al.*, 2000; Vallad *et al.*, 2003; Kavroulakis *et al.*, 2005; Conrath *et al.*, 2006; Van Loon, Rep & Pieterse, 2006). Some PR proteins exert a direct antipathogenic activity in the plant through hydrolytic action on cell walls of the pathogen and/or release of non-specific elicitors that induce defense responses in the plant (Yoshikawa, Tsuda, & Takeuchi, 1993; Van Loon, 1997; Hammerschmidt, 1999). For example, Van Loon, Bakker & Pieterse (1998) reported that certain *Trichoderma* spp. induced PR proteins in tobacco seedlings. Vallad *et al.* (2003) further reported that

Arabidopsis seedlings grown in soil amended with a compost, displayed an increase in pathogenesis related defense genes that suppressed bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (*Pst*) more than plants grown in unamended soil. The SAR-inducing activity of composts is heat labile since autoclaving destroys the SAR-inducing effect. This activity can however be restored by mixing autoclaved compost with unautoclaved compost, suggesting that the SAR-inducing activity of the compost might be of biological nature (Zhang *et al.*, 1998).

The expression of disease resistance genes is activated in plants in response to pathogen attack (Zhang *et al.*, 1996; 1998; Haggag, 2002). Zhang *et al.* (1996; 1998) found that in plants cultivated on certain compost, infection induced a more rapid expression of SAR than no infection. For example, cucumber plants inoculated with *Pythium* root rot and *Arabidopsis* plants inoculated with *Pseudomonas syringae* pv. *maculicola* expressed a stronger SAR reaction than uninoculated plants. Even though the specific compound(s) present in compost that induce SAR in plants have not yet been identified, plants expressing SAR have been shown to have higher levels of β -1,3-glucanase and peroxidase enzymes activities than those grown in peat (Zhang *et al.*, 1996; 1998).

The objectives of the present study were: (a) to evaluate the inhibitory effect of six composts on *Alternaria* leaf blight development in radish (*Raphanus sativus*) seedlings caused by *Alternaria raphani* (J.W. Groves & Skolko); and (b) whether disease suppression induced by these composts was correlated with the induction of SAR in the radish seedlings.

MATERIALS AND METHODS

The inhibitory effect of compost on *A. raphani* infection of radish seedlings was investigated by using six composts varying in composition and maturity and obtained from various commercial nurseries near Bloemfontein (Table 1). Soil was obtained from the experimental farm of the University of the Free State, Bloemfontein and compared with compost amended soil. The activity of β -1,3-glucanase and peroxidase was evaluated in plants inoculated and uninoculated with *A. raphani* conidia, grown on compost amended soil and soil.

Evaluation of pathogenicity

Preparation of inoculum: An isolate of *Alternaria raphani* (isolated from radish) was sourced from CABI (California Birth Index database), United Kingdom, and cultivated on potato carrot agar (PCA) for 14 days at 25 °C. Conidia were harvested by scraping them from sporulating colonies on PCA plates and suspended in sterile water mixed with 0.05 % (v/v) Tween 80. The conidial suspension (1×10^6 CFU ml⁻¹) was used to inoculate seedlings in the greenhouse.

Alternaria blight of radish bioassays: The SAR inducing effect of the composts was determined using a modified method of Su *et al.* (2005). A total of six composts and four soil treatments were used (Table 1). Salicylic acid (SA) (5mM) treatment on leaves was applied 2 days prior to inoculation with *A. raphani*. Eight pots, each containing 5 radish plants were used for each treatment. When secondary leaves were fully developed (~20 days), the seedlings were inoculated with *A. raphani* conidial suspension (10^6 CFU/ml) in a glucose solution (1g glucose/L sterile water). The

inoculum was sprayed onto the secondary leaves and the plants were immediately covered with a sheet of transparent plastic to create humid conditions for 48 hours.

The plants were harvested at four different sampling times (0, 48, 96 and 144 hours post inoculation). At each stage four leaves (second-leaves) were randomly harvested from each treatment, quick-frozen in liquid nitrogen and stored at -20 °C until further use. Disease development was monitored for a period of 7d following inoculation and a rating scale as described by Meena *et al.*, (2011) (Table 2) was used to evaluate disease development. A disease index was then calculated using the formula (Sharma & Kolte, 1994):

$$\text{Disease index (\%)} = \frac{\text{sum of all disease rating}}{\text{No. of leaves}} \times 100$$

The experiment was conducted twice and data were subjected to analysis of variance (ANOVA). Data and means were separated using Fischer's least significant difference ($\text{LSD}_{0.05}$) test.

β -1,3-glucanase and peroxidase activity

Extraction of total proteins: Enzymes were extracted using 10mM sodium acetate extraction buffer, pH 5.5, containing 10mM mercaptoethanol, 2mM ethylene-diamine-tetra acetic acid (EDTA) and 2mM phenyl-methane-sulfonyl-fluoride (PMSF). The leaves (0.5 g) were ground into a fine powder in liquid nitrogen using a pre-cooled mortar and pestle and homogenized in extraction buffer (plant tissue: buffer, 1:4). The homogenate was centrifuged (12 000 rpm, 4 °C) for 20 minutes. The supernatant was used as the crude enzyme extract.

Estimation of protein concentration: Protein concentration was measured according to the method of Bradford (1976), using a Bio-Rad protein assay reagent and γ -globulin as a standard. The absorbance of the coloured product was measured at 595 nm using a microplate reader (Zenyth 3100) and the protein concentration of each sample determined.

β -1,3-glucanase activity: β -1,3-glucanase activity was determined according to a modified method of Fink, Liefland & Mendgen, (1988). The reaction mixture contained: 240 μ l 50mM sodium acetate buffer (pH 4.5), 240 μ l laminarin (2 mg ml⁻¹) and 10 μ l of the enzyme extract. The mixture was incubated at 37 °C for 10 min. 500 μ l Somogy (Somogy, 1952) reagent was added and the reaction mixture heated for 10 min in a boiling (95 °C) water bath. The mixture was cooled under tap water and 500 μ l of Nelson's reagent (Nelson, 1944) added and the solution mixed thoroughly. The blank was prepared in the same manner except that the buffer solution only replaced the enzyme extract. The absorbance of the sample was read at 540 nm using a spectrophotometer (Varian 8x6 Multicell Block Water Thermostatted Series II, Australia), and specific β -1,3-glucanase activity was expressed as milligram glucose equivalent released per milligram protein.

Peroxidase activity: Peroxidase activity was determined according to the method of Zieslin & Ben-Zaken (1991). The reaction mixture consisted of the following: 830 μ l 40mM potassium phosphate buffer, pH (5.5), 20 μ l enzyme extract, 100 μ l 5mM guaiacol, and 50 μ l H₂O₂ (8.2mM). A blank was prepared in the same manner but lacked enzyme extract. Change in absorbance was read at 470 nm for 180s at 30 °C using a

spectrophotometer (Varian 8x6 Multicell Block Water Thermostatted Series II, Australia). Specific peroxidase activity was expressed as mmol tetraguaiacol per milligram of protein per second.

Compost evaluation

Chemical composition of the composts: A chemical analysis of composts was conducted using the method by Hesse (1971) to evaluate the nutrient composition of composts and their pH values. The procedure was carried out at Department of Soil, Crop and Climate Sciences of the University of the Free State, Bloemfontein.

Fungal diversity of composts: A sample of compost (1 g) was placed in 10 ml of sterile distilled water in a water flask and shaken mechanically for 10 min. The suspension was diluted (10^{-1} to 10^{-6}) and 0.1 ml of each dilution aliquoted onto separate petri-dishes (50 % malt extract agar) in 4 replications. The plates were incubated for 48 hours at 22 °C and the total number of fungal colonies growing in each plate was recorded. Dilution plates that had colonies between 5- 200 were selected. The total number of fungal colonies/or number of colony forming units (CFUs) was estimated (Pugliese *et al.*, 2008; Sharma, 2010; Sharma, 2011). Hyphal tips were removed from each colony with a sterile dissecting needle and placed onto malt extract agar supplemented with 0.5 mg of streptomycin sulphate. After incubation (4-10 days) at 25 °C, the fungi were classified into genera using standard mycological procedures and available literature. Isolates from certain genera that could not be classified into species were transferred onto specific agar media and/or sent for sequencing at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, to identify to species level.

RESULTS

Evaluation of pathogenicity

The severity of *Alternaria* blight on radish plants grown in different growth media was significantly different among treatments (Table 3). Disease severity was significantly lower on radish plants grown in compost #2 and #6 compared to those grown in composts #1, #3, #4, and #5 and soil treatment #2 and #4, in both experiments. Severity of blight on radish plants grown in compost #1, #3, #4, and #5 was low compared to that in soil (soil treatment #2) inoculated with *A. raphani* conidia in experiment 1, but not significantly different in experiment 2. Disease severity in radish plants treated and not treated with SA before inoculation (*A. raphani* conidia) did not differ significantly in both experiments. There were no disease incidences in radish plants grown on soil and not inoculated with the conidia spores.

In general, the severity of *Alternaria* blight in radish plants grown in the majority of the composts (#1-3, #5 and #6) was significantly lower ($P < 0.05$) than in plants grown on soil and not pre-treated with SA before *A. raphani* inoculation. The diameter of lesions ranged between 2-5 mm on plants cultivated in composts but increased to >6 mm on plants grown in soil. Radish plants cultivated in compost #6 were the least infected (35 % and 42 %) followed by radish plants cultivated in compost #2 (42.5 % and 47.5 %). Plants cultivated in compost #4 (65 % and 67 %) were severely infected and did not differ from radish plants cultivated in soil and not pre-treated with SA before inoculation with *A. raphani* conidial spores. SA treatments of plants did not have any impact on disease severity of *Alternaria* blight on radish plants.

β -1,3-glucanase and peroxidase activity

β -1,3-glucanase activity: β -1,3-glucanase activity was measured in plants grown in both composts and soil (Figure 1). Activity of β -1,3-glucanase in radish plants grown on composts #2, #4, and #6 was higher than that of plants grown in composts #1, #3 and #5, and in soil #1 and #2. The β -1,3-glucanase activity of plants grown on soil treatment #3 and #4 was also higher than in plants grown on soil treatment #1 and #2, probably due to the effect of the SA pre-treatment. Activity of β -1,3-glucanase in inoculated and non-inoculated plant (SA pre-treated) was not significantly different at all sampling times in experiment 1. In experiment 2 there was a slight difference evident in β -1,3-glucanase activity at 96 and 144 hours post inoculation (hpi). *A. raphani* significantly increased β -1,3-glucanase activity at 48 and 96 hpi in plants cultivated in compost #6 in experiment 1 and 2. There was also a significant increase in β -1,3-glucanase activity in plants cultivated in compost #2, but only at 48 and 96hpi in experiment 1, and at 48, 96 and 144hpi in experiment 2. A significant increase in β -1,3-glucanase activity was also observed in plants cultivated in compost #4 at all sampling times in both experiment 1 and 2. There was no significant increase in β -1,3-glucanase activity in plants cultivated in compost #3 and #5 at all times in experiment 1 and 2, with the exception being at 144hpi for compost #5 in experiment 2. All treatments inoculated with *A. raphani* rapidly increased the β -1,3-glucanase activity compared to uninoculated treatments (Figure 3).

Peroxidase activity: Peroxidase activity in *A. raphani* inoculated plants was measured in plants grown in composts and soil (Figure 2). A combination of *A. raphani* inoculation

and compost #2 and #6 induced high activity of peroxidase in radish plants at 48hpi which was not evident in the absence of inoculum (Figure 3). A combination of *A. raphani* inoculation and compost #1, #3, #4 and #5 did not induce any increase in peroxidase activity in radish plants throughout the 144hpi trial. The activity levels were not any different from those of radish plants grown in soil. Radish plants cultivated in soil pre-treated with salicylic acid and inoculated with *A. raphani* conidial spores did not exhibit any increased levels of peroxidase activity throughout the trial period. The trends were similar in both experiment 1 and 2.

A combination of *A. raphani* inoculation on plants in composts #2 and #6, induced higher activities of peroxidase and β -1,3-glucanase than in plants in the same growth media but not inoculated. These composts (#2 and #6) in addition to inoculation with *A. raphani*, rapidly induced peroxidase and β -1,3-glucanase activities at 48hpi, whereafter activity decreased but still remained higher than in the other treatments (Figure 4). Compost #6 induced the highest activity of the two enzymes. Compost #4 on the other hand induced an increase in β -1,3-glucanase in both experiments that was higher than that expressed in plants grown in compost #2. This level of induction occurred only at 96 and 144hpi in experiment 1, and at all hpi in experiment 2. Nevertheless, composts #2 and #6 correlated well in terms of enzyme activity with greenhouse bioassays, which showed lower disease severity (mean 45 % and 38.75 % respectively) than other treatments (mean >60 %). The majority of treatments followed the same pattern of rapidly increasing activity at 48hpi and decreasing thereafter (Figure 4).

Compost evaluation

Chemical composition of composts: The chemical composition (Table 4) showed high nutrient levels (Ca, K, Mg, Na, P, N, and C) in all composts with the exception of composts #4, which had high blight infection and low activity of peroxidase. The chemical composition of composts (#2 and #6) that induced high activity of β -1,3-glucanase and peroxidase and lower blight infection, was higher than of other composts. High nutrient levels in composts was not consistently correlated with high activity of β -1,3-glucanase and peroxidase or low blight infection as composts (#1, #3, and #5) with high nutrient levels failed to induce β -1,3-glucanase and peroxidase activity or suppress *Alternaria* blight. The pH values of composts were also measured and were in an optimum range (6.2-8.5).

Fungal diversity: Fungal diversity differed markedly amongst composts of which compost #4 was the highest (Table 5). Compost #4 failed to suppress *Alternaria* blight, however, induced high activity of β -1,3-glucanase slightly but failed to induce peroxidase activity. Compost #6 which suppressed *Alternaria* blight ranked 2nd in terms of fungal density and consistently induced high activity of β -1,3-glucanase and peroxidase. Compost #2 which also consistently induced high activity of β -1,3-glucanase and peroxidase and showed some significant suppression levels to *Alternaria* blight, was the lowest in terms of fungal density. Nine genera of fungi were isolated in the composts of which *Aspergillus niger* (van Tieghem) was the most common species occurring in four composts followed by *A. flavus*, *Emericella nidulans* (Winter) and *Fusarium solani* (Mart.) Sacc. occurring in three composts (Table 6). It is not clear if

any of these species played a role in inhibiting growth of *Alternaria* blight or in the induction of PR proteins; however, *Trichoderma harzianum* (Rifai) and *T. viridae* are reported to induce PR proteins in certain plants. Only compost #6 possessed both these species and induced activity β -1,3-glucanase and peroxidase while compost #4 exhibited only *T. viridae* and failed to induced β -1,3-glucanase and peroxidase activity significantly. Compost #2 which induced activity of β -1,3-glucanase and peroxidase exhibited *Penicillium decumbens* and *P. raistricki* which are not known to induce PR proteins.

DISCUSSION

Disease suppression of foliar pathogens by composts is reportedly associated with the induction of resistance mechanisms in plants (Zhang *et al.*, 1996; 1998; Abbasi *et al.*, 2002; Kavroulakis *et al.*, 2005; Sang, Kim & Kim, 2010; Yogev *et al.*, 2010; Sang & Kim, 2011). The mechanisms by which composts induce disease resistance are reportedly due to factors such as pH levels and the availability of nutrients (Zhang *et al.*, 1996; Hoitink & Boehm, 1999), as well as the activity of beneficial microorganisms (Abbassi *et al.*, 2002). Plants can also acquire enhanced resistance to pathogens after treatment with natural or synthetic compounds such as salicylic acid (SA). This is because such compounds enhance the capacity of plants to mobilize infection-induced cellular defense responses known as “priming” (Conrath, Pieterse & Mauch-Mani, 2002).

In the present study, composts apparently induced a primed state of resistance in radish plants, which enabled them to express defence responses much faster and more

intense when challenged by *A. raphani* (Conrath *et al.*, 2002). This phenomenon was evident from the high activity of β -1,3-glucanase and peroxidase following infection (Figure 1 and 2). However, composts exhibited lower levels of induced PR proteins in the absence of infection, which strengthens the argument that the effect of composts on peroxidase and β -1,3-glucanase activity was more pronounced after infection. This is consistent with findings by Zhang *et al.* (1996; 1998) and Sang & Kim (2011). Consequently, the reduction of blight (Figure 5) in radish plants expressing high levels of β -1,3-glucanase and peroxidase activity was significantly greater ($P < 0.05$) than in plants expressing low levels of activity. The correlation between *Alternaria* blight disease suppression and the rapid infection-induced increase of β -1,3-glucanase and peroxidase activity indicates that induced plant resistance played a role in the suppression of the disease. These results are consistent with previous reports where compost-induced suppression of anthracnose and bacterial speck development in cucumber and *Arabidopsis* leaves was associated with high levels of β -1,3-glucanase and peroxidase activity (Zhang *et al.*, 1996; 1998). Kavroulakis *et al.* (2005) also reported that compost induced SAR was correlated with suppression of the foliar pathogen *Septoria lycopersici* (Speg) in tomato.

Salicylic acid (SA) has been reported to induce SAR by priming plants (Conrath *et al.*, 2002). In the present study, SA sprayed onto leaves slightly induced increased levels of defence responses but failed to suppress *Alternaria* blight development. In order for defence responses to be effective, their expression must be fast and strong (Pieterse & van Loon, 1999). Furthermore, β -1,3-glucanases (PR-2) may not be sufficient on their own to protect plants from diseases (Tuzun & Bent, 2006). However,

they are crucial in disease resistance due to their antimicrobial properties and their ability to generate elicitors (oligosaccharides active in signaling defence responses) upon encountering a pathogen (Ryan, 1987). Peroxidases (PR-9) have an indirect antimicrobial effect by synthesizing lignin which is involved in restricting the entry of pathogens into plant cells and therefore prevent infection (Tuzun & Bent, 2006). Present studies results thus indicate that SA dosage in the absence of compost was probably not sufficient to induce responses strong enough to suppress infection by *A. raphani*.

The relationship between microbial populations in compost, induced reduction of disease incidence and the activation of plant resistance responses remains contradictory. Composts which induced high resistance responses (β -1,3-glucanase and peroxidase activity) had variable fungal population densities. It is not clear if any of the fungal species present in the composts such as *T. harzianum* and *T. viridae* in compost #6 played any role in the induction of the enzyme activities. Compost #2, which also induced high enzyme activity, had a low fungal population density however. The latter was also true for compost #5 although it exhibited a low level of enzyme activity. However, composts #1, #3 and #4 which had high fungal population densities failed to activate an increase in defence response. The fungi, *T. asperellum* and *T. hamatum* 384 have been associated with the induction and accumulation of PR proteins in cucumber roots and other plants (Yedidia, Benhamou & Chet, 1999; Khan, 2004; Shores, Yedidia & Chet, 2005). Bacterial species present in composts have also been reported to play a role in the induction of resistance in plants. For example, *P. agglomerans* was found to induce SAR against bacterial foliar spot of radish caused by

Xanthomonas campestris (Abbasi *et al.*, 2002). Vallad *et al.* (2003) further reported that *Arabidopsis* seedlings grown in soil amended with composts, displayed a higher increase in pathogenesis related defense genes that suppressed bacterial speck caused by *Pseudomonas syringae* *pv.* *tomato* (*Pst*) than plants grown in unamended soil.

Nutrients in composts have been reported to affect many plant diseases and to induce SAR (Hoitink & Boehm, 1999). These nutritional factors provide energy that, in addition to growth and development, may be used for supporting plant defenses against pathogens (Heil, 2002). In the present study, the chemical composition of compost #2 and #6 that induced high levels of enzyme activity was high. However, the chemical composition was also high in composts that failed to induce activity of enzymes. Thus no clear relationship between nutritional composition and enzymes activity could be derived.

High pH values in compost have also been considered a contributing factor in the suppression of diseases such as *Fusarium* wilt (Cotxarrera *et al.*, 2002). Increase in pH reduces the availability of micronutrients such as iron, copper and zinc which are essential for plant growth and subsequently limit growth, sporulation and pathogenicity of pathogen. Compost pH ranging between 6.5 and 8.5 has been reported to favour disease suppression and promote plant growth (Hoitink, Boehm & Hadar, 1993). The pH of compost #2 and #6 in this study was thus close to the optimum pH range that favours disease suppression.

The present study indicated that compost amendments to soil induced SAR in radish plants as a pre-conditioning resistance mechanism to disease caused by *A. raphani*. Resistance was however more pronounced after *A. raphani* infection occurred on radish plants. This phenomenon only occurred in plants grown on compost amended soil compared to unamended soil. This indicates that compost probably primed the plants by rapidly inducing the activity of β -1,3-glucanase and peroxidase enzymes thus enabling them to induce defence responses very quickly upon challenge. Even though SA sprayed on plants induced some defence responses, the levels were quantitatively lower than thresholds required to suppress pathogen development, consequently failing to suppress *Alternaria* blight development. It is not clear if SA is associated with compost-induced *A. raphani* resistance. The endogenous levels of SA during compost induced *A. raphani* resistance were not determined. This however represents an important topic for future research. The chemical composition and fungal density did not consistently predict compost induction of defence responses in radish; however, the mechanisms of compost induces SAR remain a possibility. Compost induction of SAR in plants is accompanied by interactions between microorganisms and the plant and also the presence of important nutrients and other factors that may be present in the compost. A clearer understanding of how composts induce resistance to disease in plants is therefore an important avenue of research for disease management practices.

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Table 1: Characteristics of composts used and treatments on composts and soil performed in the study

Growth medium	Composition	Brand	Treatment of plants	Age (months)
COMPOST 1	Garden refuse + leaves + horse manure	Homemade	Inoculated with <i>A. raphani</i> conidial spores	12
COMPOST 2	Sawdust from <i>Eucalyptus granis</i> + horse manure	Homemade	Inoculated with <i>A. raphani</i> conidial spores	22
COMPOST 3	Garden refuse + 25% cattle + chicken manure	Homemade	Inoculated with <i>A. raphani</i> conidial spores	24
COMPOST 4	<i>E. grandis</i> leaves	Homemade	Inoculated with <i>A. raphani</i> conidial spores	24
COMPOST 5	Culterra organic compost	Commercial	Inoculated with <i>A. raphani</i> conidial spores	>24
COMPOST 6	Terra microbe enriched compost	Commercial	Inoculated with <i>A. raphani</i> conidial spores	>6
SOIL1	Soil	NA [#]	Uninoculated	NA
SOIL2	Soil	NA	Inoculated with <i>A. raphani</i> conidial spores	NA
SOIL3*	Soil	NA	Sprayed with 5mM salicylic acid and uninoculated	NA
SOIL4*	Soil	NA	Sprayed with 5mM salicylic acid and Inoculated with <i>A. raphani</i> conidial spores	NA

* Salicylic acid (5mM) was sprayed onto the leaves 2 days before conidial suspension inoculation

Not applicable

Table 2: Disease development scale used to rate the infection

Rating	Symptoms	Susceptible/resistant
0	no lesions/healthy	Immune
1	non-sporulating pin point size or small brown necrotic spots, less than 5% leaf area covered by lesion	Highly resistant
2	small roundish slightly sporulating larger brown necrotic spots, about 1-2mm in diameter with a distinct margin or yellow halo, 5-10% leaf area covered by lesions	Moderately resistant
3	moderately sporulating, non-coalescing larger brown spots, about 2-5mm in diameter with a distinct margin or yellow hallow, 11-25% leaf area covered by the spots	Resistant
4	moderately sporulating, coalescing larger brown spots about 4-5mm in diameter, 26-50% leaf area covered by the lesions	Moderately Susceptible
5	profusely sporulating, rapidly coalescing brown to dark spots, more than 6mm in diameter, without margins, covering 50-75% leaf area	Susceptible
6	profusely sporulating, rapidly coalescing brown to dark spots, more than 6mm in diameter, without margins, covering >75% leaf area. Leaves yellowish and/ or dead	Highly Susceptible

Table 3: *Alternaria* blight severity in radish grown in a compost or soil or salicylic acid treatments

	Exp 1	Exp 2
Growth medium	Disease severity (% leave blight area/ total no. of leaves)	Disease severity (% leave blight area/ total no. of leaves)
Comp 1	55 ^b ^c	60 ^a
Comp 2	42.5 ^c	47.5 ^b
Comp 3	60 ^b	65 ^a
Comp 4	65 ^{ab}	67 ^a
Comp 5	60 ^b	62.5 ^a
Comp 6	35 ^c	42.5 ^b
Soil treatment 1	0 ^d	0 ^c
Soil treatment 2	75 ^a	72.5 ^a
Soil treatment 3*	0 ^d	0 ^c
Soil treatment 4*	62.5 ^{ab}	65 ^a

Each value is a mean percentage of 8 replicates of 5 radish seeds. Means within a column not followed by the same letters are significantly different ($p=0.05$) according to Fisher's least significant difference test ($LSD_{0.05}=14.85$). *Alternaria* blight severity was expressed as percent leaves blight area per total number of leaves for each treatment.*Plants treated with SA (5mM) before inoculation.

Table 4: Chemical composition of composts

Compost	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	N %	N mg/kg	C %	C mg/kg	pH
1	4080.0	2600.0	1440.0	138.0	2868.5	0.796	7955.0	7.64	76400.0	7.05
2	6490.0	2650.0	1880.0	272.0	3468.5	1.070	10695.0	13.95	139500.0	6.20
3	7140.0	4100.0	1840.0	178.0	5309.0	0.830	8298.2	7.95	79500.0	7.29
4	345.0	1850.0	620.0	98.0	1413.0	0.529	5289.7	9.15	91500.0	6.49
5	3495.0	6000.0	800.0	228.0	2333.5	0.807	8074.5	12.30	123000.0	8.13
6	7825.0	6450.0	1980.0	168.0	5443.0	1.396	13957.0	12.80	128000.0	8.45

Table 5: Composts colony forming units at 10^{-1} dilution

Composts	Colony forming units (CFU)/ g compost
1	7.20×10^4
2	0.40×10^4
3	6.28×10^4
4	12.2×10^4
5	0.68×10^4
6	10.8×10^4

Table 6: Fungal species isolated in the respective composts

Fungal species	Composts					
	1	2	3	4	5	6
<i>Aspergillus flavus</i> Link			X		X	X
<i>Aspergillus fumigatus</i> Fresen					X	X
<i>Aspergillus niger</i> van Tieghem	X		X	X		X
<i>Aspergillus sp</i>						X
<i>Cladosporium cladosporioides</i> (Fresen) de Vries				X		
<i>Clonostachys rosea</i> (Link: Fries) Schoers, Samuels, Seifert & Gams				X		
<i>Emericella nidulans</i> Winter			X		X	X
<i>Fusarium culmorum</i> Sacc.	X					
<i>Fusarium equiseti</i> (Corda.) Sacc.	X		X			
<i>Fusarium lateritium</i> Nees				X		
<i>Fusarium nelsonii</i> Marasa & Logrieco	X			X		X
<i>Fusarium oxysporum</i> Schlecht.				X		
<i>Fusarium solani</i> (Mart.) Sacc.	X		X			X
<i>Fusarium sporotrichioides</i> Sherb						X
<i>Fusarium sp.</i>						X
<i>Fusarium virguliforme</i> O'Donnell & Aoki				X		
<i>Paecilomyces inflatus</i> Burnside Carmichael				X		X
<i>Penicillium chrysogenum</i> Thom.				X		X
<i>Penicillium decumbens</i> Thom.		X		X		
<i>Penicillium glabrum</i> Wehmer Westling	X		X			
<i>Penicillium janthinellum</i> Biourge				X		
<i>Penicillium lividum</i> Westling				X		
<i>Penicillium raistricki</i> Raistricki		X		X		
<i>Rhizopus oryzae</i> Went & Prins. Geerl	X					
<i>Trichoderma harzianum</i> Rifai						X
<i>Trichoderma viridae</i> Pers.				X		X

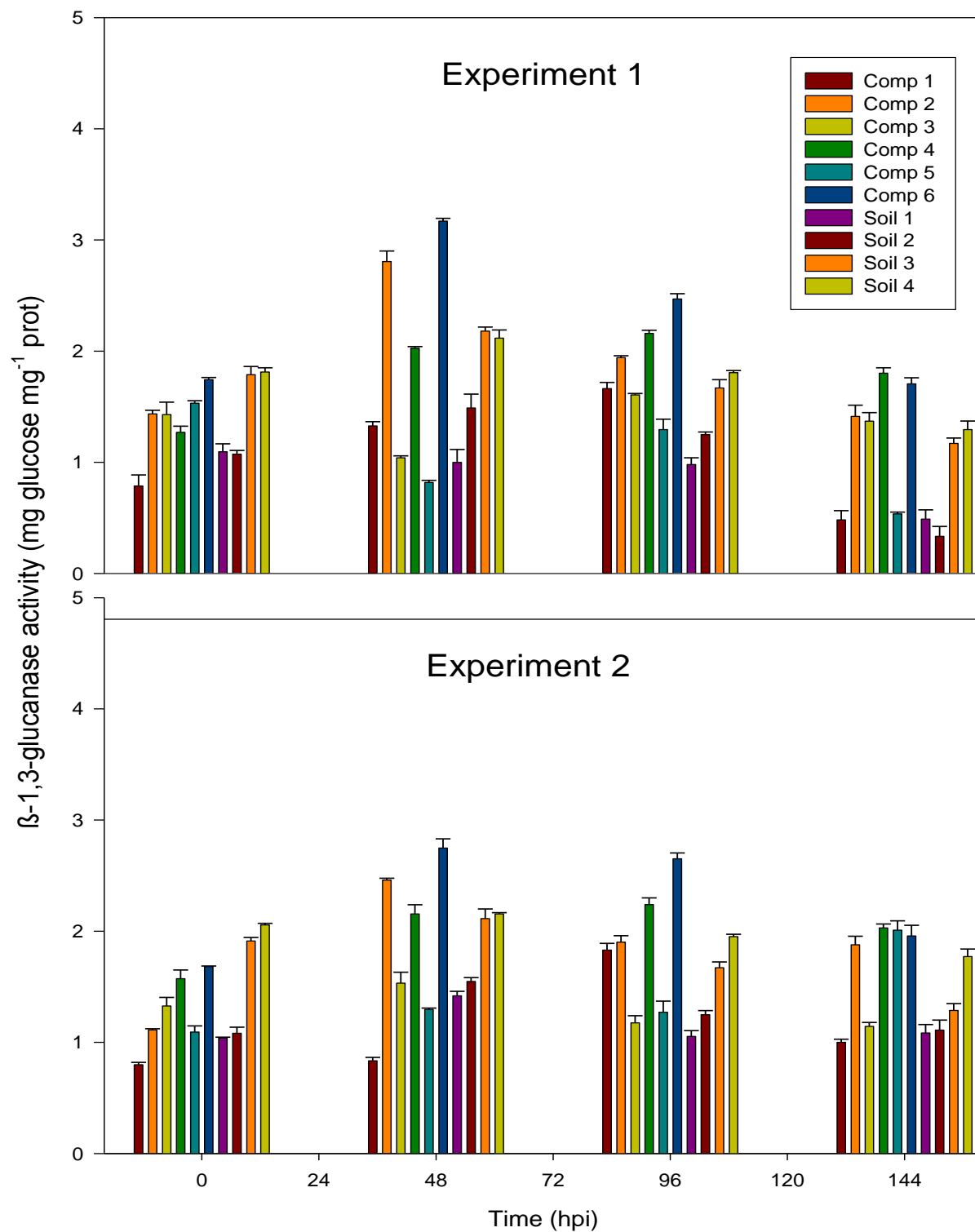


Figure 1: Effect of *Alternaria raphani* on β -1,3-glucanase activity in radish plants grown in different compost and soil media. Data presented are means \pm SD (n=3).

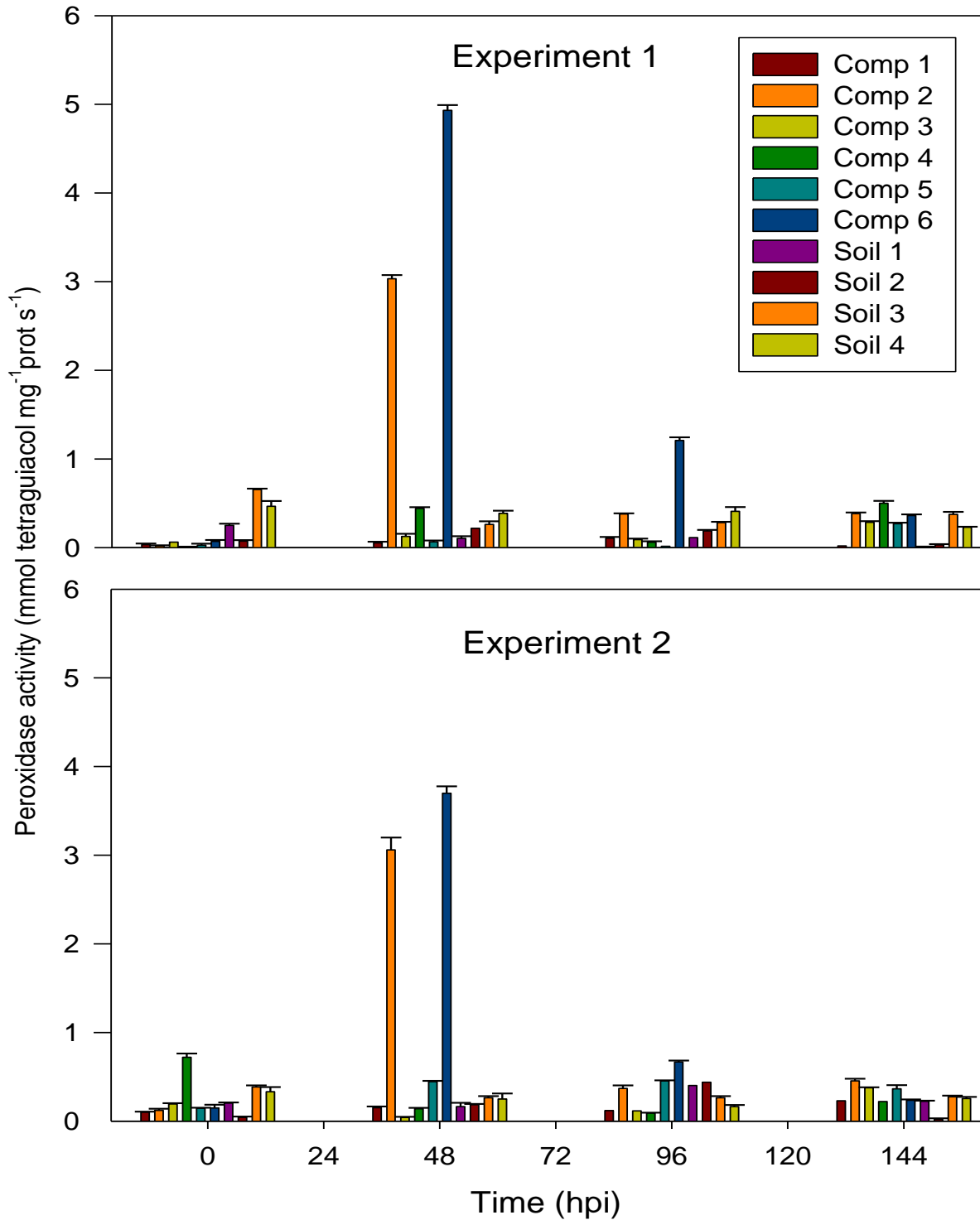


Figure 2: Effect of *Alternaria raphani* on peroxidase activity in radish plants grown in different compost and soil media. Data presented are means \pm SD (n=3).

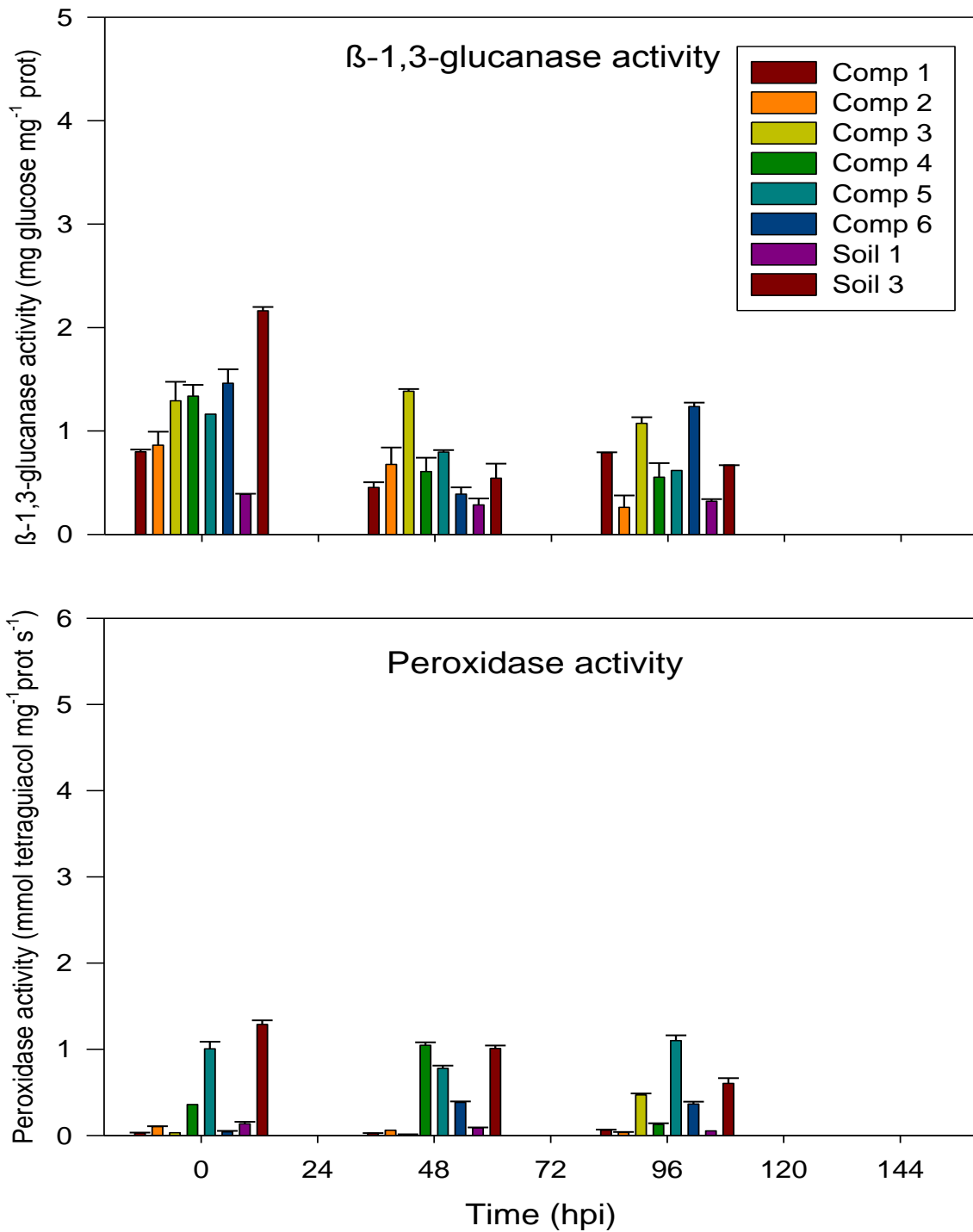


Figure 3: The β -1,3-glucanase and peroxidase activities in uninoculated radish plants grown in different compost and soil media. Data presented are means \pm SD (n=3).

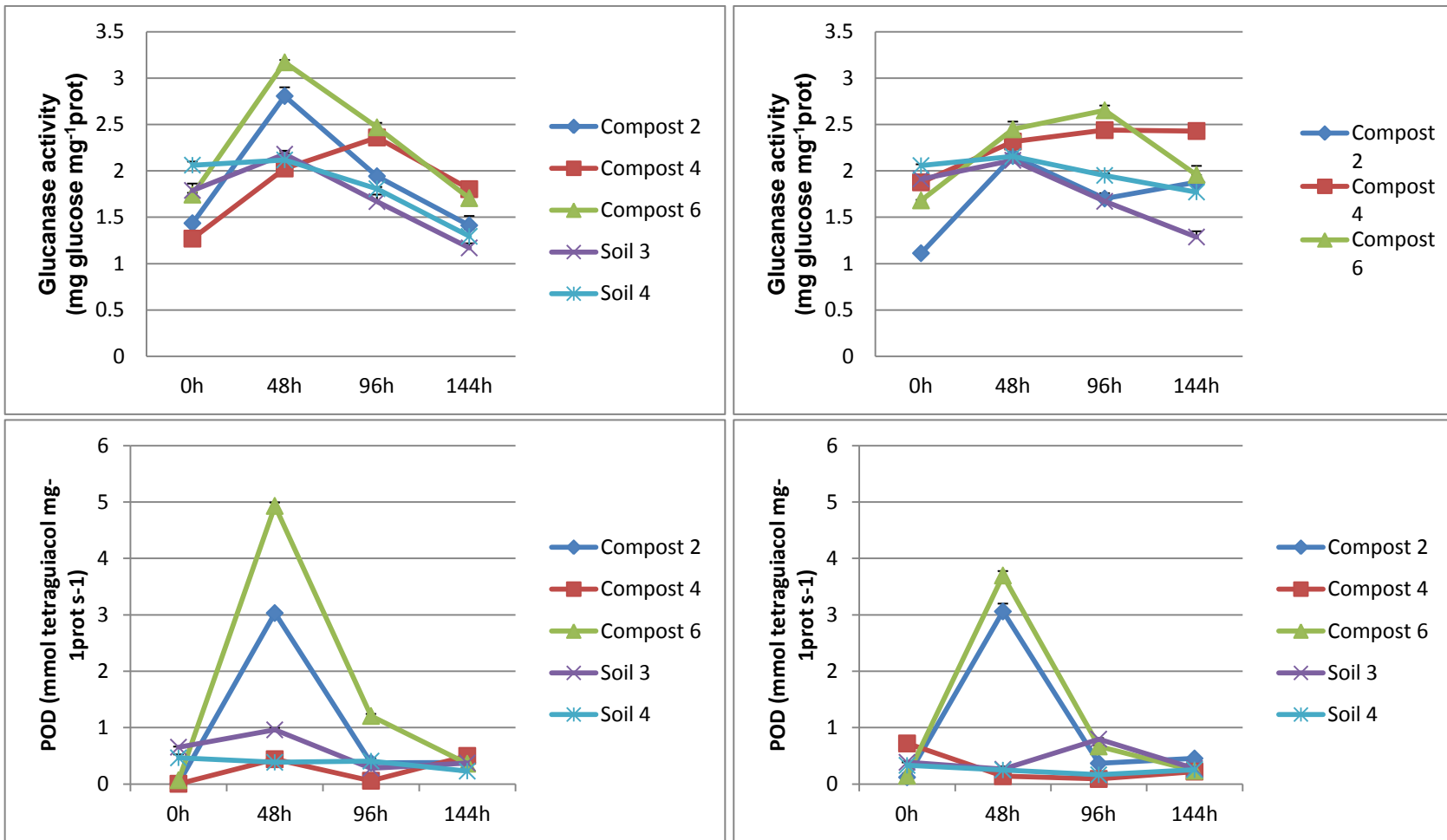
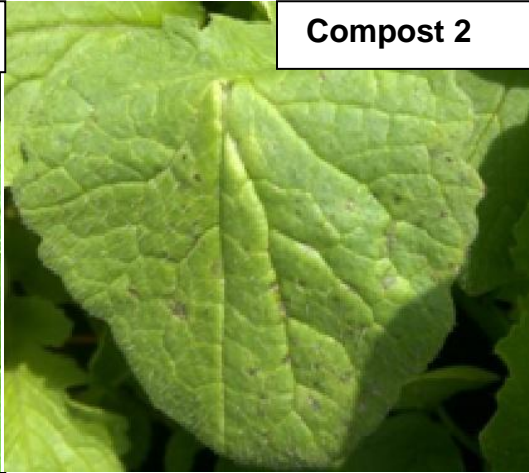


Figure 4: Comparison of enzyme activities (β -1,3-glucanase and peroxidase) for radish plants grown in compost #2, #4 and #6, and soil #3 and #4. Data presented are means +SD (n=3)

Figure 5: *Alternaria* blight severity on radish leaves grown on different media. Compost #2 and #6 rated as resistant. Yellow, dark brown to black circular leaf spots with target like, concentric rings can be seen on compost #4 rated as susceptible and highly severe symptoms on soil growth media (#2 and #4) rated highly susceptible. Plants in soil #1 were not inoculated with the pathogen.



Soil treatment 1



Compost 2



Compost 6



Soil treatment 2



Soil treatment 4



Compost 4

SUMMARY

Composts of different composition, age and maturity were evaluated for their phytotoxic impact on germination and biomass of lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) seedlings. The suppressive ability of composts on two isolates of *Rhizoctonia solani* (Kühn) was further evaluated in the glasshouse. The study also included a growth medium consisting of finely ground soil from disused termitaria which are reported to have high microbial diversity. Six batches of compost suppressed at least one isolate of *R. solani*, while nine batches, including the termitaria soil, failed to suppress the isolates. Correlations were observed in six batches of composts where two batches suppressed one or both *R. solani* isolates and were not phytotoxic to the plants, while four batches were strongly phytotoxic and failed to suppress the two isolates. Other composts gave variable results in terms of relationship between phytotoxicity and pathogenicity. The microbial diversity of the composts measured by means of various biochemical and molecular analyses gave variable results in terms of consistently predicting the phytotoxicity and disease suppressiveness of the composts.

The impact of genetic variation of pathogens on compost's inherent disease suppressiveness was studied by testing the suppressiveness of various compost batches to seven isolates of *R. solani* on radish seedlings. Various biochemical and molecular analyses were employed to evaluate the chemical and biological characteristics of composts that possibly have a relationship with their respective disease suppressive ability. The nutritional composition and fungal population densities were also studied in this regard. There was a highly significant variation between *R. solani* isolates and their interaction with compost amendments. The

chemical and biological attributes of the composts could however not explain the variation of the isolates' response to the diseases suppressive ability of the compost. Some isolates were suppressed by compost while others were not, which indicates that pathogen genotype plays an important role in the disease suppressive ability of certain composts.

Research on the suppression of *Alternaria raphani* blight of radish seedlings was tested in the glasshouse. Results confirmed that certain composts have the ability to induce systemic acquired resistance (SAR) in plants. Radish plants with reduced *Alternaria* blight symptoms exhibited higher levels of pathogenesis-related (PR) proteins compared to plants with severe symptoms. However, the levels of PR proteins β -1,3-glucanases and peroxidases in radish plants, grown on certain composts, rapidly increased after inoculation with the pathogen, suggesting that the compost primed the plant and enabled it to induce PR proteins upon pathogen challenge. Soil with or without salicylic acid (SA) applied to leaves was also studied and neither of the treatments was successful in suppressing *Alternaria* blight. However, SA sprayed onto the leaves did induce defence responses that were not significantly different from that in pathogen challenged plants.

The results of the present study confirm that there is potential for the use of compost in the management of soilborne and foliar pathogens. The challenge that remains, however, is that plants are exposed to a broad range of pathogen genotypes in their lifetime and compost that can suppress a broad range of pathogens is therefore ideal. The addition of effective antagonistic microorganisms to compost that is able to inhibit a broader range of pathogens will enhance its

potential for disease suppression. These practices should therefore be addressed in order to optimize the preparation and use of composts for disease suppression.

Keywords: Soil ecology; Disease suppressive compost; Microbial diversity; *R. solani*; Radish; Phytotoxicity; Pathogenicity; Genetic variation; Systemic acquired resistance; *A. raphani*

OPSOMMING

Kompos van verskillende samestellings, ouderdomme en vlakke van volwassenheid is geëvalueer volgens hul fitotoksiese impak op die ontkieming en biomassa van blaarslaai- (*Lactuca sativa*) en radys- (*Raphanus sativus*) saailinge. Die onderdrukkingsvermoë van kompos op twee isolate van *Rhizoctonia solani* (Kuhn) is verder in die glashuis geëvalueer. Die studie het ook 'n groeimedium, bestaande uit fynge maalde sand van ou termitehope, wat volgens bronne oor baie hoë mikrobiële diversiteit beskik, ingesluit. Ses komposmengsels het ten minste een isolaat van *R. solani* onderdruk, terwyl nege komposmengsels – insluitende termiethoop-grond, nie die isolate onderdruk het nie. Korrelasies is waargeneem by ses komposmengsels waar twee mengsels een of beide *R. solani* isolate onderdruk het en nie fitotoksies was ten opsigte van die plante nie, terwyl vier mengsels hoogs fitotoksies was en nie die twee isolate onderdruk het nie. Ander komposmengsels het veranderlike resultate opgelewer in terme van die verhouding tussen fitotoksiteit en patogenisiteit. Die mikrobiële diversiteit van die komposmengsels, wat deur middel van verskeie biochemiese- en molekulêre-tegnieke gemeet is, het veranderlike resultate gelewer in terme van die konsekwente voorspelling van fitotoksiteit en/of die siekte-onderdrukkingsvermoë van die komposmengsels.

Die impak van genetiese variasie van patogene op kompos-mengsels se siekte-onderdrukkingsvermoë, is bestudeer deur verskeie komposmengsels se onderdrukkingsvermoë van sewe *R. solani* isolate op radys-saailinge te toets. Verder is verskeie biochemiese- en molekulêre-tegnieke gebruik om moontlike chemiese en biologiese kenmerke van komposmengsels, wat met hul onderskeie siekteonderdrukkingsvermoë verband hou, te evalueer. Die komposmengsels se

voedingswaardesamestelling en swambevolkingsdigthede, is ook in diè verband bestudeer. Daar was beduidende vlakke van variasie tussen die *R. solani* isolate en hul interaksie met die komposmengsels. Die chemiese en biologiese kenmerke van die komposmengsels kon egter nie hierdie wisseling in terme van die isolate se reaksie jeens die komposmengsels se siekte-onderdrukkingsvermoë, verduidelik nie. Sommige isolate is onderdruk deur komposmengsels en ander nie, wat duidelik aandui dat patogene se genotipe 'n belangrike rol speel in die siekte-onderdrukkingsvermoë van komposmengsels.

Die onderdrukkingsvermoë van komposmengsels op blaarskroei van radys-saailinge deur *Alternaria raphani* is in die glashuis getoets. Resultate het bevestig dat sekere komposmengsels die vermoë het om sistemies-verwerfde weerstand by plante te veroorsaak. Radysplante met min skroei-simptome het hoër vlakke van patogeenese-verwanteproteïene getoon in vergelyking met plante met ernstige simptome. Die vlakke van die twee proteïene, β -1-3-glukanases en peroksidases by radysplante wat in kompos gekweek is, het egter vinnig toegeneem na inokulasie met die patoogeen. Die kompos berei dus die plante voor en stel hulle in staat om patogeenese-verwante proteïene vry te stel indien hul deur patogene aangeval word. Suiwer grond, met of sonder salisielsuur behandeling op radysblare, is ook ondersoek en beide was onsuksesvol in die onderdrukking van *Alternaria*-blaarskroei. Die sproei van salisiel-suur op die blare het egter ook verdedigingsreaksies ontlok, maar die vlakke was nie beduidend anders as by patoogeenbesmette plante nie.

Die resultate van die huidige studie het bevestig dat daar potensiaal is in die gebruik van komposmengsels in die bestuur van grond- en blaarpatogene. Die

uitdaging lê daarin dat plante in hul leeftye aan veelvuldige patogene blootgestel word. Kompos wat 'n breër reeks patogene kan onderdruk, is dus nodig. Die toevoeging tot kompos van effektiewe antagonistiese mikoorganismes wat die groei van 'n breër reeks patogene onderdruk, sal die kompos se potensiaal as siekte-onderdrukker, versterk. Hierdie soort praktyke moet aangespreek word ten einde die voorbereiding en gebruik van kompos as siekte-onderdrukker van 'n breër reeks patogene, te optimaliseer.