ORGANICALLY BASED STRATEGIES USED BY SMALL-SCALE FARMERS IN LESOTHO FOR THE SUSTAINABLE MANAGEMENT OF SOILBORNE DISEASES

By

MAPOTSO ANNA KENA

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Department of Plant Sciences (Plant Pathology)

Faculty of Natural and Agricultural Sciences

University of the Free State

BLOEMFONTEIN

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Promoter: Prof. W.J. Swart

Co-promoter: Dr S. Ralitsoele

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PREFACE

Traditional agricultural practices used by small-scale farmers in Lesotho, often provide effective and sustainable means of disease control. These practices often include the use of composted animal manure and crude plant extracts with the former proving effective in the management of soilborne diseases. The main purpose of this study was to investigate the effectiveness of the aforementioned disease management strategies in the laboratory and greenhouse. The project was conducted over a period of four years and the resulting dissertation consists of five chapters. Due to the fact that each chapter represents an independent unit, some repetition and lack of continuity between chapters is unavoidable.

Chapter One represents a literature review covering the management of soilborne plant diseases in general. Abiotic and biotic factors influencing soilborne pathogens are reviewed. Abiotic factors such as soil pH, moisture and temperature are emphasized, while microbial activities in soil as a limiting factor in pathogen survival, are also discussed. The effect of organic soil amendments using composts and crude plant extracts, and other strategies for managing soilborne diseases are also reviewed.

Chapter Two investigates the suppressive effects of four types of composted animal manure on damping-off of vegetable seedlings incited by *Rhizoctonia solani* Kuhn, *Fusarium oxysporum* Schlecht. and *Pythium ultimum* Trow. These three pathogens were identified as the main causal agents of seedling damping-off in vegetable seedbeds in Lesotho. Evaluation of the effects of the four composted animal manures was conducted on seedling damping-off under greenhouse and field conditions.

General effects of microbial activity in composts on soilborne pathogens prompted the investigation covered in Chapter Three. Fungal species in composted animal manure were also investigated with the characterization of fungal isolates based predominantly on morphology. FDA hydrolysis was used to determine microbial activity.

In Chapter Four, the extracts of *Rhamnus prinoides* L. Herit, *Artermisia afra* Jacg ex Willd, *Leucosidea sericea* Eckl & Zeyh and *Melia azedarach* L. are evaluated for their effectiveness against *R. solani*, *P. ultimum* and *F. oxysporum*. The four plants are used to control insects pests in Lesotho and farmers using them have reported low incidences of soilborne diseases. Their effectiveness is compared with those of the recommended fungicide dichlorophen.

Chapter Five examines the compatibility of composted animal manure and plant extracts in the suppression of soilborne pathogens. Composted animal manure is utilised by farmers in Lesotho as a soil conditioner, while plant extracts are applied to suppress insects pests. Fields treated with these plant extracts also have lower incidences of fungal diseases. Although there is no similarity between fungi and insects, we suspect that these plants also have suppressive effects towards soilborne fungal diseases. The use of these soil amendments simultaneously were therefore investigated.

CHAPTER ONE

THE SUSTAINABLE MANAGEMENT OF SOILBORNE PLANT PATHOGENS WITH SPECIFIC REFERENCE TO COMPOSTING, ORGANIC AMENDMENTS AND PLANT EXTRACTS

1.0 INTRODUCTION

Soilborne plant pathogens cause extensive damage to crops worldwide and often result in significant economic losses despite the fact that they often remain "hidden" and losses are therefore never quantified (Neher and Campbell, 1994). According to Bruehl (1990), the most destructive soilborne diseases are those caused by *Pythium* and *Rhizoctonia* spp. *Pythium* seedling rots, as well as roots rots and foliar blights of cuttings are among the most devastating diseases of greenhouse crops because of the high plant densities and favourable environmental conditions present in greenhouses (Ben-Yephet and Nelson, 1999).

Soilborne plant pathogens are distinguished from other phytopathogens by their unique morphological/physiological characteristics, which provide them with the ability to infect the roots of host plants (Bruehl, 1990). Intensive farming systems greatly enhance the negative impact of these pathogens. Significant losses can occur because of narrow crop rotations together with the type of tillage used, thus resulting in soil inoculum levels reaching very high densities (Blok *et al.*, 2000). The introduction of synthetic nitrogenous fertilizers can also cause a decline in the quality of soil organic matter, which in turn exacerbates disease severity (Lewis and Papavizas, 1975).

Besides causing disease, soilborne plant pathogens play many significant roles in the soil ecosystem (Christensen, 1989). Some pathogenic fungi are also responsible for the decomposition and mineralization of organic residues in soil (Harley, 1971; Christensen, 1989). Pathogenic soil fungi

thereby provide an important food source for other soil organisms (Moore *et al.*, 1988; de Ruiter and Bloem, 1994). Pathogenic soil fungi also contribute to soil quality and sustainability of the soil ecosystem (Doran and Linn, 1994; Eash *et al.*, 1994). Soilborne pathogenic fungi can also be antagonistic towards other pathogenic microorganisms in the soil. For example, *Rhizoctonia solani* Kuhn, a causal agent of damping-off of seedlings, has been found to parasitize *Pythium debaryanum* Hesse, which also causes damping-off of seedlings (Doran and Linn, 1994).

Many different methods are used to control soilborne pathogens. These include cultural, biological and chemical tactics. A crucial factor in the management of soilborne diseases is to reduce inoculum levels before a particular crop is planted (Blok *et al.*, 2000). Soil fumigation is a widely used method of managing soilborne diseases, but due to its high cost, it cannot be afforded by small-scale farmers, and its use is therefore somewhat limited (LaMondia *et al.*, 1999). Biotic and abiotic factors that affect the survival and dispersal of soilborne pathogens are therefore usually manipulated in the management of soilborne pathogens. For example, strategies such as crop rotation (Mol *et al.*, 1995; Xiao *et al.*, 1998), tillage (Paul and Clark, 1989; Bockus and Shroyer, 1998), the application of pesticides (Lewis and Papavizas, 1975) and organic soil amendments (Sun *et al.*, 1989; Nair *et al.*, 1993; Scholtze and Lootsma, 1998; Sampangiramaiah, 1997), have been shown to reduce the survival of pathogen propagules in the soil.

This review will provide a brief historical perspective of soilborne disease management as well as a brief outline of the most important biotic and abiotic factors that influence the survival, dispersal and longevity of

soilborne plant pathogens. Management strategies aimed at manipulating these factors will be discussed with special attention devoted to practices such as adding organic soil amendments, composting and the addition of plant extracts to soils.

1.1 Historical perspectives

Farmers in all parts of the world for ages have relied on the use of organic amendments to improve soil conditions and suppress plant pathogens (Cook and Baker, 1983). Islamic and Roman writers often referred to the application of manure, other organic matter and/or ashes around the roots of diseased plants (Orlob, 1973). Various types of manure were used to cure diseases of bananas, apples, peaches, citrus trees and other crops (Al-Awan, 1988). Farmers in the Far East used organic materials until the mid-nineteenth century when mineral fertilizers were first introduced (Cook and Baker, 1983). Many farmers in China and South and Central America used to apply organic amendments to soil as a sustainable means of improving soil fertility and suppressing soilborne diseases (Cook and Baker, 1983). Farming systems in seventeenth century China showed that the application of organic fertilizers or manure was the most effective means to improve soil structure and achieve sustainability, even under intensive cultivation (Cook and Baker, 1983).

In Africa, traditional farmers have also utilized organic materials to improve soil fertility and suppress soilborne diseases. A common practice is to incorporate organic plant materials into soil mounds and raised beds (Adesiyan and Adenjii, 1976). In Ghana, farmers can increase yam yield and

decrease nematode infection by adding cow dung to soil mounds (Adesiyan and Adenjii, 1976). Cook and Baker (1983) attribute the suppressive effect of this practice either to increased populations of nematode trapping fungi or to nematodes being attracted to organic matter rather than to yam roots.

The use of microbial antagonists in plant disease control dates as far back as 1937, when practical directions were given for controlling *Phymatotrichum* root rot of cotton by burying organic manure in deep furrows prior to planting (Sun *et al.*, 1989). Intensive studies regarding the use of organic amendments to enhance the suppression of root infecting fungi by soil micro flora began in the 1950's (Sun *et al.*, 1989). Soil amendments used by traditional farmers to improve soil fertility and manage plant diseases usually consisted of animal and human manure, composts, crop and plant debris, aquatic plants and mud from rivers, streams, and canals. Animal products such as blood, urine and powdered bones, horns and ivory were also incorporated into the soil, in addition to plant matter such as straw, husks, leaves, and bark shavings (Watson, 1973).

2.0 ABIOTIC FACTORS INFLUENCING SOILBORNE PLANT PATHOGENS

The soil environment is very complex, a fact that can and does significantly influence microorganisms (Bruehl, 1990). Factors such as soil texture and structure, pH, moisture and temperature have been shown to significantly affect the survival of soilborne pathogens. A few of the most important factors will be examined below.

2.1 Soil pH

Soil pH can affect soil pathogens in many different ways. Early experiments conducted by Walker (1950) on the effect of soil pH on pathogen survival, showed that *Fusarium* wilt of cotton and club root of cabbage are favored by acidity while *Phymatotrichum* root rot and potato scab caused by *Streptomyces scabies* (Thaxter) Waksman & Henrici are favored by alkaline soils. Soil pH also correlates with soil moisture to suppress or stimulate soilborne pathogens. For example, club root of cabbage caused by *Plasmodiophora brassicae* Waronin does not occur in heavily limed soil if kept continuously moist (Dobson *et al.*, 1983).

Studies conducted by Paulitz (1987a) revealed that pH does not have a major effect on the *in vitro* growth rate of *P. ultimum*, especially in the range of 5.0-7.0. Soil pH more often influences host vigour directly rather than acting on the growth of pathogens (Paulitz, 1987a). However in most cases, pH levels of amended soils may rise within a short time due to nutrients released from the amendments, thus cancelling this effect (Hoitink and Fahy, 1986). Unfortunately, disease control through the maintenance of different pH values is impractical as most plants grow at different pH levels (Hoitink and Fahy, 1986).

2.2 Soil water

Available soil water is essential for microbial activity, and any shortage of water inhibits the activities of soil organisms (Bruehl, 1990). Water stress can also disrupt intracellular compartmentation; releasing latent acid

hydrolysis, thus cause direct injury to plants and exposing them to pathogen attack (Bruehl, 1990). High soil moisture or water logging is the primary environmental factor in the development of *Phytophthora* root rot in crop production (Zentmeyer, 1980). Sterne *et al.* (1977) studied the effects of metric and solute water potential on *Phytophthora* root rot of avocado. Highest disease levels occurred near saturation, -5 to 0 kPa matric potential. Disease severity was greatest at 37 kPa and decreased as the water content dropped towards –262 kPa. The authors concluded that metric water potential above –10 kPa was required to facilitate zoospore movement.

Pathogens such as *P. ultimum* and *S. scabies* are also affected by soil water content. *Pythium ultimum* germinates and grows rapidly in wet soil in the presence of host exudates (Kerr, 1964), while *S. scabies*, which causes potato scab, is favoured by soils drier than -40 kPa. Managing soil water content by applying mulch, for example, can therefore play a significant role in the successful management of these pathogens.

2.3 Soil compaction

Soil compaction, which in most cases is the result of soil disturbance, also has an effect on disease incidence caused by soilborne plant pathogens. For example, increased *Rhizoctonia* root rot has been attributed to compacted soil (Cook, 1986; Cook and Veseth, 1991; Bockus and Shroyer, 1998) as has root rot of white beans caused by *R. solani* and *Fusarium solani* f. sp. *phaseoli* Sacc. This has been mainly attributed to the fact that the mycelial web of *R. solani* and sclerotia of *F. solani* remain undisturbed in compact soils, thus increasing their ability to attack roots (Bockus and Shroyer, 1998).

Soil compaction also affects oxygen availability to roots, thereby resulting in decreased levels of disease resistance in plants (Cook and Veseth, 1991).

3.0 BIOTIC FACTORS INFLUENCING SOILBORNE DISEASES

The survival and spread of soilborne plant pathogens is greatly influenced by biotic factors in soils. Microbial communities in soil can influence the survival of soilborne plant pathogens and many soil microorganisms affect soilborne plant pathogens either by competing with them for nutrients (Whipps, 1997), or by acting as hyperparasites (Chen *et al.*, 1988a) or antagonists (Alabouvette *et al.*, 1993).

Numerous microorganisms contribute to the mortality of plant pathogens in soil and artificial growth media (Simon and Savisithamparam, 1988; Boehm *et al.*, 1993). Microbial activity can also prevent spore germination of some soilborne pathogenic organisms thereby preventing infection (Chen *et al.*, 1988a). The mechanisms are presumed to be antagonism, hyperparasitism, antibiosis and/or competition for nutrients (Chen *et al.*, 1988b; Mandelbaum and Hadar, 1990; Hoitink *et al.*, 1991).

Antagonism is described as an interaction between species in which at least one of the interacting species is harmed (Whipps, 1997). The three modes of action during antagonism are: competition, where demand exceeds immediate supply of nutrients or space; antibiosis, where antagonists secrete metabolites harmful to pathogens; and parasitism, where nutrients from the pathogen are utilized by the suppressive agent (Whipps, 1997).

Microbiota in soil can be antagonistic towards many pathogens (De Brito Alvarez *et al.*, 1995; Liu *et al.*, 1995a; You and Savasithamparam, 1995)

and often; one antagonist may exhibit several modes of action simultaneously or sequentially (Whipps, 1997). In some cases, dormant propagules such as sclerotia, clamydospores and oospores are stimulated, but are unable to compete with active saprophytic microbiota in the absence of the host and are thus subject to nutrient stress (Whipps, 1997). Indirect mechanisms are also known where plants respond to the presence of an antagonist, resulting in induced resistance or perhaps plant growth promotion. In the case of natural biocontrol in some suppressive soils, several antagonists exhibiting different modes of action may act in concert to control a particular disease (Alabouvette *et al.*, 1993).

Many species of fungi are parasites of soilborne pathogens (Harley, 1971). For example, some species of *Gliocladium* have been reported as parasites of *R. solani*, *Pythium aphanidermatum* (Edson) Fitzp. *Sclerotinia sclerotiorum* Lib. De Bary and *Sclerotinia rolfsii* Sacc. (Tu and Vaataja, 1980; Beagle-Ristanio and Papavizas, 1985; Howell, 1987; Kenerley *et al.*, 1987; Liu, 1989; Sreenivasaprasad and Manibhushanrao, 1990). Studies of the invasion of oospores of *Phytophthora megasperma* var. *sojae* Drechsler, *Phytophthora cactorum* Libert. & Cohn., *Pythium* sp., and *Aphanomyces euteiches* Kendr. in soil showed that an array of microorganisms including oomycetes, chytridiomycetes, hyphomycetes, actinomycetes, and bacteria are capable of parasitically invading and destroying oospores of these pathogens (Sneh *et al.*, 1977).

Many microbiologists believe that in the microbial struggle for occupancy of any given environment, antibiosis is a decisive factor. Antibiosis appears to play a major role responsible for the biocontrol potential of

Gliocladium (Pachenari and Dix, 1980; Lumsden *et al.*, 1992 and 1993; Wolffhechel and Funck, 1992). Antibiotic production by soil fungi exhibiting biocontrol activity is commonly reported for isolates of *Gliocladium* species (Howell *et al.*, 1993; Wilhite *et al.*, 1994), *Taloromyces flavus* Link (Kim *et al.*, 1990) and *Trichoderma* species (Ghisalberti and Sivasithamparam, 1991; Scarcelletti and Faull, 1994; Huang *et al.*, 1995; Wada *et al.*, 1995). A large number of bacteria also produce antibiotics, which suppress fungal pathogens in the soil (Whipps, 1997). The antibiotic, pyrrolnitrin, produced by *Pseudomonas fluorescens*, and obtained from the rhizosphere of healthy cotton plants, reduced *R. solani* damping-off by 50% after seed treatment (Howell, 1982). This bacterium also controls *P. aphanidermatum* on cucumber (Lumsden *et al.*, 1983).

4.0 STRATEGIES FOR MANAGING SOILBORNE DISEASES

Crop management strategies that change soil properties or plant cover can affect soil organisms both negatively and positively (Paul and Clark, 1989). A deteriorated soil structure and impermeable layers in the soil profile, sometimes lead to unexpected disease outbreaks (Paul and Clark, 1989). Practices such as tillage (Bockus and Claassen, 1992; Reicosky and Lindstrom, 1993; Bockus and Shroyer, 1998), crop rotation (Mol *et al.*, 1995; Xiao *et al.*, 1998), and fertilization (Smiley, 1975; Murray *et al.*, 1992) can have a significant impact on soilborne plant pathogens. Competition for elements such as carbon and nitrogen, which are required by both pathogens and other soil microorganisms, may also be involved in the suppression of soilborne pathogens, as for example, the suppression of *F. oxysporum* f. sp.

melonis Snyder & Hansen and *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder and Hansen by *Trichoderma harzianum* Rifai (Sivan and Chet, 1989). Competition for thiamine has been suggested as a possible mode of action in the control of *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* Walker by a sterile red fungus in the rhizosphere of wheat (Shankar *et al.*, 1994). Competition for volatile organic materials derived from germinating seeds, which may stimulate spore germination, may also be involved in the suppression of *P. ultimum* by *Pseudomonas* sp (Paulitz, 1991). Iron competition between pathogens and rhizobacteria is another mechanism involved in the suppression of soilborne pathogens (Leeman *et al.*, 1996). The effectiveness of some of these management strategies on soilborne diseases and their causal agents are discussed in more detail below.

4.1 Tillage

Tillage is a soil management strategy practiced by farmers worldwide. It includes conservation tillage, which includes reduced, minimum or stubble-mulch tillage, no-till, and conventional tillage (Paul and Clark, 1989). Tillage practices play an important role in determining the presence of both pathogenic and beneficial microorganisms in soil. For example, a comparison of fungal populations between ploughed and minimum tilled soil indicated that in ploughed soil, there was a population increase from a depth of 10 to 30 cm (Paul and Clark, 1989). The effect of tillage on pathogens varies according to the pathogen and the tillage system used. There are as many reports of the suppression of plant disease as there are concerning the stimulation of plant disease (Roane *et al.*, 1974; Bockus and Shroyer, 1998).

Reduced tillage encourages the survival of pathogens in the previous year's crop residues, making disease occurrence more likely (Bockus and Shroyer, 1998). Reduced tillage can also favour pathogens via mechanisms such as protecting the pathogen's refuge in residue from microbial degradation, lowering soil temperatures, increasing soil moisture and leaving soil undisturbed. Observations made by Roane *et al.* (1974), on gray leaf spot of maize, showed that the disease increased with reduced tillage systems. Plant residues serve both as refuge and food source for pathogens, which are consequently able to infect the succeeding crop. In the case of *Cephalosporium graminearum* Crand. which causes showy stripes on wheat leaves, reduced tillage is very important for survival of the pathogen (Wiese, 1987). *Gaeumannomyces graminis*, which causes take-all disease of wheat, survives as mycelium in the host plant (Bockus and Shroyer, 1998). Reduced tillage favours survival of the pathogen by leaving large, infected plant fragments that last longer in the soil environment (Moore and Cook, 1984; Wilkinson *et al.*, 1985).

Pythium and *Rhizoctonia* spp. are also favoured by reduced tillage (Cook and Haglund, 1982; Cook and Veseth, 1991). *Pythium* spp. produce long-lasting resting spores and has the ability to survive saprophytically in the soil (Bockus and Shroyer, 1998). Wet soils that result from reduced tillage increase the ability of *Pythium* spp to affect wheat seedlings (Heri *et al.*, 1987). In the long-term, no-till has been shown to affect both plant growth and yield. Pathogens such as *Bipolaris sorokiniana* Sacc., *F. graminearum*, *F. culmorum* and *F. aveneacum*, and *Pseudocercosporella herpotrichoides* Deighton., are completely or partially controlled by reduced tillage (Bockus

and Shroyer, 1998). Reduced tillage can be used very successfully in the management of soilborne plant pathogens, when used in conjunction with crop rotation (Bockus and Shroyer, 1998).

4.2 Crop rotation

Crop rotation has been used very effectively to manage soilborne plant diseases for centuries (Garret, 1955). One of the aims of crop rotation is to deprive the pathogen of its host, so that it has to survive for long periods in the soil during which time it might die of starvation or be lysed by natural soil organisms (Neher and Campbell, 1994). This practice is still widely used to manage soilborne pathogens. It has been a prominent cultural practice used to reduce take-all of wheat (Yarham, 1981; Kollmorgen, 1985). The effectiveness of crop rotation in take-all management is attributed mainly to the build up of a specific group, or groups, of antagonistic microorganisms in the lesions of host plants, and to the subsequent transfer of these microorganisms in host residues (Rovira and Wildermouth, 1981; Cook *et al.*, 1986). In fact, long crop rotations permit the destruction of pathogen inoculum by antagonists residing in soil (Cook, 1985). Crop rotation is highly recommended to reduce *Verticillium dahliae* Kleb. microsclerotia in soil and to reduce *Verticillium* wilt in certain crops (Xiao *et al.*, 1998). In most cases, for successful control of this pathogen, a 5-10 year rotation is required to reduce its density in soil (LaMondia *et al.*, 1999).

4.3 Fertilization

The primary consideration in fertilizing soil is to enhance plant growth and increase crop yield (Cook and Baker, 1983). Fertilizer application has however been implicated in the natural suppression of soilborne pathogens by stimulating indigenous fungal antagonists (Cook and Baker, 1983). For example, both soluble NH_4NO_3 and slowly available uramite increases the effectiveness of corn or oat soil amendments in reducing the severity of root rot of bean caused by *R. solani* (Davey and Papavizas, 1981).

The ability of soil to suppress pathogens and support the activity of beneficial organisms depends on the organic matter in the soil (Lewis and Papavizas, 1975). The quantity of readily biodegradable organic matter present in the form of cellulotic substances determines the extent to which the suppression of pathogens will last (Lewis and Papavizas, 1975). This suppressive effect is attributed partially to the gradual release of organic nutrients from soils rich in organic matter, which support microbial activity and sustain biological control (Hoitink *et al.*, 1991).

The availability of nitrogen, potassium, magnesium, calcium and other micronutrients also plays a very significant role with regard to pathogen suppression (Rowe *et al.*, 1987; LaMondia *et al.*, 1999). High levels of nitrogen generally promote succulent growth in plants (Hoitink and Fahy, 1986) and have been shown to promote diseases such as fire blight, *Phytophthora* stem dieback and some bacterial leaf spots in plants (Hoitink and Fahy, 1986). Nitrogen application is also reported to enhance *Fusarium* wilt in certain plants (Hoitink *et al.*, 1991). High nitrogen levels have, however, also been associated with decreased levels of *Verticillium* blight of

potatoes (Rowe *et al.*, 1987) and the application of nitrogen rich media decreases inoculum *of V. dahliae* in soil (LaMondia *et al.*, 1999).

Take-all associated with wheat is suppressed by ammonium nitrogen and enhanced by nitrate nitrogen (Smiley, 1975). Simon and Sivasithamparan (1988) studied the suppression of take-all disease by ammonium sulphate. They proposed that the application of ammonium sulphate to soil resulted in increased activity of soil migroorganisms, including *Trichoderma* spp, which resulted in increased pathogen suppression.

4.4 Intercropping

Intercropping, a practice whereby different crops are grown together at same time, may also reduce disease in a susceptible crop (Whipps, 1997). In Japan, *Fusarium* wilt of bottle gourd, caused by *F. oxysporum* f. sp. *lagenariae*, is traditionally controlled by growing bottle gourd (*Lagenaria siceraria* Standl.) with Welsh onion. Control is attributed to bacteria, possibly *Pseudomonas* spp., which colonise the roots of Welsh onion and produce antifungal compounds such as pyrrolnitrin, which diffuse into the rhizosphere of the bottle gourd, inhibiting the pathogen (Whipps, 1997).

4.5 Mulching

Mulching is a practice which involves applying a covering layer of organic (fresh or dried plant material) or inorganic material (stones) to the soil surface. Mulching in semi-arid areas contributes towards the improvement of soil structure which stimulates air movement between soil and the atmosphere. In high rainfall areas, plant mulches can stimulate anaerobic

conditions, which can reduce propagules of plant pathogens (Blok *et al.*, 2000). An anaerobic condition in soil develops when oxygen consumption by soil microflora prevents the resupply of oxygen by diffusion from the atmosphere (Cook and Baker, 1983). This reduction in oxygen suppresses pathogen survival in soil through competition, thus reducing inoculum buildup (Blok *et al.*, 2000). There are numerous reports on the suppression of soilborne pathogens with mulches (LaMondia *et al.*, 1999; Blok *et al.*, 2000). Straw mulch of potato fields infested with *V. dahliae* and the nematode *Pratylenchus penetrans* Cobb, reduces the survival of both pathogens (LaMondia *et al.*, 1999). Covering potato fields with rye grass reduces populations of *P. penetrans* in *V. dahliae*-infested fields (LaMondia *et al.*, 1999). In South Africa, a yield increase in avocados was associated with a leguminous cover crop and lucerne straw mulch, alone, or in combination with cattle manure (Duvenhage *et al.*, 1993).

4.6 Organic soil amendments

The application of organic amendments to soil can supplement many management strategies discussed previously by increasing microbial activity thus resulting in a greater antagonistic effect, increased competition for available nutrients, and improved soil structure and fertility. Organic soil amendments are traditionally used to improve soil structure and plant nutrition but reports show that they can also lead to the control of soilborne plant pathogens. In many instances, the organic matter fraction of amended soils is related to their fertility and ultimately to their ability to sustain crop production. Organic matter has a major influence on the physical and

chemical properties of soil even though it usually makes up only 0.05 percent of the soil mass. Mulching soil with organic material stimulates plant root growth, increases nutrient uptake, decreases evaporation from soil, increases soil water-holding capacity, reduces surface water runoff, regulates soil temperature and provides a rich substrate for soil microbes (Chen *et al.*, 1988a; Ribeiro and Linderman, 1991). Organic amendments have been used successfully to manage plant pathogens, and there are many examples of soilborne pathogens managed by the addition of organic matter to the soil (Lewis and Papavizas, 1975; Cook and Baker, 1983; Chan and close, 1987; Thurston and Abawi, 1997). Organic amendments are said to suppress plant diseases by increasing microbial activity, which results in enhanced competition and antagonism (Workneh and van Bruggen, 1993). Amendment of soil with animal manure in particular increases the level of organic matter content, resulting in high microbial activity (Aryantha *et al.*, 2000).

Extensive studies of the use of organic amendments to enhance the suppression of root infecting fungi by soil microflora began in the 1950's (Hornby, 1992). They included the control of nematodes and root infecting fungi. Sun *et al.* (1989) reported the successful management of soilborne pathogens in Taiwan by organic soil amendments. The amendments were effective against *Fusarium* diseases of watermelons, melons, peas and radishes, clubroot of cabbage caused by *Plasmodiophora brassicae* Woronin, *Phytophthora* blight of cucumber and bacterial wilt of tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi. Stalk rot of maize caused by *Fusarium moniliforme* J. Sheld. decreased significantly in amended soil compared to non-amended control treatments (Osunlaja, 1990). High levels

of organic matter in soil amended with animal manure suppress the activity of the root rot causing pathogen *Phytophthora cinnamomi* Rands (Broadbent and Baker, 1974; Hoitink and Boehm, 1999; Aryantha *et al.*, 2000). However, although this pathogen is suppressed by addition of animal manure in soil, reports show that it is not manure, which is suppressive towards this pathogen but rather associated microorganisms (Aryantha *et al.*, 2000). Promising results obtained in reducing *Rhizoctonia* stem canker of potato with farmyard manure were attributed to higher populations of mycophagous soil mesofauna, which feed on *R. solani* (Scholtze and Lootsma, 1998).

An evaluation of the effectiveness of different organic amendments on *Fusarium* wilt of muskmelon showed that amendments had an inhibitory effect on mycelial growth of *F. solani in vitro*. Of all the amendments evaluated, margosa cake and mustard cake were most effective for the control of *F. solani* on melons (Chakrabarti and Sen, 1991). In experiments conducted by Nair *et al.* (1993) on the suppression of foot rot of black pepper caused by *Phytophthora* spp. with organic amendments, disease incidence was significantly reduced in amended fields compared to non-amended fields.

Many authors have reported the inactivation of fungal pathogens in soil by amending soil with cruciferous plant tissues (Lewis and Papavizas, 1971; Muelchen *et al.*, 1990; Gamliel and Stapleton, 1993; Kirkegaard *et al.*, 1996; Smolinski *et al.*, 1997). Amendments such as stems and leaves of cabbage, kale, mustard, brussel sprouts added to soil consistently can reduce pea root rot, with disease suppression lasting for at least 15 weeks (Lewis and Papavizas, 1975). Air-dried cabbage amendments are said to be more suppressive than water extracts of decomposing cabbage leaves and stems.

The latter, in most cases, does not suppress or prevent mycelial growth, sexual reproduction or zoospore production of *Pythium* spp. and *Phytophthora* spp. (Lewis and Papavizas, 1975).

The inactivation of fungal pathogens by cruciferous amendments is attributed to toxic, volatile products of glucosinolates present in these amendments (Lewis and Papavizas, 1971; Smolinska *et al.*, 1997). Differences in the content and type of glucosinolates in plant material can affect suppression of the pathogen (Smolinska *et al.*, 1997). Blok *et al.* (2000) noted that glucosinolate products were not effective in broccoli-amended, non-covered plots, but effective in covered plots due to higher concentrations of volatiles trapped under plastic covers.

Dry, mature amendments such as barley straw, corn stover, sudan grass, oat straw and soybean tissue, and green immature amendments such as timothy, oats, corn, wheat and sudan grass, can effectively suppress hypocotyl root rot of beans caused by *R. solani* (Lewis and Papavizas, 1975). Green or dry corn and oats were the best amendments for the control of *R. solani* root rot of beans (Lewis and Papavizas, 1975). Time of application plays an important role in disease suppression. For example, mature grain straws imparted considerable protection to beans from *R. solani* soon after incorporation, but rapidly decreased in effectiveness with time (Lewis and Papavizas, 1987). Easily decomposable amendments gave best control when beans were planted 1-3 weeks after incorporation, while less easily composable amendments gave protection only after 3-7 weeks. However, organic soil amendments that suppress plant disease can also suppress saprophytic activity (Lewis and Papavizas, 1987). Corn and oats

amendments have the ability to stimulate the highest number of streptomycetes antagonistic to *R. solani* thus resulting in the suppression of its saprophytic activity in soil (Lewis and Papavizas, 1987; Lewis *et al.*, 1991).

V. dahliae and potato scab caused by *Streptomyces* spp. are also suppressed by organic amendments, such as, fish meal and soy meal (Lazarovits and Kritzman, 1999). Organic amendments suppress *V. dahliae* by reducing the viability of microsclerotia (Lazarovits and Kritzman, 1999). Potato plants in amended soils are much greener, more vigorous and survive the entire season, compared to those in untreated soils (Lazarovits and Kritzman, 1999).

Organic soil amendments have also been successfully used to manage nematodes. Mojtahedi *et al.* (1993) were able to suppress *Meloidogyne chitwoodii* Golden, O'Bannon, Santo & Finley populations by amending infested soil with leaves of rapeseed (*Brassica napus* L.). Glucosinolates in rapeseed leaves; seed and roots were cited as the major substances that induced suppression of the nematodes.

Studies on corky root of tomato caused by *Pyrenochaeta lycopersici* Hansen showed that microbial activity was higher in organically treated plots (Workneh and van Bruggen, 1994), but that high nitrogen concentrations in organically managed soils affected tomato rhizosphere populations antagonistic to *P. lycopersici* and negated the effect of microbial activity on disease suppression (Workneh and van Bruggen, 1994). The suppression of corky root in organically amended soil is therefore associated with both soil nitrogen status and microbial activity (Workneh and van Bruggen, 1994).

Organic soil amendments can sometimes be responsible for increased soilborne plant diseases (Moubasher and Abdel-Hafez, 1986). Fresh organic material that is not fully colonized by soil microorganisms capable of inducing microbiostasis also supports plant pathogens, which can increase disease incidence (Aryantha *et al.*, 2000). The addition of immature green crop debris (Watson, 1973), such as, sucrose, cornmeal or oatmeal, results in increased severity of *Pythium* diseases (Paulitz and Baker, 1987b). Because *Pythium* spp. are excellent pioneer colonists due to their rapid germination and high growth rate, the availability of a readily utilizable food source could increase the inoculum potential of this pathogen (Paulitz and Baker, 1987b). Vetch incorporated into soil as a green manure stimulates populations of *Pythium* spp. and increases *Pythium* damping-off of lettuce if the crop is planted immediately after incorporation of the amendment (Watson, 1973). The use of *Sesbania* spp. green manure is also reported to increase damping-off severity caused by *Pythium* and *Rhizoctonia*, (Neher and Campbell, 1994). *Sesbania* itself does not appear to act as a stimulant for the pathogen. The effect is attributed to the plants' general biocidal effect, and its interaction with soil fungi (Neher and Campbell, 1994).

4.7 Composting

Solid organic amendments in the form of composts are considered valuable agricultural resources because they improve the structure and moisture retention properties of soil, supply plants with nutrients and suppress soilborne plant pathogens (Hoitink and Fahy, 1986). In many countries, such as China and Japan, composts have been beneficially used in agriculture for

hundreds of years (Cook, 1986). Composting is also an easy way of treating organic wastes such as sludge thus reducing their hazardous effects on the environment (Hoitink and Fahy, 1986).

Organic soil amendments in the form of composted substrates are suppressive to a wide range of soilborne plant pathogens (Nelson and Craft, 1992). The following factors affect the quality of compost and its ability to suppress plant pathogens: 1) chemical properties which affect nutrient retention; 2) physical properties which affect aeration, water-holding capacity and bulk density; and 3) microbial activity which directly affects soilborne pathogens by competing with them or acting as antagonists (Farrel, 1993). Depending on the rate of decomposition and quality, composts may result in the amended soil becoming either conducive or suppressive to soilborne diseases (Hoitink *et al.*, 1996; Hoitink and Boehm, 1999).

Suppressive compost is defined as an environment in which disease development is reduced despite a pathogen being introduced in the presence of a susceptible plant (Hadar and Mandelbaum, 1992). Several composts have proven to be suppressive to a number of plant pathogens. For example, composted grape marc and composted separated cattle manure have shown positive results on the physical, chemical and biological properties of container media, and suppression of soilborne plant pathogens including *R. solani* and *P. ultimum* (Inbar *et al.*, 1991). Composted chicken and cow manure is reported to suppress root rot, dieback and plant death caused by *P. cinnamomi* (Aryantha *et al.*, 2000).

Container media used in nurseries are often amended with composts in order to suppress soilborne plant pathogens such as *P. aphanidermatum, P.*

ultimum, R. solani F. oxysporum, S. rolfsii and *P. cinnamomi* (Hadar and Mandelbaum, 1986; Hoitink and Fahy, 1986; Mandelbaum and Hadar, 1990; Hadar and Gorodeski, 1991; Hadar and Mandelbaum, 1992). Composts made from waste hardwood and pine bark, are suppressive to several soilborne pathogens including *P. cinnamomi, P. cactorum, Pythium* spp. and *R. solani* (Lumsden *et al.*, 1983).

Both chemical and physical factors such as particle size, nitrogen content, cellulose and lignin content, electrical conductivity (salinity), pH and inhibitors released by compost have been implicated in the suppressive effect of composts towards soilborne plant pathogens (Hoitink and Fahy, 1986). The effectiveness of composts in the management of soilborne pathogens is also associated with increased numbers of microorganisms. Organic matter provided by composts, especially animal manure, has high levels of available nutrients and supports the growth of both plants and microorganisms (Hoitink and Boehm, 1999). These microorganisms can be highly antagonistic towards many pathogens in soil (De Brito *et al.*, 1995; Liu *et al.*, 1995a; You and Savisithamparam, 1995). Bark composts, which are quite suppressive towards a number of soilborne pathogens, appear to be suppressive, at least in part, due to their biological and/or chemical characteristics rather than physical factors (Hoitink, 1980).

Sewage sludge has also been shown to suppress soilborne plant diseases (Lumsden *et al.*, 1983; Hoitink *et al.*, 1997). For example, the addition of composted sewage sludge to soil can significantly reduce the severity of *Aphanomyces* root rot of peas; *Rhizoctonia* root rot of beans, cotton and radish; *Sclerotinia* lettuce drop, *Fusarium* wilt of cucumber; and

Phytophthora crown rot of pepper (Lumsden *et al.*, 1983). Examination of the long-term effects of composted municipal sludge in field soil on lettuce drop caused by *Sclerotinia minor* Jagger showed reduced incidences of lettuce drop over a 4-year period in both spring and autumn plantings (Lumsden *et al.*, 1983).

The amendment of container media with composted liquorice roots and composted separated cattle manure (Hadar *et al.*, 1992) effectively suppressed seedling damping-off caused by *P. aphanidermatum* (Hadar and Mandelbaum, 1992). Composts prepared from a number of different feed stocks are suppressive to *P. graminicola* diseases on creeping bent grass, in both laboratory and field experiments (Craft and Nelson, 1996). Higher microbial activity, particularly of fungi and actinomyces, was also observed by Kuter *et al* (1988) and Lewis *et al* (1991), in composts made from municipal biosolids suppressive to *P. ultimum.* Microbial activity was shown to increase as the level of decomposition increased. Damping-off of cucumber caused by *Pythium* spp. was successfully controlled with composted municipal biosolids and composted leaves of mustard (Ben-Yephet and Nelson, 1999).

Composted separated cattle manure and composted grape marc have a strong suppressive ability towards *R. solani.* When poultry-cow manure, sludge compost and organic fertilizers composed of animal and plant meals were compared with the fungicide iprodione to control dollar spot of creeping bent grass and annual bluegrass turf, only poultry-cow manure and organic fertilizer were effective in reducing the disease (Nelson and Craft, 1992). In studies to control early dying disease of potatoes caused by *V. dahliae*, spent mushroom compost was able to reduce disease severity and dramatically

increase tuber yields. Symptoms of foliar senescence were delayed by up to 10 days in compost-amended plots and a delay in the decline of photosynthesis over time in compost-amended plots compared to non-amended plots was noted (LaMondia *et al.*, 1999).

Many factors are involved in the successful suppression of soilborne plant pathogens by composted materials. Most important are those involved in the eradication of pathogens from organic wastes during composting (Hoitink and Fahy, 1986). These factors include nutrient availability, exposure to high temperatures, release of toxic products during or after the self-heating process, and microbial antagonists present in the sub-lethal outer temperature zone of compost piles during curing (Hoitink and Fahy, 1986). Hoitink *et al.* (1996), proposed composting to high temperatures to ensure that pathogens are killed, followed by the amendment of mature composts with exotic biocontrol agents as a way of improving the reliability of compost.

Nutrients play a very significant role in the suppression of soilborne pathogens in composted media (Rowe *et al.*, 1987; LaMondia *et al.*, 1999). Suppression of early dying disease of potatoes caused by *V. dahliae* with spent mushroom compost was attributed to high levels of nitrogen in the compost (LaMondia *et al.*, 1999). Spent mushroom compost amendments have been shown to alter the mineral composition of root and leaves thus affecting plant nutrition and altering the response of potato plants towards the pathogen (LaMondia *et al.*, 1999). Nutrients can induce the susceptibility of crops towards soilborne pathogens (Hoitink and Fahy, 1986). Composts high in nitrogen content, such as municipal sludge, which have a low C: N ratio,

release considerable amounts of nitrogen and enhance *Fusarium* wilt (Hoitink *et al.*, 1991).

Composted hardwood bark supplies nutrients for antagonistic organisms, micronutrients for plant growth but removes nitrogen from the soil if used prior to being composted for at least six months (Hoitink and Fahy, 1986). In practice, the immobilization of nitrogen necessitates fertilization with high levels of N (Hoitink and Fahy, 1986). Composts from tree bark can consistently suppress *Fusarium* wilt of plants (Hoitink *et al.*, 1997). This is attributed to the fact that composts made from high C: N materials, such as tree bark, immobilize nitrogen only if colonized by appropriate microflora. *Fusarium* wilts are suppressed by composted pine bark if approximately 40 % wood remains attached to the bark before the onset of composting (Hoitink and Fahy, 1986).

Pine bark has a high lignin (Hoitink and Fahy, 1986) and low cellulose content and is therefore resistant to decomposition (Hoitink and Fahy, 1986; Hoitink *et al.*, 1991). Because pine barks do not immobilize significant amounts of nitrogen in the absence of appropriate microorganisms, comparatively little nitrogen has to be added to compensate for immobilization process (Hoitink *et al.*, 1991). Apart from nitrogen, other nutrients, e.g., micronutrients and sources of Ca and Mg must, however, be provided for the adequate growth of most plants. High ammonium and low nitrate nutrition increases *Fusarium* wilt, which is probably why, a low C: N ratio in predominately ammonium-releasing sludge compost enhances *Fusarium* diseases (Trillas-Gay *et al.*, 1986).

Composted biosolids may enhance disease because they release nitrogen in the form of ammonium (Hoitink *et al.*, 1997). Composts with high levels of nitrogen have been shown to promote fire blight, *Phytophthora* stem dieback and some bacterial leaf spots in plants (Hoitink and Fahy, 1986). A decline in the carbohydrate content of compost correlates with a loss of suppressiveness because bacteria capable of biological control are replaced by ones that cannot provide control (Wu *et al.*, 1993). Hadar and Mandelbaum (1992) demonstrated that compost media amended with glucose / asparagine was more conducive to bacterial growth and less conducive to fungal growth. The adding of a glucose / asparagine mixture of 10 carbon units to one nitrogen unit (C : N ratio = 10 : 1) to container media can result in a rapid increase in microbial respiration rate and enzymatic activity in a composted, separated cattle manure medium (Hadar and Mandelbaum, 1992). However, leaching and volatization of ammonia during composting enables the growth of fast growing saprophytes as well as pathogens which utilize readily available sugars resulting in the suppression of pathogens such as *P. cinnamomi* (Aryantha *et al.*, 2000).

Chen *et al.* (1988b) examined the effects of readily available nutrients in composts on the suppression of root rot caused by *P. ultimum.* By monitoring the concentration of free glucose in a compost-amended potting mix prepared with a mature but high temperature compost (60 ^{0} C), high concentrations of free glucose were shown to accumulate after the compost was incubated at low temperatures. After 45 hours of incubation at 25 ^{0} C, concentrations of readily available nutrients declined rapidly. During the short period when the high glucose concentration prevailed, populations of *P.*

ultimum increased and *Pythium* damping-off developed. High saline composts have also been shown to enhance *Pythium* and *Phytophthora* diseases unless they are applied months ahead of planting to allow for leaching (Hoitink *et al.*, 1997). Fresh chicken manure compost, which is reported to suppress *P. cinnamomi* (Aryantha *et al.*, 2000), is rich in soluble nitrogen, phosphorus and potassium (Casale *et al.*, 1995). Three different forms of nitrogen (ammonium, nitrate and nitrite) have been reported to suppress *P. cinnamomi*, both in soil and *in vitro* (Broadbent and Baker, 1974). Roy and Newhook (1970) found that more sporangia were produced by *P. cinnamomi* when soils were treated with fresh cow dung and urine and postulated that this contributed to increased tree death in farm shelter belts.

Compost maturity level is important in its ability to suppress soilborne plant pathogens. In mature composts, where concentrations of free nutrients are low (Chen *et al.*, 1988a), sclerotia of *R. solani* are killed by hyperparasites (Nelson *et al.*, 1983). Mature composts are able to release nutrients slowly and support the activity of microflora thus sustaining biocontrol (Hoitink *et al.*, 1991). This explains the ability of *R. solani* to cause more disease in fresh undecomposed organic matter than in adequately stabilized composts. For the suppression of *R. solani* to occur in composted growth media, concentrations of readily available nutrients must be low enough to allow competition and the production of lytic enzymes and antimicrobial compounds involved in hyperparasitism of the pathogen (Chen *et al.*, 1988a, Chung *et al.*, 1988). The duration of the suppressive effect is also dependent on the availability of biodegradable carbon as a substance for the growth of antagonistic organisms (Hoitink and Boehm, 1999).

Another important factor affecting nutrient availability and suppressiveness of composts in growth media is pH (Hoitink and Fahy, 1986). For compost, this ranges between 5.5 and 6.0, which is the range in which most plants grow well. In nursery practice, pH levels of growth media may rise within weeks and thus cancel this effect (Hoitink and Fahy, 1986). During short production cycles pH may still, in practice, bring about disease control, as pH values remain low over these short periods. Unfortunately, disease control through the maintenance of low pH values is impractical as few plants grow optimally at a pH lower than 4.0 (Hoitink and Fahy, 1986). The pH of amended soil influences other factors such as the solubility of minerals and ionization of salts and acids (Paulitz, 1991). Plants adapted to high pH levels become susceptible to *P. ultimum* when grown at low pH levels (Paulitz, 1991). The pH in compost-amended soil presumably influences the vigor of the host plant directly, rather than affecting the development of the pathogen (Paulitz and Baker, 1987b).

A number of other factors affect the suppression of diseases in compost-amended media. They include type of compost (Craft and Nelson, 1996; Ringer *et al.*, 1997), organic matter quality (Boehm *et al.,* 1993, 1997), and microbial activity (Mandelbaum and Hadar, 1990; Theodore and Toribio, 1995; Hoitink and Boehm, 1999). Factors such as temperature, moisture, compost dosage, and the target pathogen, can also contribute to variation in disease suppressiveness (Ben-Yephet and Nelson, 1999).

The amount of water in composted materials is a significant factor that can affect decomposition rate and the disease suppressiveness of such compost when used as a container medium. Maximum water retention in a

medium is best referred to as the water holding capacity and is the percentage of the total volume in a medium occupied by water after saturation with water and subsequent drainage (Hoitink and Fahy, 1986). The moisture content of compost critically affects the potential for bacterial mesophiles to colonize the substrate after peak heating. These bacterial mesophiles play an important role as biological control agents during planting. For example, dry composts (<34% moisture w/w) have lower bacterial populations and are more conducive to *Pythium* diseases (Hoitink *et al.*, 1991). To induce disease suppression, the moisture content must be high enough, between 40-50% to stimulate the colonization by both fungi and bacteria (Hoitink *et al.*, 1997).

A large number of microorganisms contribute to the biological control of plant pathogens such as *Phytophthora* and *Pythium* spp. in composted media (Boehm *et al.*, 1993). This is attributed to the fact that microbial activity of the general soil micro flora in compost is able to prevent spore germination of pathogenic organisms (Chen *et al.*, 1988a). Microbial activity in compost is supported by the gradual release of organic nutrients, which, in turn, sustain biological control (Hoitink *et al.*, 1991). Fungal and bacterial activities have been found to be most active in composted separated cattle manure which is suppressive to many *Pythium* diseases, with bacteria probably most important (Hadar *et al.*, 1992). Aryantha *et al.* (2000) reported increased microbial activity in soil amended with composted animal manures. Chicken manure compost increased the number of endospore forming bacteria, while cow and horse manure composts increased populations of actinomycetes, fungi and fluorescent pseudomonads. Suppression of dollar spot of creeping bent grass

and annual bluegrass by poultry-cow manure and sludge compost was attributed to microbial antagonists contained in composted medium.

A decrease in *Fusarium* brown rot in plots treated with composted larch bark was attributed to increasing populations of *Trichoderma* spp. (Sekiguchi, 1977). However, composts prepared from sawdust and composted hard bark, and enriched with microorganisms, delayed symptom development but did not control *Fusarium* wilt of tomato (Kato *et al.*, 1981). In composted media, suppression of soilborne pathogens is the result of microbial activity developing immediately after the thermophillic phase of composting (Hadar and Mandelbaum, 1992). This explains how alterations in compost composition can affect the compost's suppressiveness to pathogens. Composted animal manure can induce suppression of different pathogens if applied at conventional rates. Voland and Epstein (1994) were able to suppress damping-off of radish caused by *R. solani* at low inoculum levels using composted dairy manure. However, suppression was not obtained at high inoculum levels. Disease suppression of lettuce drop caused by *S. minor* was also attributed to increased microbial activity even though survival of the pathogen was unaffected (Lumsden *et al.*, 1983).

The mechanism involved in the suppression by compost of *R. solani* differs from that involved in the suppression of *Pythium* and *Phytophthora* spp. (Hoitink and Fahy, 1986; Hoitink *et al.*, 1991). *Rhizoctonia solani* produces sclerotia, which are independent of nutrients (Hoitink *et al.*, 1991). Variation in the suppression of damping-off caused by *R. solani* in growth media amended with mature composts is due, in part, to random recolonization by microorganisms that vary in their degree of efficacy towards

the pathogen during the mesophilic phase of composting (Hoitink *et al.*, 1997). Composts, which are produced in the open and near a forest, support a wider variety of microorganisms. This is said to be the main reason for their suppressiveness towards *R. solani* (Hoitink and Fahy, 1986).

Inoculation with antagonistic organisms is regarded as being particularly important in the production of *Rhizoctonia* suppressive composts (Hoitink *et al.*, 1991). *Trichoderma* and *Gliocladium* have been identified as antagonistic agents of *R. solani* (Hoitink and Fahy 1986; Chung *et al.*, 1988). In fresh organic matter, both *Trichoderma* and *R. solani* grow as saprophytes but the medium remains conducive to disease as *R. solani* retains its ability to cause disease (Hoitink *et al.*, 1991). In mature composts with low cellulose content, however, sclerotia of *R. solani* are killed due to hyperparasitism (Hoitink and Fahy, 1986). According to Chung *et al.* (1988), the high concentrations of glucose in fresh composts may repress chitinase activity required for biological control. As the degree of suppressiveness in composted media is contingent to the degree of which organic matter has been decomposed, it is important that compost be stabilized to the correct level of decomposition (Hoitink *et al.*, 1991).

Nursery growers in the eastern United States have consistently obtained *Fusarium* suppression through their use of composted pine bark mixes (Hoitink *et al.*, 1991). Diffirent biological agents are associated with the suppression of Fusarium wilt (Ariel *et al.*, 1987; Baker *et al.*, 1987; Ringer *et al.*, 1997). *Pseudomonas* spp isolated from onion roots have been found to be suppressive to *Fusarium* wilt of onion (Ariel *et al.*, 1987). Increased microbial population in composted animal manure results in the suppression

of diseases caused by *Fusarium* spp., *Verticillium* spp. and *R. solani* (Ringer *et al.*, 1997).

4.8 Plant Extracts

Extracts from many plant species are reported to have the potential to inhibit pathogens including nematodes (Mojumber and Mishra, 1992); bacteria (Vijai-Pal *et al.*, 1994); viruses (Balasubrahmanyam *et al.*, 2000) and fungi (Kaushal and Paul, 1989; Kishore *et al.*, 1989) including soilborne pathogenic fungi.

4.8.1 Nematode suppression

Mojumber and Mishra (1992), noted that extracts of *Azadirachta indica* A. Juss, and *Brassica rapa* L, have the ability to reduce the hatching of nematode egg masses and inhibit the penetration of juveniles into the roots of chickpea. Soaking of nematode-infected seeds in aqueous extracts of *Cannabis sativa* L. and other plants extracts such as *Datura metel* L., *Argemone mexicana* L. and *A. indica* reduces the penetration of *Meloidogyne incognita* (Kofoid & White) Chitwood. juveniles in chickpea. Extracts of *Chromolaena odorata* (L.) King & Robinson, *Mimosa invisa* L, and *Ananas comosus* Merr. have been reported to kill the nematode, *Radopholus similis* Cobb (Sundararaju *et al.*, 1998). Extracts of these plants exhibited a high degree of nematicidal action against the adults and larvae of this nematode. Other plant extracts effective in the control of this nematode are reported by Jasy and Koshy (1992).

4.8.2 Bacterial suppression

A large number of bacteria that cause diseases in both plants and animals are successfully controlled by extracts obtained from plants. The growth of *Erwinia carotovora* (Jones) Bergey, which causes soft rot of potatoes, can be inhibited by extracts of common weeds such as *Calotropis procera* (Ait) R. Br., *Solanum surratense* L. and *Cannabis sativa* (Vigai-Pal *et al.*, 1994). *Staphylococcus aureus* is suppressed by flavanoids of *Geranium* spp (El-Gammal and Mansour, 1986).

4.8.3 Fungal suppression

Numerous fungal pathogens of plants have been successfully suppressed by botanical extracts (Kaushal and Paul, 1989; Kishore *et al.*, 1989; Awuah, 1994; Wilson *et al.*, 1997; Ramirez-Chavez *et al.*, 2000). Awuah (1994) was able to control black pod disease of cacao, caused by *Phytophthora palmivora* Butler, by spraying infected pods with a crude steam distillate of *Ocimum gratissimum* L. Results obtained with the extract were the same as those obtained with the fungicide Kocide 101, a mediocre fungicide. Singh (1999), reported the successful control of *R. solani* and *R. bataticola* (Taubenh) Butler (*Macrophomina phaseolina* (Tassi) Goid.) with leaf extracts of *A. indica, Mentha arvensis* L., *Eucalyptus globules* Labill, *Allium sativum* L. and *Allium cepa* L., among others. The extracts exhibited an inhibitory effect towards all pathogens even at very low concentrations.

Most higher plants, which possess antifungal activities, generally show very few symptoms of fungal disease either on the leaves or other parts of the plant (Srivastava and Kediyal, 1983). Certain chemical constituents in these

plants are presumably responsible for the suppression of some plant pathogens. Oleoresin, which occurs in the family *Pinaceae,* is a hydrophobic mixture composed largely of resin and fatty acids in turpentine or a volatile oil, consisting mainly of mono and sesquiterpenes and a few alkanes (Cobb *et al.*, 1968). Although there are some conflicting reports of the antifungal abilities of this chemical (Scheffer and Cowling, 1966; Fugii *et al.*, 1991), the weight of evidence strongly favors the presence of antifungal components in oleoresin (Wood, 1967; Biehn *et al.*, 1968). Some of the volatile components of oleoresin were shown to have a fungistatic and even fungicidal effect, on a number of coniferous pathogens and non-pathogens. A compound of oleoresin, heptane, was found most inhibitory and was considered fungistatic to *Fomes annosus* (Fr.: Fr) Cooke. and *Ceratocystis pilifera* (Fries) Monroe (Wood, 1967; Cobb *et al.*, 1968). In those cases where oleoresin had a non-inhibitory effect towards *F. annosus* (Fugii *et al.*, 1991), escape of volatiles, as well as the low solubility of oleoresin in aqueous media, was suggested to be the cause (Fugii *et al.*, 1991).

Many plant species which have been used for medicinal purposes also exert an inhibitory effect on plant pathogens (Fugii *et al.*, 1991). Leaves of *Geranium* spp are reported to have flavonoids, which exhibit an inhibitory effect on the growth of *R. solani*, and *F. oxysporum* (El-Gammal and Mansour, 1986). *Rhizoctonia solani* was also suppressed by leaf extracts of *Geranium pratense* L. and *Sanguisorba officinalis* L., both medicinal plants (Ushiki *et al.*, 1996). The inhibitory effect of *Eupatorium* spp. root extracts towards *R. solani* has been attributed to the antimicrobial effect of certain chemicals contained in this plant (Rao and Alvarez, 1981). The same

compound shows a suppressive effect towards *Bacillus subtilis* (Ushiki *et al.*, 1996). Homogenates from seedlings and cotyledons of Norway spruce exhibit the strongest antimicrobial effect on *P. ultimum* and *P. irregulare* (Kozlowski and Metraux, 1999). In other reports (Cobb *et al.*, 1968; Fugii *et al.*, 1991), five phenolic compounds isolated from the inner bark of Norway spruce (*Picea abies* L.) inhibited the growth of *F. annosus* in vitro.

Certain botanical extracts result in a detrimental effect on the morphology of fungi (Singh, 1999). High concentrations of the rhizome extract of *Cyperus rotundus* L., are reported to impose a characteristic bulging of *Fusarium udum* Butler spores before germination. Morphological modifications are also induced by garlic extracts on *P. ultimum* and *R.solani,* including undulations of the plasmalemma, the accumulation of lipidic osmophilic bodies and the thickening of cell walls (Bianchi *et al.*, 1997). In fact, the accumulation of lipid bodies and the thickening of cell walls are similar to the effects produced by certain synthetic fungicides (Hippe, 1991). The high level of growth inhibition of *V. dahliae* and *R. solani* by root extracts of *Euphorbia fortunei* L., make this plant an ideal candidate for suppressing soilborne plant diseases (Ushiki *et al.*, 1996). The root extracts of *S. officinalis,* which also inhibits the growth of *F. oxysporum* and *R. solani*, is reported to possess high levels of tannin (Nonaka *et al.*, 1982), which is the cause of fungal inhibition and phytotoxicity (Ushiki *et al.*, 1996).

Fungal inhibition by aqueous extracts from *Brassica* plants has been reported by Smolinski *et al.* (1997). The precise compound responsible is unclear but the detrimental effect of cruciferous tissues on other microorganism has been attributed mainly to water-soluble and volatile

degradation products of gluocosinolates (Drobnica *et al.*, 1967; Lewis and Papavizas, 1970; Dawson *et al.*, 1993; Angus, 1994; Kirkegaard *et al.*, 1993 and 1996). The glucosinolate product allyl isothiocyanate released from macerated cabbage tissues is toxic towards *Perenospora parasitica* Pers.:Fr. (Smolinski *et al.*, 1997), and isothiocyanates and aldehydes in solarized soil amended with cabbage residues correlate with a reduction of *P. ultimum* and *S. rolfsii* propagules (Smolinski *et al.*, 1997). *Brassica* meal extracts also contain 5-vinylloxazolidine-2-thione, a water-soluble volatile compound with fungitoxic effects towards different soilborne pathogens (Smolinski *et al.*, 1997).

The treatment of soil with seaweed extract results in the suppression of soilborne plant pathogens (Dixon and Walsh, 1998) which is the result of benign organisms such as *Ralstonia putita* having the capacity to form fungitoxic substances in seaweed treated soils. The latter reduce the metabolic efficiency of pathogenic organisms (Dixon and Walsh, 1998). Extracts of another plant, *Ascophyllum nodusum* L., when applied to soil, may alter the mode of activity of microorganisms. This may result in a direct or indirect alteration in pathogen behaviour such as root colonization and penetration, competition with other organisms, and microbiostatis and antibiosis (Dixon and Walsh, 1998).

Extracts of *Reynoutria sachalinensis* Houtt have a slight suppressive effect on the germination of conidia of *Sphaerotheca fuliginea* (Schltdl.:Fr.) Pollacci. but no fungitoxic effects on *Botrytis cinerea* Pers.:Fr. (Daayf *et al.*, 1995). Investigations have shown that proteins, terpenoids, phenolics and regular sugars act as active ingredients and that the resistance-inducing

factor is most likely a carbohydrate with a hydrophobic tail. *Reynoutria sachalinensis* is reported to induce a rapid and abundant accumulation of glycosidically-bound phenolics, which are in turn responsible for pathogen suppression (Daayf *et al.*, 1995).

Some plant extracts are reported to have selective suppressiveness towards certain pathogens, while others show a wide biocidal effect (Bowers and Locke, 2000). Pepper and mustard extracts, which are very effective towards *F. oxysporum*, act as a general biocide and also kill other microflora in soil, while cassia extracts act only on *F. oxysporum* (Bowers and Locke, 2000). Ethanol extracts from the aerial parts of *Sophora alopecuroides* Turner inhibit conidial germination of *Glomerella cingulata* (Stoneman) Spauld. & Schrenk. (Zhao and Jiang, 1999). Alkaloid fractions had the strongest inhibitory effect and seven monomers isolated from the alkaloid were identified as sophocospine, matrine, sophoramine, lehmanine, sophoradine, aloperine and cytosine. All 7 alkaloids had a strong inhibitory effect on the conidial germination of *G. cingulata* (Zhao and Jiang, 1999).

Botanical products in the form of essential oils and derived from both medicinal and aromatic plants have also been found to exhibit fungicidal, bactericidal, insecticidal and nematicidal effects (Singh, 1999). They have proved their usefulness in controlling many postharvest diseases of fruits (Singh, 1999). Despite their high cost, essential oils and their constituents are therefore a potent source of environmentally safe biocides (botanical pesticides) that could be explored for commercial application (Singh, 1999).

4.9 Induced systemic acquired resistance (SAR)

Host plant resistance is the most important single element in the control of plant diseases, including those caused by soilborne pathogens (Cook and Baker, 1983). Certain mechanisms can be used to induce resistance in plants to soilborne pathogens. Cross protection has been defined as the inhibition of disease symptoms resulting from the prior or simultaneous inoculation of the host with a close relative of the pathogen (Cook and Baker, 1983). Systemic acquired resistance (SAR), also termed systemic induced resistance by some authors (SIR), is a non-specific resistance that is induced and transmitted through the plant following infection by a necrosis-causing pathogen, or by treatment with abiotic agents such as, chitosan, salicylic acid and isonicotinic acid (Leon, 1995). In most cases, development of SAR is accompanied by the expression of a set of genes within the plant including those which encode for pathogenesis-related proteins such a chitinases glucanases and thymidine-like proteins with antifungal activities (Linthorst, 1991). SAR also triggers the synthesis of phytoalexins (van Peer *et al.*, 1992).

Numerous factors are thought to play a role in the induction of systemic resistance by beneficial microorganisms in soil. The lipopolysaccharide O-antigen of rhizobacterium *Pseudomonas fluorescens* strain WCS374 can play a role in the induction of systemic resistance in radish against *Fusarium* wilt (Leeman *et al.*, 1996). The degree of resistance induced in radishes is said to be affected by the levels of iron available in growth media (Leeman *et al.*, 1996). Since many rhizobacteria triggering SAR can also inhibit the growth of

pathogens directly, their capacity to suppress disease may involve more than one mechanism. For example, bacterial siderophores inhibit plant pathogens through competition for iron, while production of antibiotics suppresses competing microorganisms (van Loon *et al.*, 1999). Enzymes chitinases and glucanases lyse cells of pathogenic microorganisms, resulting in their death (van Loon *et al.*, 1999). From experiments of Maurhofer *et al.* (1994), SAR induced in tobacco by strains of *P. fluorescens* against tobacco necrosis virus coincided with the accumulation of glucanase and chitinase proteins. Reports also show that plant growth promoting rhizobacteria and fungi also have the ability to induce SAR against foliar diseases of plants (Liu *et al.*, 1995b; Wei *et al.*, 1996).

As mentioned previously, SAR is always accompanied by an increase in the concentration of salicylic acid and induction of pathogenesis-related proteins in plants (Ryals *et al.*, 1996; Sticher *et al.*, 1997). The same mechanism is involved in systemic resistance induced by *Trichoderma* spp. Mycelial extracts of *Trichoderma,* besides inducing systemic resistance, also induce pathogenesis related proteins (PR-proteins) in tobacco seedlings (van Loon *et al.*, 1999). Some of these PR proteins, such as glucanases and chitinases, have the ability to hydrolyze fungal cell walls (van Loon *et al.*, 1999).

Studies have also been made of the effect of rhizosphere growth promoting bacteria in disease suppression. A strain of *Pseudomonas fluorescens,* WCS417, was found to be twice as effective as WCS358 in suppressing *Fusarium* wilt in carnation. Similiarly, a Sid-mutant of the same strain was as effective as the wild type in suppressing the disease (Duijff *et*

al., 1993). In this case, competition for iron was responsible for protecting carnation against *Fusarium* wilt by WCS417 (Duijff *et al.*, 1993). The application of WCS417 and *Fusarium oxysporum f. sp dianthi* (Prill & Delarcr.) to different parts of carnation plants by treating the roots with the bacterium and introducing the fungus one week later, resulted in disease reduction (Lemanceau *et al.*, 1992; van Peer *et al.*, 1992). Similar results were obtained by Leeman *et al.* (1996), while treating radish root tips with *P. fluorescens* strains WCS417 or WCS374 and inoculating with *F. oxysporum.* The strain WCS417 was also found to induce resistance against *Rhizoctonia* root rot, but only if the inoculum density of the causal pathogen *R. solani* was relatively low (van Loon *et al.*, 1999). In contrast, the induction of systemic resistance toward *P. aphanidermatum*, by growth promoting rhizobacteria *P. corrugata* 13 and *P. fluorescens* C15, was obtained when both pathogen and rhizobacteria were applied at the same time, instead of applying bacteria one week after inoculation with the fungus (Zhou and Paulitz, 1994).

The concentration and availability of nutrients within soil organic matter plays a critical role in regulating SAR in plants (Zhang et al., 1996b; Hoitink *et al.*, 1997; Hoitink and Boehm, 1999). There are several reports that a broad spectrum of chemicals, plant extracts and composts may induce resistance in plants, thus reducing disease incidence (Trankner, 1992; Weltzien, 1992). A report by Trankner (1992), states that powdery mildew of wheat and barley, which was less severe in compost-amended than in non-amended soils because of SAR. Composted spruce and pine bark amended mixes induced SAR in cucumber against *Pythium* root rot and anthracnose caused by *Colletotrichum orbuculare* (Berk & Mont.) Arx. (Zhou and Paulitz, 1994).

Fortifying compost mixes with biocontrol agents, induces systemic resistance towards cucumber anthracnose and bacterial speck *of Arabidopsis* caused by *Pseudomonas syringe* pv *maculina*. Autoclaving destroys the SAR-inducing effect of compost, and the effect can only be restored by mixing with unautoclaved compost, suggesting that the resistance inducing activity of the compost is biological in nature (Zhang *et al.*, 1998).

Growth promoting *Pseudomonads* from composted mixes were also shown to provide control of *Pythium* root rot of cucumber by inducing SAR (Loper, 1988; Zhou and Paulitz, 1994). Experiments with an acidic peroxidase isozyme, which has been identified as a molecular marker of SAR in plants (Albert and Anderson, 1987; Smith *et al.*, 1991), which showed a significant increase in enzyme activity in cucumber grown in compost amended mixes compared to a peat mix (Zhang *et al.*, 1996a). There are also factors in composts which complement the activity of *C. orbiculare* in inducing SAR, which might also explain higher peroxidase isozyme activity in leaves of cucumber plants that were inoculated and grown in composted media. This activity does not occur in plants until they are infected with the pathogen (Zhang *et al.*, 1996a, 1998). Some rhizobacteria, which are capable of inducing SAR, also induce the accumulation of PR proteins in plants (Maurhofer *et al.*, 1994). The interaction of compost and pathogens is critical for the rapid activation of systemic acquired associated gene expression in cucumber plants grown in composted mixtures (Zhang *et al.*, 1996a). According to Zhang *et al* (1998), compost-induced disease suppression involves low-level induction of resistance responses rather than high-level activation. To verify this, Zhang *et al.*, (1998), used a split-root system, where

only part of the system of cucumber plants needs to be exposed to compost amended potting mix suppressive to *Pythium* root rot to induce protection to the disease in the entire root system.

Several microorganisms which are capable of inducing systemic resistance in radish and cucumber in suppressive compost, have been identified by Han *et al.* (2000). This included *Trichoderma hamatum* Rifai 382 and bacterium *Pantoea agglomerans* 278A which induces systemic resistance to foliar bacterial spot caused by *Xanthomonas campestris* (Pammel) Dowson *pv, armoraceae,* when applied to the roots of radish seedlings (Han *et al.*, 2000). Some types of rhizosphere microorganisms are also suspected to induce systemic resistance in plants (van Loon *et al.*, 1998). These results have led to the conclusion that active batches of composts seem to prime plants to better defend themselves against a given pathogen (Hoitink and Boehm, 1999).

5.0 CONCLUSION

Many factors in soil are affected by either biotic or abiotic factors introduced in the course of crop management practices. These factors could lead to stress, which in turn might predispose plants to soilborne pathogens. Examples sited above indicate that numerous aspects pertaining to the pathogen, host plant and their interactions with other organisms in the soil and surrounding environment should be considered when developing sustainable management strategies for soilborne pathogens.

In this review, a few issues affecting the survival and management of soilborne plant pathogens have been discussed. Many others remain to be

discussed and elucidated. The question that remains is, can the use of organic soil amendments and the manipulation of cropping practices become a realistic alternative to chemical control of soilborne plant diseases? Taken in its broadest sense, the answer is affirmative. Most of the methods, especially organic soil amendments, were the only method used traditionally by farmers to increase crop yield and control diseases before the development and introduction of synthetic chemicals.

Organic amendments in the form of composts can be used successfully in conjunction with other management strategies to suppress the inoculum of soilborne pathogens and soils. For example, where crop rotation cannot be practised frequently, compost can be used to increase competition between pathogens and microorganisms available in composted materials. This can be achieved by means of increased microbial activity, which prevails in composts.

Organic amendments can also be used to supplement chemical fertilizers, thus resulting in improved soil fertility and structure. As mentioned in this review, composts are considered valuable agricultural resources because they can improve the structure and moisture retention properties of soil, supply plant with nutrients, and suppress soilborne plant pathognes (Hoitink and Fahy, 1986).

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CHAPTER TWO

THE EFFECT OF FOUR COMPOSTED ANIMAL MANURES ON DAMPING-OFF SEVERITY AND PLANT BIOMASS OF VEGETABLE SEEDLINGS

ABSTRACT

Seedling diseases are a continuing problem of vegetables grown in seedbeds of small-scale farmers in Lesotho. The development of cheap and effective methods to control these diseases is therefore important. Strains of *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* were isolated from diseased vegetable seedlings. Artificial inoculation of three indicator plants (cabbage, tomato and amaranth) were conducted with these pathogens in the greenhouse to confirm their pathogenicity. Sheep manure compost prepared by a local farmer and three other animal manure composts were tested for their suppressive effect on disease of the indicator plants caused by the three pathogens. Composts were also tested for their ability to increase the biomass of plant seedlings. Compost prepared from cattle, poultry or pig manure were most suppressive and also significantly increased the biomass. Disease severity was lowest in pig manure compost. In compost made from sheep manure however, few seeds germinated and total biomass was consequently very low. The observed suppression of damping-off and increase in plant biomass indicates that animal manure derived composts can play an important role in cost-effective, organically based management strategies for reducing diseases of vegetables and thereby increasing crop production in Lesotho.

INTRODUCTION

More than 85% of arable land in Lesotho is cultivated by farmers who primarily engage in traditional farming practices (King, 1989). Much of this land is used for vegetable production with crucifers (especially cabbage), tomato and cucurbits (pumpkin) most commonly cultivated. Although farming systems are not necessarily high yielding, they are generally dependable and stable in yielding a reasonable harvest under all but the poorest of conditions (King, 1989). The growing of vegetables usually first involves growing seedlings in seedbeds before they can be transferred to the field. Seedlings are usually grown on soils amended with composted animal manures or non-amended soils.

Seedling diseases, especially soilborne, are an important problem on many types of vegetables grown in seedbeds and greenhouses worldwide (Zhang *et al.*, 1990). These diseases include damping-off and root rot caused by species of *Fusarium*, *Rhizoctonia* and *Pythium*. The problem is also common in Lesotho, however, pesticides are rarely used by small-scale farmers in Lesotho due to their high cost. Traditional methods, such as the application of animal manure, green manure, and the application of plant extracts to expel insects and kill pathogens are commonly practiced by Lesotho farmers (King, 1989). Efficient management strategies for animal manure (Voland and Epstein, 1994) can greatly reduce the adverse effect of conventional production practices while at the same time providing for sustainable productivity (Workner and van Bruggen, 1994).

Increasing interest in composting as a waste management strategy has led to research efforts being directed toward the utilization of composts in agricultural crop production (Craft and Nelson, 1996). Composts have been widely accepted as soil amendments or as amendments to container growing media for boosting the yield of agronomic and horticultural crops (Chen *et al.*, 1988; Dick and McCoy, 1993). There is considerable evidence that various types of compost also suppress soilborne diseases (Hoitink *et al.*, 1991; Hadar *et al.*, 1992; Voland and Epstein, 1994). For example, composts prepared from waste materials, including animal manure, control certain soilborne plant pathogens when used as a component of plant growth media in containers or as a soil amendment for field crops (Hoitink and Fahy, 1986).

Most of these methods are used to improve soil fertility and structure, and their effect on soilborne diseases have not been well documented. The objectives of this study were firstly, to determine the major fungal pathogens causing damping-off and root rot of three types of vegetable seedlings in Lesotho. Secondly, to evaluate the suppressiveness of four animal manure derived composts to damping-off caused by isolates of *Pythium ultimum* Trow, *Rhizoctonia solani* Kuhn and *Fusarium oxysporum* Schltdl. obtained from vegetable plots in Lesotho. Thirdly, the influence of these composts on seedling biomass were determined. Finally, the chemical composition of composts and the possible relationship of this to disease suppression and seedling biomass was investigated.

MATERIALS AND METHODS

Isolation of pathogens. Isolations were conducted from diseased plant material and soil obtained from seedbeds and greenhouses in three different locations in Lesotho (Lesotho Agricultural College farm and experimental plots, Maseru and Leribe campuses). Five seedlings, each of tomato and cabbage, showing symptoms of either damping-off or root rot were washed under running water for 20 minutes. Small pieces (5 ml) were cut from diseased stem and root tissues showing discolouration, surface sterilized with 10% NaOCl for 1 min, rinsed three times in sterile water, and plated onto a selective medium for each of the three target pathogens. The plates were incubated for five days at 25° C. Hyphal tips growing from diseased plant tissue were cut and transferred to the same selective media. After seven days of incubation at 25° C, examination of morphological structures under a light microscope was conducted to confirm the identity of isolated fungi.

For the isolation of *Pythium* spp., a selective medium consisting of 5mg pimaricin, 300 mg vancomycin hydrochloride, 100 mg PCNB, 10 mg rose bengal, 20 g sucrose, 1 mg $ZnCl_2$ 0.02 g of $CuSO_4.5H_2O$, MoO_3, MnCl2 and $FeSO_4.7H_2O$, 10 mg $MgSO_4.7H_2O$, 1g K_2HPO_4, and 17 g corn meal agar (CMA) (Difco) in 1000 ml sterile distilled water was used (Mircetich, 1971). After 48 hours of growth at 24°C, fungal colonies were transferred to corn meal agar (CMA) (Difco) in order to obtain pure cultures for identification. For the identification of *Pythium* species, the formation of sporangia and zoospores was induced by taking 3-4 discs (diameter 6-10) from the margin of 2 –3 day old *Pythium* cultures and placed onto sterile carrot discs (diameter

10 mm). Discs were placed in petri dishes containing 5 ml sterile water and incubated at 20° C, 25° C and 30° C. The water was changed at least three times per day to promote the production of sporangia and discharge of zoospores (Chamswarng and Cook, 1985).

Plant material presumed to be infected by *R. solani* was surface sterilized as described above and plated onto a selective medium consisting of 1g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 10 mg $FeSO_4.7H_2O$, 0.2 g $NaNO_2$, and 20 g agar in 1000 ml sterile distilled water. The following ingredients were added after autoclaving for 20 minutes at 121° C: 0.4 g gallic acid, 90 mg Dexon, 50 mg chloramphenicol and 50mg streptomycin. Fungal colonies were allowed to develop for 48 hours at 24° C before hyphal tips were removed and plated onto potato dextrose agar (PDA) (Difco) to obtain pure cultures.

For the isolation of *F. oxysporum,* diseased plant tissue was sterilized and plated onto a selective medium for *Fusarium* spp.. The medium consisted of 10 g glycerine, 1 g urea, 0.5 g L-alanine, 1 g PCNB, 0.5 g rose bengal, 15 g agar in 1000 ml sterile distilled water to which, 50 mg of streptomycin was added after autoclaving for 20 minutes at 121° C. For species identification, single spore isolates were transferred onto Spezieller-Nahrstoffarmer Agar overlaid with sterile filter paper to enhance sporulation. Identification was based on morphological characteristics after incubation for 7-10 days at 24° C.

Isolations from soil samples were performed by adding 1 g of soil to 100 ml sterile distilled water. The soil-water suspension was diluted 10 times and 1 ml from the final dilution was transferred to Petri plates containing water agar (WA). The plates were incubated for 48 hours at 25° C after which

hyphal tips of fungi were transferred to culture media and incubated for 7 days at 24° C. Isolates of *F. oxysporum* and *R. solani* obtained from seedlings and soil samples were stored on PDA, while *P. ultimum* was stored on CMA until further use.

***Inoculum preparation*:** A mixture of wheat (50 g) and barley grains (50 g) were soaked in 100 ml distilled water in 1 liter Ehrlenmeyer flasks for 15 hours. Excess water was removed and the seed mixture was autoclaved for 20 min at 121° C. After being allowed to cool down, 10 small blocks of agar containing either *R. solani* or *F. oxysporum* were added to each flask, which was then shaken and sealed with alluminium foil and incubated at 25° C for 2 weeks. The inoculum mixture was then removed from flasks and air dried at 30° C for 72 hours before being ground to a fine powder in a Waring blender and stored in sterile containers at 5° C. Inoculum viability was tested before use by thawing stored inoculum powder before placing a few grams onto water agar. *Pythium ultimum* was grown on autoclaved oats kernels (50 g oats kernels in 50 ml distilled water) and inoculum powder was prepared as described above.

Artificial inoculation: The pathogenicity of *F. oxysporum* (2 isolates), *P. ultimum* (1 isolate) and *R. solani* (3 isolates) were screened on cabbage (cultivar Cape Spitz), tomato (cultivar Floridade) and amaranth (*Amaranthus hybridus* L.), under greenhouse conditions. A soil growth medium was prepared by mixing 10 g of inoculum powder of each pathogen with 1 kg of steam-sterilized clay loam soil in polyethylene bags (200 ml), which were then shaken vigorously to ensure uniform distribution. Infested soil was then placed in 10 x 10 cm pots and five surface disinfected seeds of each indicator

plant were planted per pot and replicated four times. Plants were watered every second day for three weeks. Disease severity in each compost was rated as an index from 1 to 3: 1= pre-emergence damping-off, 2 = diseased but not damped-off, 3= healthy. For each rating, number of diseased or healthy plants was expressed as a percentage of total plants or seeds infected (Lumsden *et al.*, 1983). A randomized complete block design was used and mean disease severity was based on four replicates (pots) per treatment. The experiment was conducted twice and data were pooled. An analysis of variance (ANOVA) (Table 2) was performed on the pooled data using NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) for each treatment and means were separated using the Newman-Keuls test.

To verify the presence of pathogens in diseased plants, 10 seedlings were randomly selected from each respective treatment, surface sterilized in NaOCl and washed three times in sterile distilled water. Plants infected by *P. ultimum* were sterilized in a 5% NaOCl and those infected by *R. solani* and *F. oxysporum* in 10% NaOCl. Plants were then plated onto suitable selective media and pathogens were identified after incubation for 7 days at 25° C.

Seedling biomass. For the assessment of seedling biomass, three healthy seedlings per pot were uprooted after two-three weeks of planting and their roots washed in running water to remove excess soil. Care was taken not to destroy the root system. Plants were then dried in an oven at 60° C for five days before the dry material was weighed to determine its biomass.

Compost preparation. Four types of animal manure were used for the preparation of composts. Poultry, cattle and pig manure were obtained from the Lesotho Agricultural College farm, Maseru. The compost piles of 0.5

m x 1m length x 0.5 m high were formed in September 1999 from layers of animal manure and plant materials including garden wastes (cabbage, weeds and tomato plants). Fresh piles were turned with a pitch fork after two weeks, followed by turning once a week. Composts piles were watered once every other week. Temperature inside the compost piles was also monitored once every two weeks during the composting process by using a thermometer, which was inserted in the compost hip (Figure 1). Sheep manure compost was obtained from a local farmer employing an organic farming system. The compost was prepared by mixing household garbage for example, egg shells, garden garbage e.g. leaves of vegetables and rotten crops and fresh sheep manure at a ratio of 3:1:2 volume. A chemical analysis of all composts used in the study was conducted. Due to high temperatures in compost heaps during composting (Figure 1), composts took only three months to decompose, followed by a one month curing period.

Greenhouse bioassay. The suppression of damping-off caused by pathogenic isolates of *R. solani, P. ultimum* and *F. oxysporum* by four composted animal manure was determined on three crops, tomato, cabbage and amaranthus, under greenhouse and field conditions. Preparation of pathogen inoculum and artificial inoculation was done using the same procedure as described previously. Infested compost, or sterilized loam soil (control treatment), was then placed into 10 x 10 cm pots and five seeds of each plant were planted in each pot. A randomized complete block design was used for the experiment and each treatment was replicated four times. Disease severity was rated three weeks after planting by determining the

percentage of diseased and healthy plants in both compost and soil treatments (Lumsden *et al.*,1983).

Seedbed bioassay. The outdoor seedbed experiment was a factorial design with four composts (poultry, cattle, pig and sheep manure) and control (soil with no compost application) treatments each infested with *R. solani, P. ultimum* or *F. oxysporum*. The 12 compost-pathogen combinations for each indicator plant were randomly arranged among three blocks resulting in 36 treatments plus three control treatments per block. The size of each seedbed was 0.6 m by 1.2 m. Each seedbed within a block was inoculated with one pathogen and planted with 24 tomato, cabbage and amaranthus seeds respectively, per seedbed.

For each seedbed 25 kg of sandy loam soil, with organic matter of 3.58 %, was hand mixed with 4 kg of each composted manure (an equivalent of 55 ton per ha) and infested with 20 grams of either *R. solani*, *P. ultimum* or *F. oxysporum* inoculum. Soil was analyzed for chemical composition before planting (Table 1). Planting of cabbage, tomato and amaranthus seeds was done after 14 days to allow for the establishment of pathogens in the soil-compost mixture. The seedbeds received no fertilizers during the run of the experiment and each seedbed was watered with 20 liters of tap water twice daily before germination of seeds and once daily after germination. A calculation of disease severity was done two-three weeks after seed germination for each crop. The experiment was conducted during February 2001 and repeated during March 2001. Biomass of the seedlings was also evaluated using the same procedure as described before. Analysis of

variance (ANOVA) was performed on the data and means of each treatment were compared using Duncan's multiple comparison test.

RESULTS

Artificial inoculations: *R. solani*, *F. oxysporum* and *P. ultimum* differed significantly ($P < 0.05$) in their pathogenicity towards each of the three indicator plants (Table 2). The isolates of *P. ultimum* and *R. solani* 1 were generally the two most virulent isolates. There were significant differences ($P < 0.05$) between the three isolates of *R. solani* (12-87%) and the two isolates of *F. oxysporum* (6-70%) on all three vegetables (Figure 2a). There was a significant interaction ($P < 0.05$) between the six isolates and the three crop species. For example, *R. solani* 1 and *F. oxysporum* 3 were significantly more virulent on tomato and amaranthus than on cabbage (Figure 2a).

Seedling biomass reduction. The seedling biomass of all three crops per pot was significantly ($P < 0.05$) reduced by most of the isolates compared to the control treatment. This reduction was generally consistent with levels of damping-off severity measured for each respective isolate (Figure 2 b).

Greenhouse bioassay of composts

Disease suppression. Composted animal manure showed variable effects on the suppression of damping-off of vegetable seedlings caused by the three pathogens for each of the three crops (Figure 3). A significant decline in damping-off ($P < 0.01$) was observed with cattle, pig and poultry manure treatments compared to sheep manure and the non-amended (control) treatment. There were few differences in disease severity between the means of cattle, pig and poultry manure for the three pathogens ($P<0.01$).

However, in sheep manure, more plants had damping-off symptoms than in the control treatment (Figure 3).

Each of the three crops showed varying degrees of damping-off severity in compost amended growth media. Seedlings of tomato, cabbage and amaranthus grown in poultry, cattle and pig manure generally displayed less damping-off than plants grown in sheep manure compost and the non-amended control treatment. Poultry and pig manure compost were most suppressive towards *F. oxysporum* for all three crops. In composted sheep manure, no seed germination was observed for all three crops and therefore no differences were observed between pathogens in terms of disease severity.

Seedling biomass. Application of composted animal manure, with the exception of sheep manure had a positive effect on the dry weight of seedlings of all three crops (Figure 4). Amaranthus, tomato and cabbage seedlings grown in composted cattle, poultry and pig manure had a significantly greater biomass than the control treatment. Three composts (poultry, pig and cattle manure) significantly increased seedling biomass ($P>0.01$) in comparison to sheep manure and the control treatment.

Seedbed bioassay. The addition of composted animal manure to infested soil had a variable effect on the disease response of the three different pathogens on three different hosts (Figure 5). For tomato seedlings, a significant decrease in disease ($P>0.01$) was observed for all three pathogens compared to the control treatment. Damping-off caused by *P.ultimum* in tomato seedlings was reduced from a mean of 58.9% in the control treatment to 5.3 and 5.1% in pig and poultry manure composts,

respectively. A significant reduction in disease severity was also observed with the application of sheep manure.

Cabbage and amaranthus seedlings displayed the same general trend as tomato. In cabbage seedlings, poultry manure was less effective towards *Pythium* damping-off compared to tomato seedlings. The same trend was evident regarding the effectiveness of sheep manure on *P. ultimum*, *R. solani* and *F. oxysporum*. Amaranth seedlings also responded very well to poultry, pig and cattle manure composts.

DISCUSSION

Composted manure of cattle, pig and poultry gave better control of seedling damping-off and root rot caused, by *R. solani*, *P. ultimum* and *F. oxysporum* than composted sheep manure. Disease severity was significantly suppressed in both greenhouse and seedbeds trials. Low incidences of diseased plants in these three composts correlated with increased seedling biomass. Seedling biomass in composted sheep manure was significantly low due to very poor seed germination, suggesting that the suppression of damping-off due to *P. ultimum* in compost-amended soil is mainly due to competition between *P. ultimum* and other microorganisms as determined by other factors (Mandelbaum and Hadar, 1990; Bruns *et al.*, 1995; Hoitink and Boehm, 1999). Previous reports indicate that *F. oxysporum* can be suppressed by composted cattle and poultry manure (Lockwood, 1985; Raviv *et al.*, 1998). In the present study, cattle and poultry manure were able to suppress not only *F. oxysporum*, but also *P. ultimum* and *R. solani*. The suppression of *R. solani* damping-off and *Fusarium* root rot in soil

substrates amended with compost has been associated, in part, with colonization by biocontrol agents such as *Trichoderma* spp. *Gliocladium* spp. and *Pseudomonas* spp. (Hoitink *et al.*, 1997).

Composted animal manure can apart from suppressing soilborne pathogens also stimulate plant growth (Lee and Battlet, 1977; Pitt *et al.*, 1998). By comparing the incidence of damping-off and seedling biomass in various animal manure composts, certain trends regarding the suppression of *R. solani*, *P. ultimum* and *F. oxysporum* in these composts were observed. Suppression of *F. oxysporum* was significantly higher in poultry, pig and cattle manure composts. Very little information on the mechanisms involved in the suppression of *Fusarium* diseases in compost amended growth media exixts. According to Hoitink *et al.* (1991), nutritional and biological factors play a role in the suppression of *Fusarium* spp. However, these have yet to be described from compost-based media.

Cattle, poultry and pig manure composts were also suppressive to disease caused by *R. solani* and *P. ultimum*. Composted animal manure is rich in organic matter, which stimulates high microbial activity resulting in the suppression of *R. solani*. Voland and Epstein (1994) were able to suppress damping-off of radish caused by *R. solani* with composted cattle manure. In our study, cattle manure compost also significantly suppressed seedling damping-off in all three tested plants. Cattle manure compost was also suppressive towards *P. ultimum*. Damping-off of seedlings caused by *P. ultimum* and other *Pythium* spp. has been reported to be suppressed by composted separated cattle manure (Hadar *et al.*, 1992). Suppression of *Pythium* in compost amended growth medium is attributed to increased

populations of bacteria and fungi (Hadar *et al.*, 1992; Ben-Yephet and Nelson, 1999).

Besides suppressing disease, composts of cattle, pig and poultry manure also increased seedling biomass. This can probably be attributed to high levels of available nutrients in these composts, which supports the growth of both plants and microorganisms (Hoitink and Boehm, 1999). These nutrients are slowly released during the growing period of the plant, thus also resulting in an increase in biomass (Chen *et al.*, 1988). Stimulation of plant growth can result in disease escape by plants (Hoitink and Fahy, 1986). Increased seedling biomass in cattle, pig and poultry manure composts obtained in our study are consistent with these reports. Our findings also indicate that increased disease suppression and seedling biomass was positively correlated with high levels of nutrients in these composts. The composts used in our study also contained high nutrient levels, especially N, P and K (Table 1). Some microorganisms in composted animal manures are also reported to promote both plant growth (Hoitink *et al.*, 1991) and induce systemic resistance (Duijff *et al.*, 1993; Liu *et al.*, 1995; Wei *et al.*, 1996).

The pH that is most favourable for seed germination, seedling growth, and increased water retention in composted animal manure can also be involved in plant growth and disease reduction (Dick and McCoy, 1993). The optimum pH for compost to suppress plant disease and promote plant growth ranges from 6.5 to 8.5 (Hoitink *et al.*, 1993) and the pH of cattle, poultry and pig manure composts in this study fell within this range. While sheep manure compost displayed a very low pH of 5. 5. Results obtained with sheep manure were inconsistent with the other three composts. Application of

composted sheep manure caused phytotoxicity in cabbage, tomato and amaranthus seeds and seedlings. Despite higher nutrient levels in this compost (Table 1), seeds failed to germinate and grow. Sheep manure compost had high levels of potassium, zinc and calcium, and the lowest pH. These factors are also probably responsible for phytotoxicity. High levels of potassium were reportedly responsible for phytotoxicity of chicken manure compost in *Banksia spinulosa* seedlings (Aryantha *et al.*, 2000). Though sheep manure compost was less effective in controlling seedling damping-off in the greenhouse, a slight increase in the number of healthy plants was observed in infested seedbeds amended with this compost. This is possibly due to the dilution of its phytotoxicity by biological, chemical and physical factors in the soil used for seedbeds.

The results presented here provide sound evidence of the growth-promoting and disease suppressive nature of composted cattle, poultry and pig manures. However, manures may also cause phytotoxicity as was the case with sheep manure compost, a phenomenon which may be related to the state of decomposition or quality of the compost (Hoitink *et al.*, 1993). Provided care is taken to avoid such negative traits, composted animal manures can nevertheless contribute to cost-effective, organically based management strategies for reducing diseases of vegetables and increasing crop production in Lesotho.

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Table 1. Chemical composition of poultry, cattle, pig and sheep manure compost and soil from Lesotho

	Types of compost				
Chemical component	Poultry	Cattle	Pig	Sheep	Soil
pH	7.84	8.02	7.82	5.47	6.64
P (%)	1.83	1.6	1.45	2.47	2.2
N (%)	0.14	1.68	1.32	2.33	3.15
Ca (%)	0.35	0.31	0.32	1.37	0.45
Mg (%)	0.81	0.74	0.68	0.47	0.7
K (%)	0.09	0.08	0.07	0.17	0.13
Na (%)	1.58	1.55	1.18	1.33	1.25
Org C (%)	9.18	7.6	13.19	9.83	6.16
Zn (dpm)	525	425	450	925	890
Fe (dpm)	8100	7600	7250	4500	6550
Mn (dpm)	425	387	435	200	364
Cu (dpm)	110	75	125	27	178

Figure 1. Temperature in compost heaps during composting period (0-20 weeks).

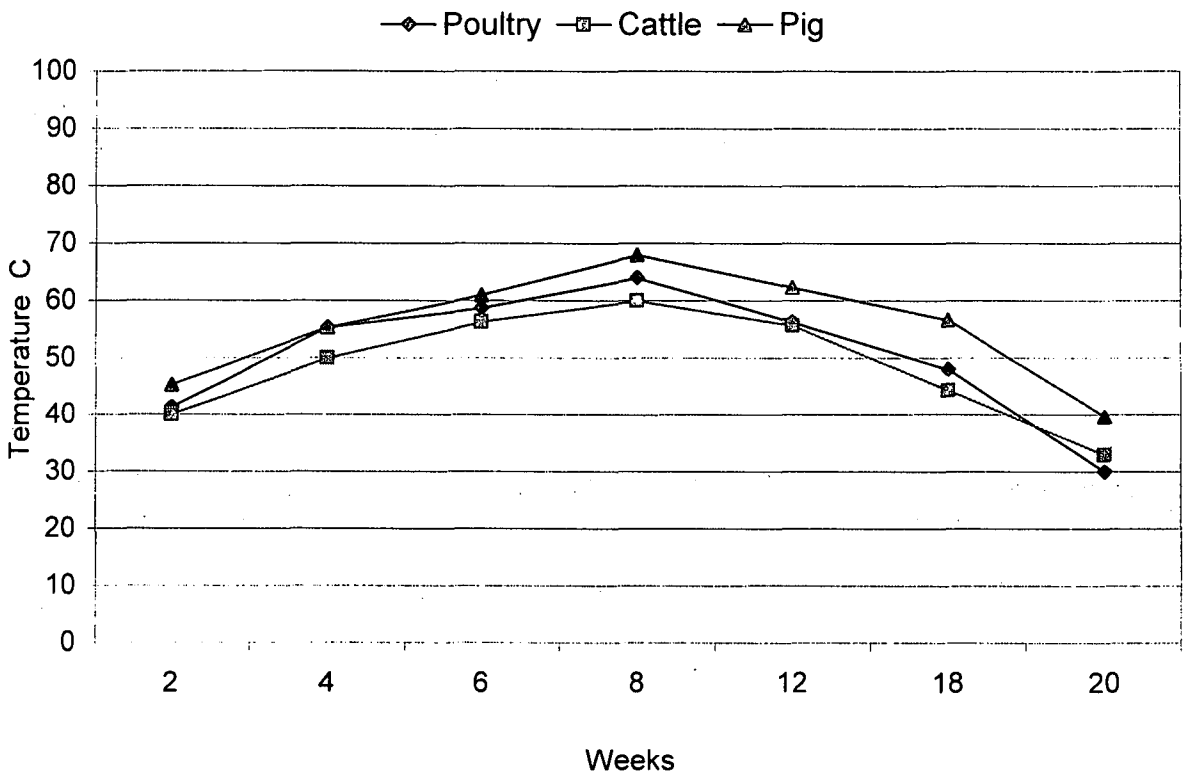

Poultry
Cattle
Pig
100
90
80
70
60
50
40
30
20
10
0
Temperature C
2
4
6
8
12
18
20
Weeks

Figure 2 A. Effect of *P. ultimum*, *R. solani* and *F. oxysporum* on seedling damping-off of tomato, cabbage and amaranthus. Bars denoted by different letters are significantly ($P < 0.05$) different for each main treatment.

Figure 2 B. Effect of *P. ultimum*, *R. solani* and *F. oxysporum* on seedling biomass of tomato, cabbage and amaranthus. Bars denoted by different letters are significantly ($P < 0.05$) different for each main treatment.

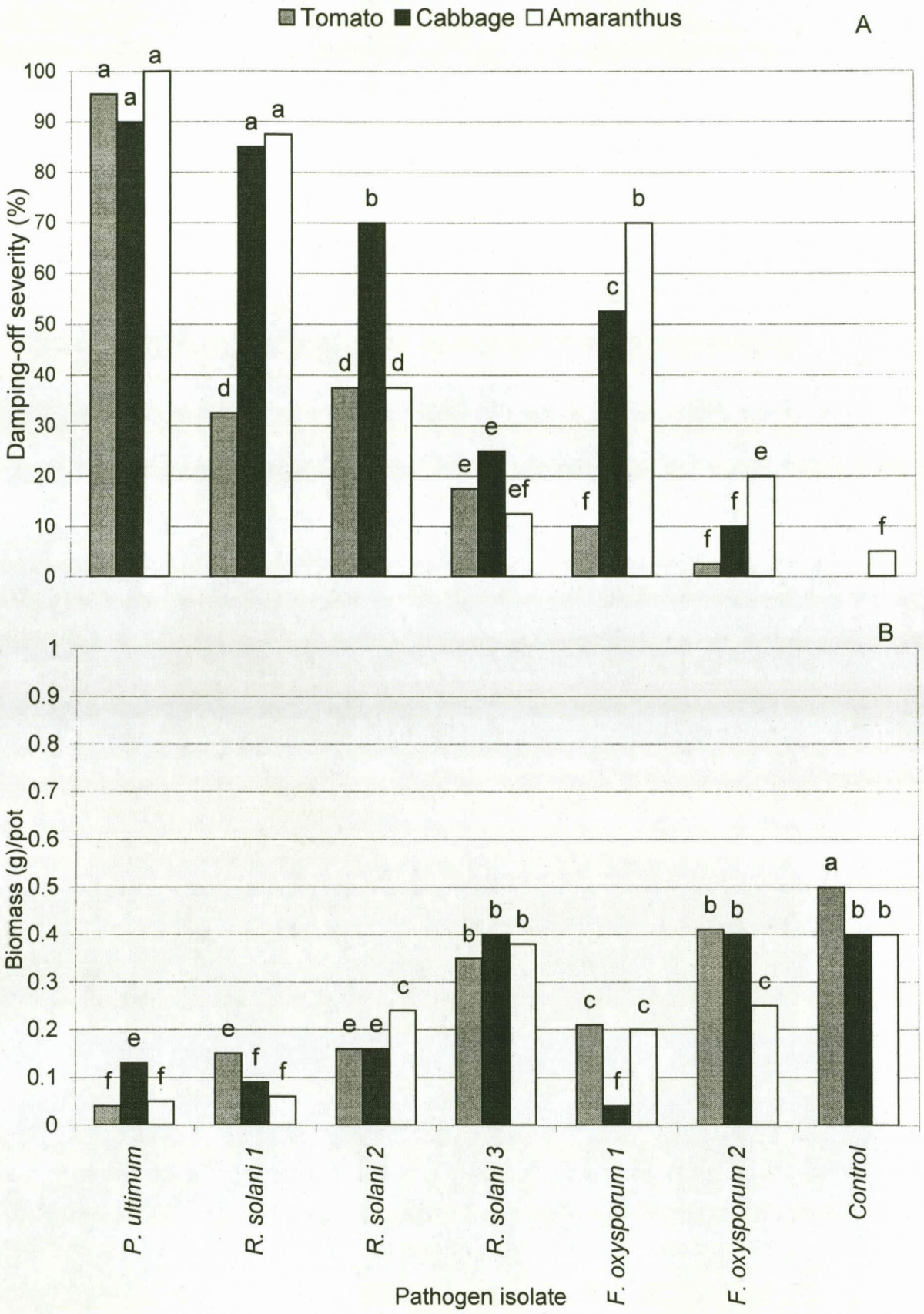

Tomato
Cabbage
Amaranthus
A
Damping-off severity (%)
100
90
80
70
60
50
40
30
20
10
0
a a a
a a a
d b d
e e ef
f c b
f f e
f
B
Biomass (g)/pot
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
f e f
e f f
e e c
b b b
c f c
b b c
a b b
P. ultimum
R. solani 1
R. solani 2
R. solani 3
F. oxysporum 1
F. oxysporum 2
Control
Pathogen isolate

Figure 3. Effect of composted animal manure on damping-off caused by *P. ultimum*, *R. solani* and *F. oxysporum* in seedlings of amaranthus (A), cabbage (B) and tomato (C) in the greenhouse. Bars denoted by different letters are significantly ($P < 0.05$) different for each main treatment.

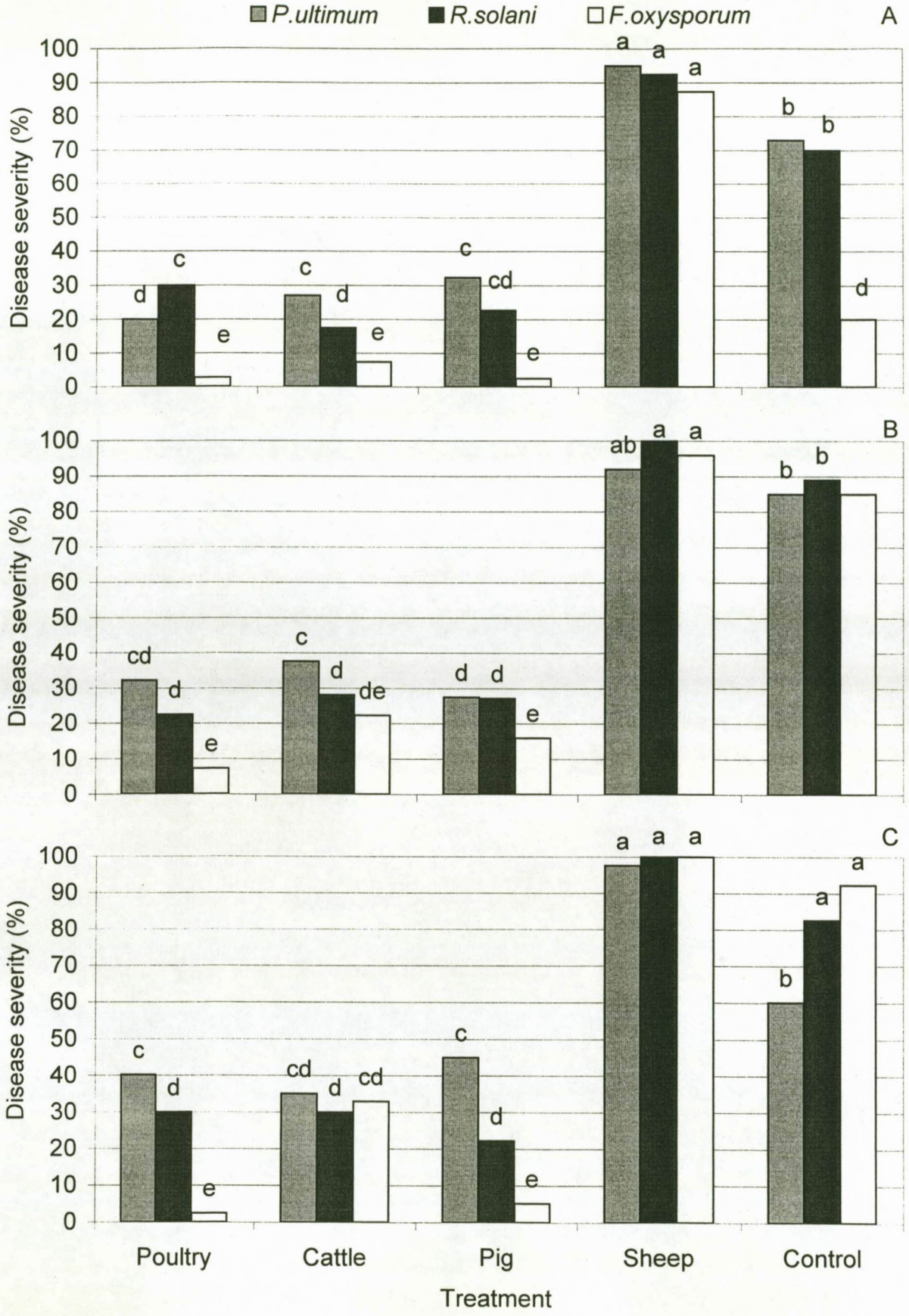

P.ultimum
R.solani
F.oxysporum
A
B
C
Disease severity (%)
Treatment
Poultry
Cattle
Pig
Sheep
Control

Figure 4. Effect of composted animal manure on seedling biomass of amaranthus (A), cabbage (B) and tomato (C). Bars denoted by different letters are significantly ($P < 0.05$) different for each main treatment.

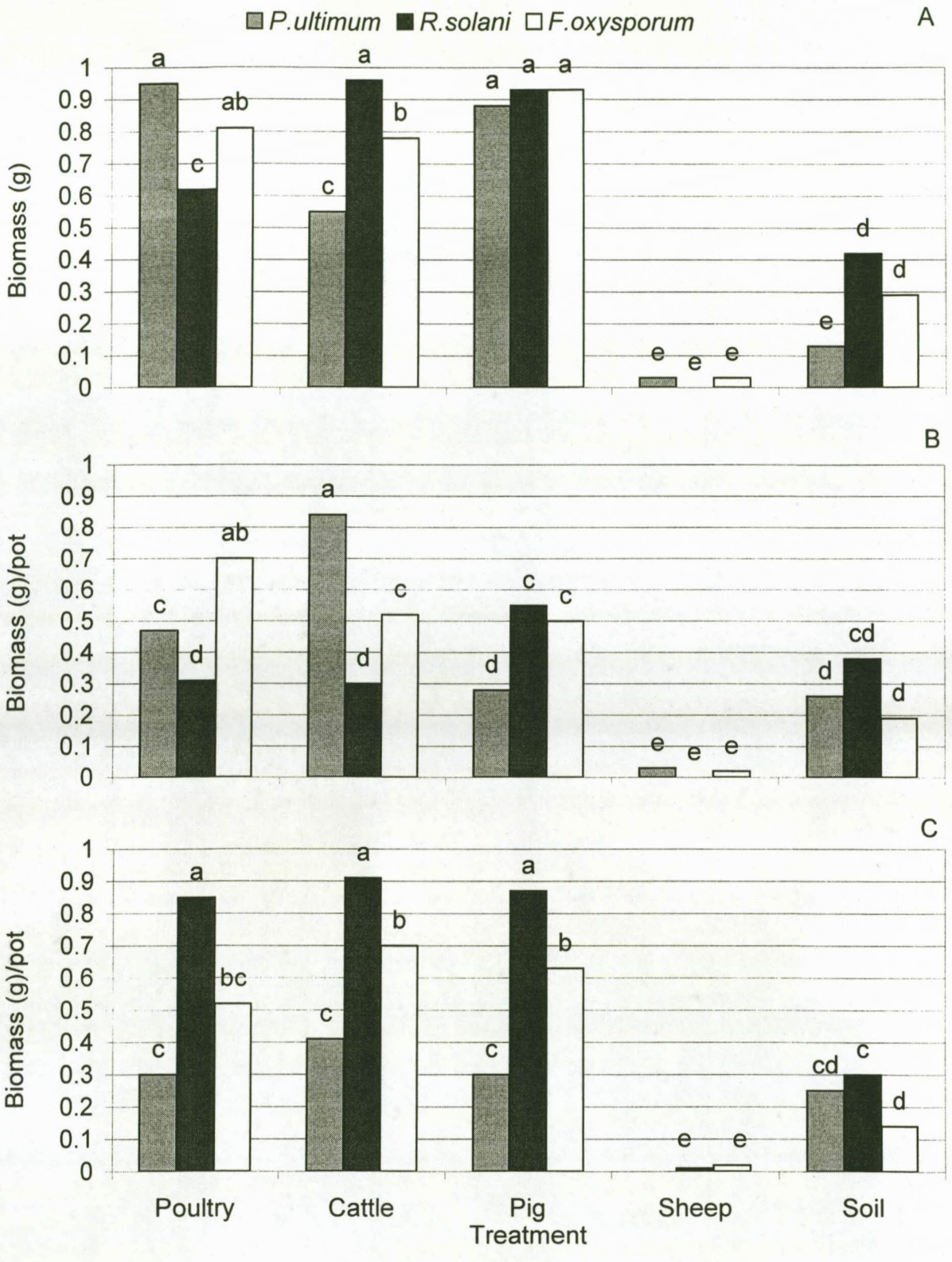
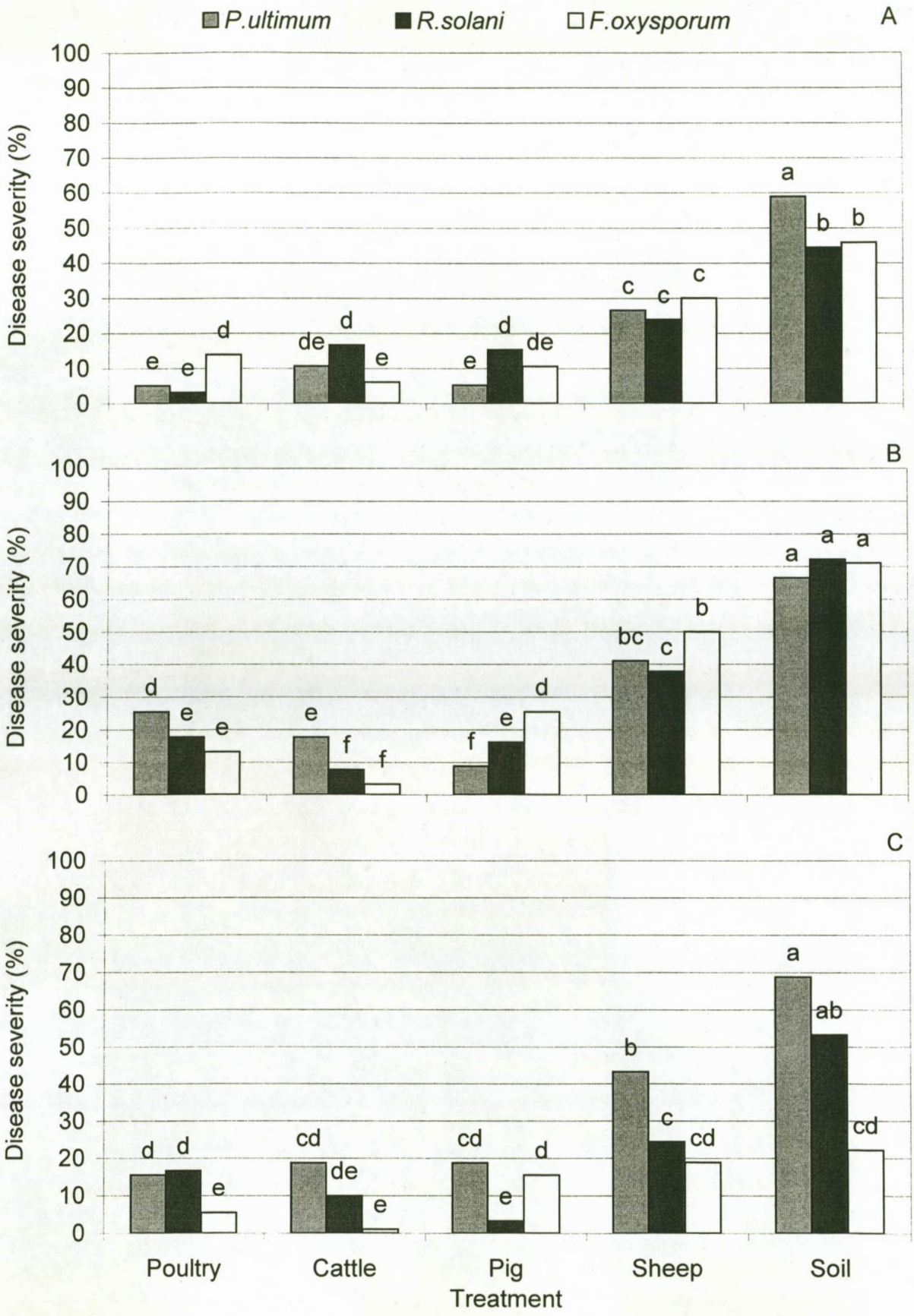

P.ultimum
R.solani
F.oxysporum
A
B
C
Biomass (g)
Biomass (g)/pot
Biomass (g)/pot
Poultry
Cattle
Pig
Sheep
Soil
Treatment

Figure 5. Effect of composted animal manure on damping-off caused by *P. ultimum*, *R. solani* and *F. oxysporum* in seedlings of amaranthus (A), cabbage (B) and tomato (C) in seedbeds. Bars denoted by different letters are significantly ($P < 0.05$) different for each main treatment.


P.ultimum
R.solani
F.oxysporum
A
B
C
Disease severity (%)
100
90
80
70
60
50
40
30
20
10
0
e e d
de d e
e d de
c c c
a b b
d e e
e f f
f e d
bc c b
a a a
d d e
cd de e
cd e d
b c cd
a ab cd
Poultry
Cattle
Pig
Sheep
Soil
Treatment

CHAPTER THREE

MICROBIAL ACTIVITY AND COMPOSITION OF COMPOSTED ANIMAL MANURE

ABSTRACT

General microbial activity of composted cattle, pig, poultry and sheep manure and loamy field soil was tested by means of a technique utilizing the hydrolysis of fluorescein diacetate (FDA). Fungal populations in these composts were also studied by serial dilution technique. Microbial activity was far higher in poultry, pig and cattle manure compost as well as field soil compared to composted sheep manure which had the lowest microbial activity. Different fungal species were recorded in composted pig, poultry, cattle and sheep manure. *Trichoderma harzianum*, *T. viride*, *Talaromyces trachyspermum* and *Penicillium verrucosum* were the most common species isolated from cattle, poultry and pig manure composts. Significantly less species were observed in composted sheep manure, *Humicola* sp. being the only species isolated frequently. This species was also isolated from pig manure compost, albeit at a lower frequency.

INTRODUCTION

Use of animal manure as a soil amendment is a farming practice traditionally used by farmers in Lesotho. Although this type of farming system is not necessarily high yielding, it is generally dependable and stable in yielding a harvest under all but the poorest of conditions (King, 1989). In most cases, animal manure is applied directly to the soil as an organic amendment the prime purpose being to increase soil fertility, and thereby plant growth.

Increasing interest in composting as a waste management strategy has led to more research efforts being directed toward the utilization of composts in agricultural crop production (Craft and Nelson, 1996). Composting of organic wastes prior to agricultural use reduces odor, decreases undesirable physical properties and increases nutrient availability (Voland and Epstein, 1994). Compost is also an excellent soil conditioner, which can provide nutrients to plants in an easily available form. Composts are known to improve the physical and chemical properties of soil, and generally enhance the activity of soil microorganisms (Cook and Baker, 1983; Hideaki et *al.*, 1990). Besides directly enhancing plant growth, some composts are also known to suppress plant diseases (Voland and Epstein, 1994).

The control of soilborne plant pathogens with compost is a relatively recent horticultural approach, which has been explored in by numerous researchers (Boehm and Hoitink, 1992; Hadar *et al.*, 1992). The addition of composts prepared from tree bark (Hoitink, 1980; Chen, Hoitink Schmitthenner and Tuovinen, 1988a), municipal sewerage sludges (Lumsden Lewis and Millner, 1983; Hoitink and Kuter, 1986) and various types of animal manure reportedly suppresses many soilborne plant pathogens including

Pythium ultimum Trow, *Rhizoctonia solani* Kuhn and *Phytophthora cinnammomi* Rands (Chen and Hadar, 1986; Chen, Hoitink and Madden, 1988b; Aryantha, Cross and Guest, 2000). Hadar and Mandelbaum (1992), described suppressive compost as an environment in which disease development is reduced despite the introduction of pathogens in the presence of susceptible plants. Disease suppression in this case, is the direct result of the activity of antagonistic microorganisms (Hadar and Mandelbaum, 1992).

The suppression of soilborne plant pathogens by composts is mainly due to increased microbial activity (Hoitink and Fahy, 1986), with a characteristically high bacterial-to-fungal ratio (Suzuki and Ishizawa, 1985). Many fungal and bacterial antagonists of plant pathogens have been isolated from organic composts (Kwok *et al.*, 1987; Mandelbaum and Hadar, 1990) and direct relationships between microbial activity and pathogen suppression have been established (Chen *et al.*, 1988b; Mandelbaum and Hadar, 1990). Compost amended growth media are able to suppress soilborne pathogens such as *P. ultimum* and *R. solani* (Boehm and Hoitink, 1992). Numerous species of *Trichoderma* have been isolated from composts suppressive to *R. solani* (Nelson *et al.*, 1983). Certain bacteria, including species of *Enterobacter, Flavobacterium, Xanthomonas* and various fluorescent *Pseudomonas* species have been isolated from composts suppressive to *R. solani* (Nelson *et al.*, 1983; Nelson and Craft, 1992). The combination of bacteria with certain *Trichoderma* species results in the increased suppression of *R. solani* (Kwok *et al.*, 1987). An increase in microbial activity results in increased competition for nutrients between pathogens and other soil microorganisms (Cook and Baker, 1983).

Microbial activity in composts can be determined by examining the concentration of different groups of microorganisms e.g. aerobic bacteria, anaerobic bacteria, fungi, actinomycetes, pseudomonads and nitrogen fixing bacteria by dilution plating (Hoitink *et al.*, 1991). However, microbial activity, based on the rate of hydrolysis of fluorescein diacetate (FDA) by microorganisms, has afforded new and useful insights into the mechanisms by which microorganisms suppress the activity of soilborne pathogens (Chen *et al.*, 1988a; Marull *et al.*, 1997). FDA, a non-fluorescent substrate, is hydrolyzed by various enzymes of living cells to yield fluorescein (Inbar *et al.*, 1991). Fluorescein remains in the cells causing intracellular fluorescence, which can be visualized by fluorescence microscopy (Rotman and Papermaster, 1966). Fluorescein can also be quantified spectrophotometrically. Swisher and Caroll (1980), used this technique to determine the amount of active microbial biomass in Douglas fir needles.

The diversity of fungi in compost can be indicative of the compost being either conducive or suppressive to soilborne plant diseases (Kuter *et al.*, 1983). Hyphomycetes, ascomycetes and zygomycetes usually dominate fungal populations isolated from growth media that is either suppressive or conducive to disease (Kuter *et al.*, 1983). A high density of *Trichoderma hamatum* (Bonorden) Bainier and *T. harzianum* Rifai are characteristic of growth media suppressive to *Rhizoctonia* spp. In contrast, populations of *Penicilium verrucosum* Westling Samson and *Geomyces* spp, are high in media conducive to diseases caused by *Rhizoctonia* (Kuter *et al.*, 1983).

The objectives of this study were firstly, to measure the microbial activity of four animal composts utilized frequently in Lesotho and secondly, to identify and quantify species of fungi present in these composts.

MATERIALS AND METHODS

Compost preparation. Cattle, poultry, pig and sheep manures were used for the preparation of composts. The compost piles of 0.5m x 1m length x 0.5m high were formed in September 1999 from layers of animal manure and plant materials utilized by local farmers in Lesotho and included garden waste (cabbage leaves, weeds and tomato plants). Fresh compost piles were turned after two weeks, followed by turning once a week. Compost piles were watered once every other week depending on moisture content. Temperature inside the compost piles was also monitored once every two weeks during the composting process (Figure 1). Sheep manure compost was obtained from a local farmer employing an organic farming system. The compost consisted of household garbage (vegetable, fruits, eggshells), garden wastes and sheep manure at a ratio of 3:1:2.

Topsoil samples of three different soil series Maseru (sandy clay with 3.95 % organic matter), Sephula (sandy clay loam; 3.68 % organic matter) and Leribe (clay loam; 2.34 % organic matter) were collected from tilled fields in Lesotho. Ten samples each from three fields previously cultivated with peas (*Pisum sativum* L.), cabbage (*Brassica oleracea* L.) and tomato (*Lycopersicon esculentum* Mill.), were passed through a 6 mm sieve and stored at room temperature until further use. A sub-sample (500 g) was air dried at 40 $^{\circ}$C for 1 hour.

Microbial activity. Microbial activity in four types of composted animal manure and field soil (control treatment) was determined by measuring the rate of hydrolysis of fluorescein diacetate (FDA) (Schnurer and Rosswall, 1982). FDA was dissolved in acetone (2.0 mg/ml) and stored as a stock solution at -20 ° C. Twenty milliliters of sodium phosphate buffer (pH 7.6) was added to individual soil and compost samples (0.5 g) placed in 250 ml Erlenmeyer flasks and. The FDA stock solution was added (0.2 ml) to the mixture, which was then incubated for 1 hour in a rotary shaker (90 rpm) at 25 ° C. Each treatment was replicated three times. A control sample not containing organic matter was used to correct background absorbance for each soil and compost sample. Compost residues were removed from the FDA-buffer mixture by filtering the suspension through filter paper (Whatman No.1). The filtrate was collected in a test tube, covered with Parafilm®, and placed in an ice bath for 30 minutes. The concentration of free fluorescein after hydrolysis was determined by reading the optical density at 490 nm using spectrophotometer after 30 minutes.

Standard curves for each compost and soil was prepared by adding various quantities of FDA (0, 100, 200, 300, 400 μl in the stock solution) to 5 ml of phosphate buffer (pH 7.6) in test tubes. Tubes were tightly closed and placed in a boiling water bath for 60 min. After cooling for 10 min, hydrolyzed fluorescein was added to 250 ml Erlenmeyer flasks containing 0.5 g (dry weight) of each compost and soil. In order to remove all remaining traces of fluorescein, test tubes were rinsed with 15 ml of phosphate buffer, which was then also poured into the flasks. The flasks were placed on a rotary shaker for 20 minutes and the reaction was stopped by adding 20 ml of acetone to all

samples. Compost and soil samples were filtered through a No 1 Whatman filter paper. The filtrate was then collected into test tubes, covered with Parafilm® and placed into an ice bath to prevent acetone volatization. The concentration of free fluorescein in the filtrate was then determined as described above.

Fungal population density. Fungal populations in the four composted animal manures and three soil samples were isolated and enumerated by means of dilution plating. Compost or soil (1 g) was placed in 99 ml of 0.1% water agar and the mixture was agitated for 45 seconds on high speed in a Waring blender. The suspension was then diluted (10^{-1}) and 0.1 ml of the final dilution was aliquoted onto 25 plates each containing water agar amended with 300μg/ml streptomycin sulphate (Novostrip). Plates were incubated at 22 ° C and the total number of fungal colonies growing in each plate was recorded after 48 hours. Hyphal tips were removed from 50-70 colonies on each plate with the aid of a dissecting needle and microscope, placed onto potato dextrose agar (PDA) (Difco) plates and incubated for 7-10 days. The resulting cultures were then sorted into presumptive groups based on cultural morphology. Representative cultures of each group were given identification numbers and set aside for detailed examination and identification. Standard mycological procedures were used to identify genera which could not readily be classified to species level by means of morphological characteristics. A mean of relative frequency of fungal genera or species from each compost and soil sample was calculated (total colonies of fungal species / number of replicates). Each experiment was conducted three times and an analysis of variance (ANOVA) was performed with the

combined data after conducting Bartlett's test for homogeneity of variances on fungal population densities. Means were separated using Duncan's multiple range test at the 95 % confidence level.

RESULTS

Microbial activity. Significant differences in microbial activity were observed between the four composted animal manures and field soil (combined mean of three samples). Significantly higher levels ($P < 0.05$) were observed in composted pig, poultry and cattle manure than in field soil (Figure 2). The highest level of microbial activity was observed in composted pig manure. Microbial activity in cattle and poultry manure was significantly lower than pig manure but was still significantly ($P < 0.05$) higher than field soil and composted sheep manure. Microbial activity in sheep compost, was lower than that recorded for field soil. Field soil displayed a significantly lower microbial activity than pig, cattle and poultry manure.

There was a significant difference ($P < 0.05$) in the absorbance of fluorescein between the four composted animal manures and field soil. A good calibration curve could however not be obtained from all four composts (Figure 3 A). Fluorescein absorbance was high in poultry, cattle and pig manure composts at all FDA concentrations. Soil samples had a lower rate of FDA absorbance than the above mentioned three composts (Figure 3 B). FDA absorbance in field soil was however higher than in composted sheep manure, the latter not differing significantly in this respect from the blank sample (control treatment).

Fungal populations. Various species of fungi were isolated from the four animal manure composts and three soil types (Table 2). Most fungal species were isolated from cattle, pig and poultry manure compost. *Trichoderma viride* Pers. was most frequently isolated from cattle, poultry, pig manure and soil, with pig manure having the highest population of this species.

Sheep manure yielded the lowest number of fungal isolates (Figure 4), with *Humicola* sp. virtually the only species isolated from this compost (34). The same species also occurred in relatively high numbers from composted pig manure but was virtually absent in the other two composts and field soil. *Fusarium oxysporum* Schlecht. and *Penicillium purpurogenum* Pitt were not isolated from sheep compost but were isolated from the other three composts and soil.

Several dominant fungal species were isolated from the four compost and combined soil types. These included *Talaromyces trachyspermum* (Stolk & Samson), *Trichoderma* spp, *Penicillium verrucosum* Dierckx, *Rhizopus* spp, *Aspergillus repens* Link. and *Humicola* sp. In cattle manure compost, *Rhizopus oryzae* Wenr & Frinsen Geerlings was the most frequently isolated fungus followed by *A. repens* and *Trichoderma harzianum* Pers. The most common fungi in composted poultry manure compost were *A. repens T. harzianum* and *P. verrucosum* .

DISCUSSION

Microbial activity in composted organic wastes, measured by the rate of fluorescein diacetate hydrolysis and fungal populations, is an important criterion in determining the conduciveness or suppressiveness of composts to plant disease (Kuter *et al.*, 1983; Mandelbaum *et al.*, 1988; Inbar *et al.*, 1991; Klamer and Søchting, 1998). In the present study, microbial activity and fungal population densities of four composted animal manures were assessed. High rates of fluorescein diacetate were absorbed in composted cattle, poultry and pig manure, which was consistent with the population density of fungi in these composts. Similarly, low population densities of fungi in sheep manure were consistent with a low rate of fluorescein diacetate hydrolysis.

The application or incorporation of organic products into soil creates a dynamic soil ecosystem by promoting microbial activity, which as a result increases soil enzyme activity (Alabouvette *et al.*, 1985; Martens *et al.*, 1992; Press *et al.*, 1996). Microorganisms in soil and compost produce enzymes such as protease, lipase, and esterase, which are responsible for the hydrolysis of FDA (Press *et al.*, 1996). Spectrophotometry measurements at an optical density of 490 nm, can determine the amount of free fluorescein in compost and soil (Inbar *et al.*, 1991). High rates of free fluorescein were observed in composted sheep manure and control soil samples, compared to a significantly lower rate in composted cattle, poultry and pig manure. Since various enzymes in living cells are responsible for FDA hydrolysis, high rates of free fluorescein in sheep manure is an indication of low microbial activity.

Low rates of free fluorescein in composted cattle, poultry and pig manure thus indicates a higher presence of microorganisms in these composts.

Composted animal manure has been shown to display higher populations of fungi than field soil (Kuter *et al.*, 1983). By comparing the fungal species isolated from the various composts and field soil, certain trends regarding the distribution of fungi in these substrates were evident. The dominant species of fungi in sheep manure compost was *Humicola* sp. This genus has the ability to withstand unfavourable conditions such as high temperatures and toxicity (Straatsman *et al.*, 1994). Sheep manure compost had high levels of zinc and phosphorus and low pH levels (Table 1). Aryantha *et al.* (2000), attributed toxicity of chicken manure to high levels of phosphorus. High populations of *Trichoderma* spp. were isolated from cattle, pig and poultry manure compost (Figure 5), but not from composted sheep manure. Hoitink and Fahy (1986), reported that *Trichoderma* spp. colonize bark composts and are a good indication of the ability of this compost to suppress *R. solani.* *Aspergillus* spp. are reported to be common colonizers of spent mushroom compost and composted municipal waste (Hoitink and Fahy, 1986). In our study, *Aspergillus* spp. were frequently isolated from cattle and poultry manure compost, and also from soil. Many of the fungi isolated from compost were also isolated from soil (Table 2).

Numerous microorganisms found in composted growth media act as antagonists of soilborne plant pathogens (Boehm *et al.*, 1993). A higher microbial activity, particularly of fungi and actinomycetes was observed by Kuter *et al.* (1983) and Lewis *et al.* (1991), in composts made from municipal biosolids and antagonistic fungi were isolated from these composts. Kuter *et*

al. 1983 and Nelson *et al.* 1983, studied the genera of antagonistic organisms in composted hardwood bark. Their results showed that genera of *Trichoderma*, *Gliocladium*, *Penicillium*, *Mortiella*, *Paecilomyces*, *Geomyces* and *Ophiostoma* were dominant. Of the seven genera, *Trichoderma* and *Gliocladium* were presumed to be the most important antagonists of pathogens. In our study, genera of *Trichoderma*, *Penicillium*, *Paecilomyces* and *Geomyces* were also isolated from all four composts and soil.

Microbial activity in compost is supported by the gradual release of organic nutrients, which, in turn, sustains biological control (Chen *et al.*, 1988b; Hoitink *et al.*, 1991). The ability of compost to suppress pathogenic organisms is attributed to the fact that microflora in compost is able to prevent spore germination of pathogens due to competition for nutrients, lysis or hyperparatism (Chen *et al.*, 1988a; Hadar *et al.*, 1992). Composted animal manure is a good source of organic nutrients (Table 1), which can significantly affect the microbial populations in soil. In our study, composted poultry, pig and cattle manure, which had high levels of nutrients also displayed high levels of microbial activity.

High bacterial populations are known to occur in soils amended with composted poultry litter (Press *et al.*, 1996). The present study confirmed that composted poultry manure had a high level of microbial activity. The low microbial activity observed in composted sheep manure can probably be attributed to high rates of toxic material which is lethal to microorganisms (Table 1). The composition of fungi in cattle, pig and poultry manure composts was similar (Table 2). Sheep manure appeared to differ from the other three composts both quantitatively and qualitatively.

Microbial activity and fungal populations in composted manure can determine the ability of such composts to suppress soilborne plant pathogens (Alabouvette *et al.*, 1985; Chen *et al.*, 1888b; Martens *et al.*, 1992; Press *et al.*, 1996; Aryantha *et al.*, 2000). Studying the association between high microbial activity, fungal populations and disease in compost amended soil media is the first step in understanding the concept of disease suppression by the four composted animal manure evaluated in this study. A better understanding of the dynamics involved in the suppressive effects of composted animal manure used by farmers in Lesotho will hopefully provide more effective and economical production methods in Lesotho.

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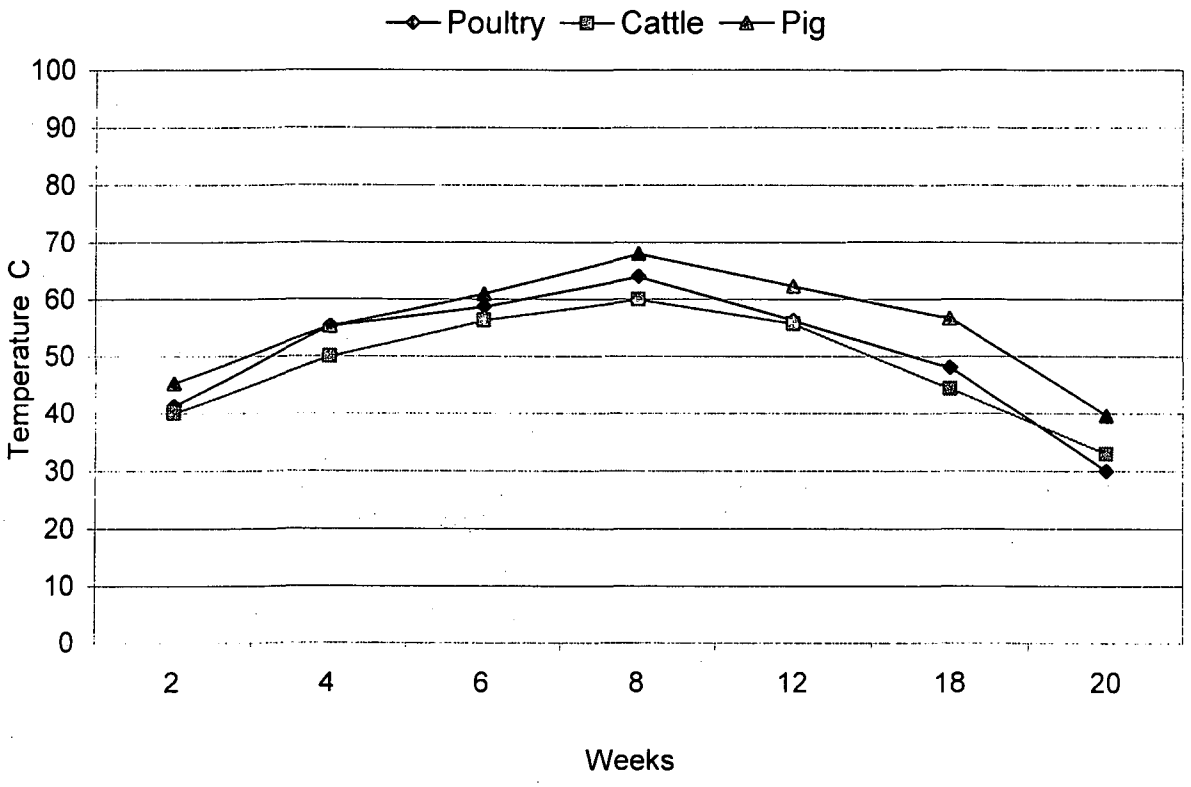
Table 1. Chemical composition of poultry, cattle, pig and sheep manure compost and soil from Lesotho

	Types of compost				
Chemical component	Poultry	Cattle	Pig	Sheep	Soil
pH	7.84	8.02	7.82	5.47	6.64
P (%)	1.83	1.6	1.45	2.47	2.2
N (%)	0.14	1.68	1.32	2.33	3.15
Ca (%)	0.35	0.31	0.32	1.37	0.45
Mg (%)	0.81	0.74	0.68	0.47	0.7
K (%)	0.09	0.08	0.07	0.17	0.13
Na (%)	1.58	1.55	1.18	1.33	1.25
Org C (%)	9.18	7.6	13.19	9.83	6.16
Zn (dpm)	525	425	450	925	890
Fe (dpm)	8100	7600	7250	4500	6550
Mn (dpm)	425	387	435	200	364
Cu (dpm)	110	75	125	27	178

Table 2. Fungal species isolated from composted animal manure and field soil

	Number of colonies				
Fungal species	Cattle	Poultry	Pig	Sheep	Soil
Aspergillus fumigatus Fresen	21.2	35.6	29.6	2.4	11.2
Aspergillus repens Link	33.2	32.8	3.6	0.2	30.2
Fusarium oxysporum Schltdl.	12.7	8	4.2	0	75.1
Geotrichum sp.	17.7	34.4	35.3	7.2	5.4
Humicola sp.	4.2	2.9	52.3	34.1	6.3
Paecilomyces inflatus Burnside Carmichael	12.2	22.9	35.6	5	24.2
Penicillium citrichum Thom	4.8	27.4	45.2	0.6	22
Penicillium griseofulvum Dierctx	42.7	12.9	7.2	0.7	36.5
Penicillium ochrochloron Biourge	13.3	27.8	25.4	1.7	31.8
Penicillium purpurogenum Thom	8.7	7	6	1.3	25.8
Penicillium verrucosum Westling Samson.	23.9	25.2	32.7	1.1	16.9
Rhizopus oryzae Went & Prinsen Geerlings	36.9	18.8	7.7	3.2	33.5
Sphaeronaemella fimicola Darchl.	35.8	20.9	14.4	1.4	27.6
Talaromyces trachyspermum Frisvad *et al*.	25.7	19.8	31.7	3.4	19.3
Torulamyces lagana Delitsch	32.3	43.4	7.7	0.1	16.4
Trichoderma harzianum Rifai	30.5	28.5	29.3	0.1	11.6
Trichoderma viride Pers.	24.8	26.5	26	0.3	17.3
Unidentified species	19.3	9.6	27.7	6.6	36.8

Figure 1. Temperature in compost heaps during composting period (0-20 weeks).


Poultry
Cattle
Pig
100
90
80
70
60
50
40
30
20
10
0
Temperature C
2
4
6
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12
18
20
Weeks

Figure 2. Microbial activity in composted animal manure and soil as measured by FDA hydrolysis. Control treatment was a blank sample, which contained neither soil nor compost. Each data point is the mean of four replications per treatment.

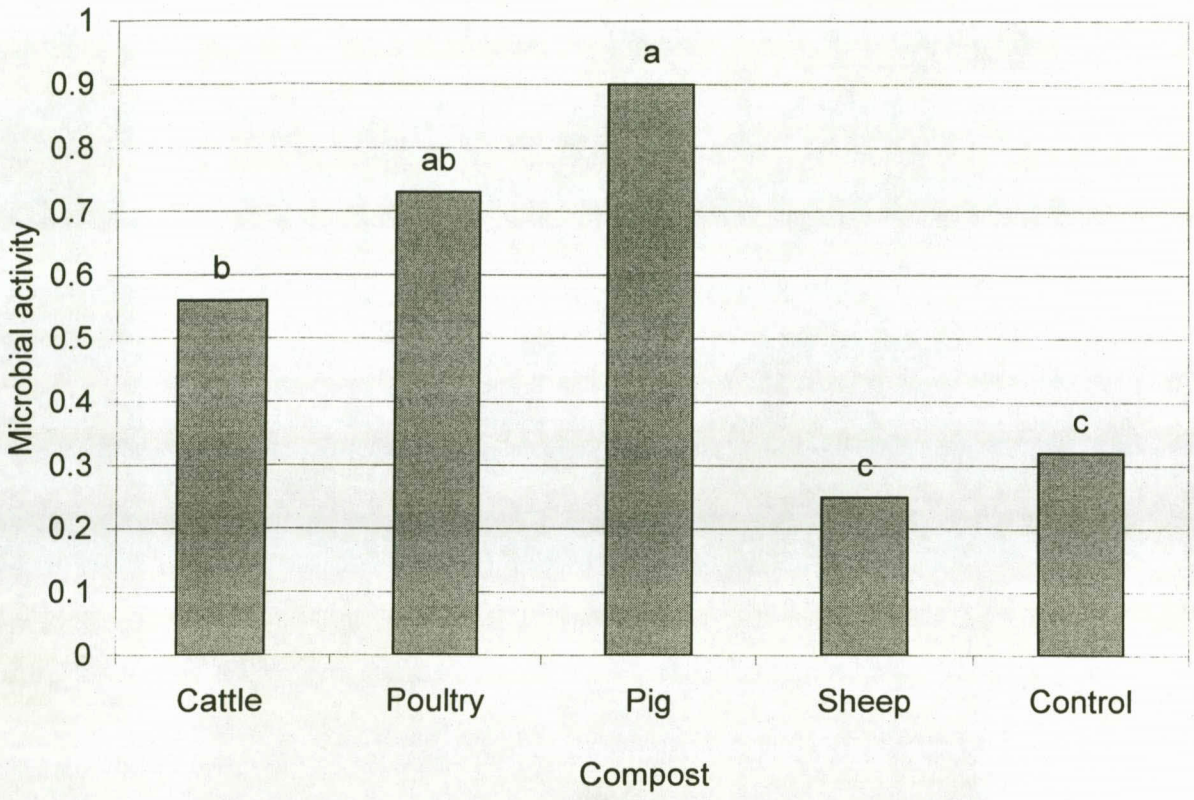
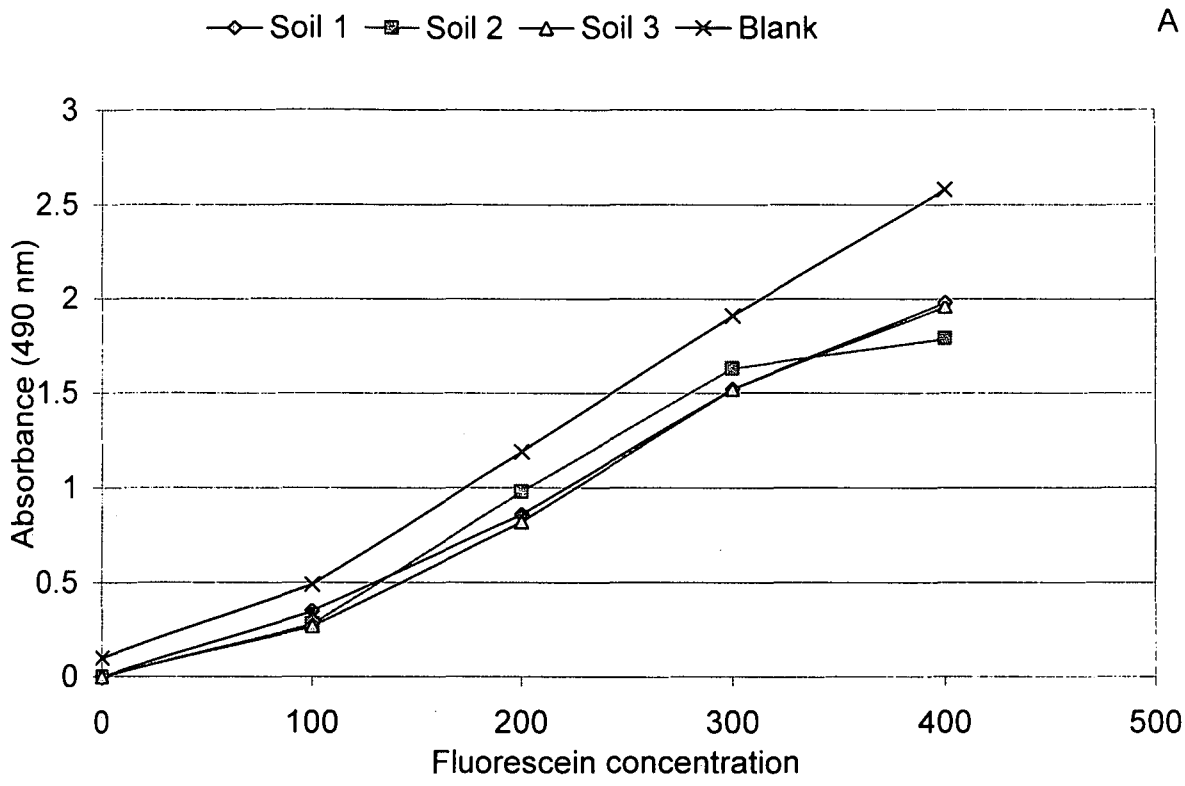

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Microbial activity
b
ab
a
c
c
Cattle
Poultry
Pig
Sheep
Control
Compost

Figure 3. Relationship between hydrolysed FDA concentration and optical density (490 nm) for soil (A), and composted animal manure (B). Blank samples did not contain soil or compost.


Soil 1
Soil 2
Soil 3
Blank
A
Absorbance (490 nm)
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Fluorescein concentration

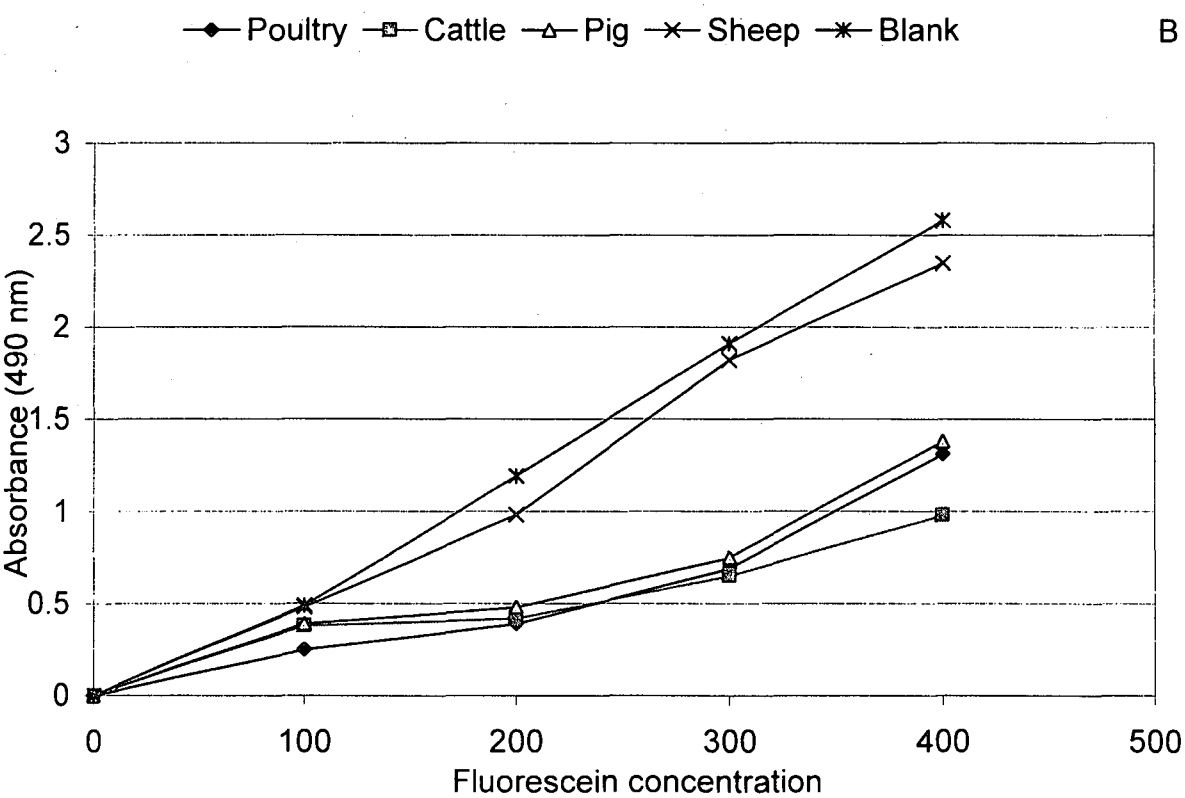
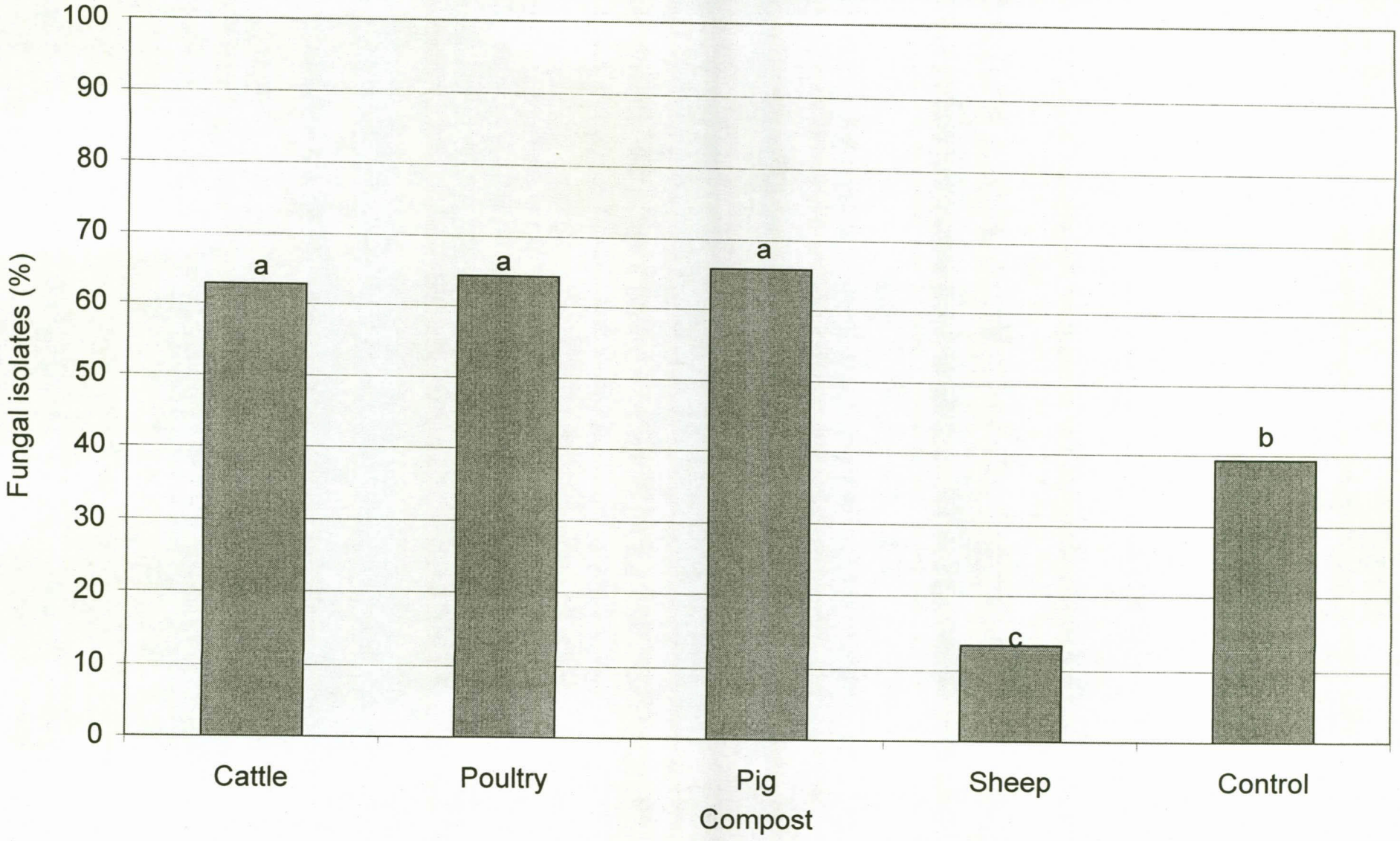

Poultry
Cattle
Pig
Sheep
Blank
B
Absorbance (490 nm)
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Fluorescein concentration

Figure 4. Population densities of fungal species isolated from different composted animal manure and field soil (control). Each datum point is a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).


100
90
80
70
60
50
40
30
20
10
0
Fungal isolates (%)
a
a
a
c
b
Cattle
Poultry
Pig
Sheep
Control
Compost

CHAPTER FOUR

DISEASE SUPPRESSIVENESS OF PLANT EXTRACTS TO THREE SOILBORNE PATHOGENS OF VEGETABLE SEEDLINGS IN LESOTHO

ABSTRACT

Four plant species (*Leucosidea sericea*, *Rhamnus prinoides*, *Artemisia afra* and *Melia azedarach*), obtained from Lesotho were tested for their efficacy in suppressing disease caused by *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*. A fungicide, dichlorophen 200 g/l was used as a control treatment. Oven dried plant materials were soaked in methanol for 12 hours, after which methanol was evaporated to obtain a powdery substance. An amount of 0.2, 0.4, 0.6, 0.8 and 1 gram plant extract/100 ml sterile distilled water was added to 2 % malt extract agar (MEA). Extracts of *L. sericea*, *R. prinoides* and *A. afra* significantly inhibited mycelial growth *in vitro* and reduced pathogen inoculum in soil. *Pythium ultimum* was highly susceptible to the three extracts with 100 % inhibition on mycelium growth recorded at a concentration of 1g plant extract/100 ml malt extract agar. Extracts from *M. azedarach* displayed very little inhibition towards *R. solani* and *F. oxysporum*. In a dilution plate bioassay, *R. solani* was significantly reduced by extracts of *A. afra* and *R. prinoides* but was not affected by *M. azedarach*. However, *P. ultimum* was strongly inhibited by extracts of *M. azedarach*. In soils treated with dichlorophen, both population densities and mycelial growth were very low, especially in soil infested with *R. solani* and *F. oxysporum*. The three pathogens were recovered more readily from soil treated with extracts (< 14 days) than from soil treated with dichlorophen (> 21 days).

INTRODUCTION

Many plant extracts exhibit inhibitory effects to a wide range of microorganisms (Kaushal and Paul, 1989; Mojumber and Mishra, 1992; Pandey and Bhargava, 1994; Vijai- Pal *et al.*, 1994) and the suppression of phytopathogenic organisms, especially fungi, using these extracts has often been reported (Wilson *et al.*, 1997; Bowers and Locke, 2000). Plants used for human medicinal purposes, generally, exhibit strong inhibitory effect towards plant pathogens (Fujii *et al.*, 1991). Many of these plants are also naturally resistant to fungal pathogens (Srivastava and Kediyal, 1983; Bianchi *et al.*, 1997) and numerous attempts have been made to identify their level of fungitoxicity in vitro (Rao and Alvarez, 1981; El-Gammal and Monsour, 1986; Awuah, 1994). Many of these plants contain toxic compounds that are biodegradable (Kumar and Tripathi, 1991; Shenk *et al.*, 1991; Bowers and Locke, 2000) and selective in their toxicity (Wilson *et al.*, 1997) and can therefore play an important role in suppressing fungal diseases.

In Lesotho, many traditional farmers combat plant diseases by using water extracts of plants that are also used for traditional medicinal purposes. They include, *Rhamnus prinoides* L. Herit, *Leucosidea sericea* Eckl. & Zeyh. and *Artemisia afra* Jacg. ex Willd (Mei, 2000). *Melia azedarach* has also been used in most African countries to combat human ailments, plant pests and diseases (Heinkens, 1991) and although it is not considered a traditional medicinal plant in Lesotho, it grows extensively in this country as an exotic weed.

The objectives of this study were to evaluate: (i) the antifungal properties of the four plants to three soilborne fungal pathogens; (ii) their

ability to reduce fungal populations in soil; (iii) their ability to suppress damping-off of seedlings when used as a soil amendment under greenhouse conditions and their phytotoxicity to seedlings of cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* Mill.) and amaranthus (*Amaranthus hybridus* L.).

MATERIALS AND METHODS

Preparation of plant extracts. Healthy, fresh leaves of *R. prinoides, L. sericea*, *A. afra,* and *M. azedarach* were collected from different locations in Lesotho during the summer of 1999. The leaves were oven dried at 40° C and then crushed with a grinder. The resulting powder was kept at 5° C until further use. Hundred grams of powder from each plant was placed in a 1 liter bottle to which was added 500 ml of methanol. The bottles were then tightly sealed and placed on a rotary shaker for 12 hours. Methanol was filtered, and then vacuum dried. The resulting powder was stored at 5° C until further use.

Preparation of pathogen inoculum. Isolates of three fungal pathogens (*Rhizoctonia solani* Kuhn, *Pythium ultimum* Trow and *Fusarium oxysporum* Schltdl) isolated from diseased vegetable seedlings (see Chapter 2) were grown on potato dextrose agar (PDA) (Difco) (*R. solani* and *F. oxysporum*), and on corn meal agar (CMA) (Difco) (*P. ultimum*) for 10 days at 25° C. Small pieces of colonized agar were subsequently transferred to McCartney bottles containing 5 ml sterile distilled water. Bottles were sealed with Parafilm and stored at 5° C until further use. Before being used in bioassays, fungi were aseptically removed from sterile water and grown on 2% malt extract agar (20 g malt extract + 15 g technical agar) for 8 days.

Bioassay of antifungal ability. The antifungal activity of four plant extracts was determined *in vitro* as follows: Different amounts of extract powder (0.2, 0.4, 0.6, 0.8 and 1 gram) from each plant was added to warm (40° C) 2% malt extract agar (MEA) (20 g malt extract + 15 g technical agar/L + 300 µl streptomycin sulphate). Twenty ml of the extract-agar mixture was poured into a Petri dish and left to solidify. A mycelium plug cut from an 8-day -old culture of each pathogen was then placed in the center of each dish, mycelium side down. Each treatment (extract concentration + fungus) was replicated three times. The plates were incubated for 72 hours at 25° C, and colony diameter was measured along two perpendicular lines drawn on the base of the plate. The mean of the two measurements was used to calculate the mean colony diameter. The experiment was conducted twice and data were pooled.

Phytotoxicity test. A phytotoxicity test was performed to determine the effect of the plant extracts on seed germination. Seeds of tomato (Floridade), cabbage (Cape Spitz) and amaranthus were surfaced sterilized with 0.4% NaOCL solution for 1 minute and rinsed 3 times in sterile water. Ten seeds of each plant were placed in a Petri dish on sterile filter paper (Whatman No. 3). Plant extract dilution (1 g/100 ml sterile distilled water) (2 ml) was added to each Petri dish at the start of the experiment and 2 ml of sterile water was added to the control treatment. A further 1 ml of extract (water for control) was added after three days. Each treatment was replicated three times. Petri dishes were incubated at 25° C under illumination for seven days after which the percentage of seeds that had germinated was determined. The experiment was conducted twice.

Damping-off bioassay. Twenty milliliters of pathogen inoculum, was added to pots containing 200 g of growth medium (2 soil : 1 vermiculate) in pots and incubated for five days. Five seeds of each indicator plant (tomato, cabbage and amaranthus) were planted in each pot and pots were treated with extracts of *R. prinoides*, *L. sericea*, *A. afra* and *M. azedarach*, repectively. Plant extracts 100 ml (1 g powder / 100 ml sterile distilled water) were added to each pot. Five ml of dichlorophen was dissolved in 2.5 liters (0.2/1ml) tap water and 100 ml of this was used to treat infested pots. Treatment of the pots with plant extract and dichlorophen was repeated after one week with the same concentrations. Each treatment was replicated four times. Disease severity was determined after three weeks. The experiment was conducted twice and data were pooled.

Inoculum survival. Cultures of *R. solani* and *F. oxysporum* were grown in a potato dextrose broth and *P. ultimum* in a corn meal broth in a vibrating incubator at room temperature for seven days. Inoculum was homogenized in a Waring blender for I min and 10 ml of the inoculum suspension was added to 100 g of loam soil sifted through a 2 mm sieve. The plant extract emulsion (10 ml of 1 g plant extract + 100 ml sterile distilled water) was mixed with the pathogen-infested soil in plastic bags by shaking vigorously to distribute the inoculum evenly. The treated soil was placed in sterile 400 ml beakers, covered with alluminium foil, and incubated at 25° C for 3 months.

The density of *R. solani*, *F. oxysporum* and *P. ultimum* inoculum in each treatment was determined using the dilution plate technique (before treatment, and at 1, 3, 7, 14 and 21 days after soil treatment). Twenty five

grams of soil treated with the plant extract and pathogen, were placed in 100 ml sterile distilled water and stirred vigorously. Ten milliliters was taken from each soil suspension and transferred to 90 ml sterile water. The dilution was repeated ten times and one milliliter from the last dilution was transferred to a Petri dish containing a selective medium for the respective pathogen. For the isolation of *P. ultimum,* a selective medium consisting of 5 mg pimaricin, 300 mg vancomycin hydrochloride, 100 mg PCNB, 10 mg rose bengal, 20 g sucrose, 1 mg $ZnCl_2$ 0.02 g of $CuSO_4.5H_2O$, MoO_3, MnCl2 and $FeSO_4.7H_2O$, 10 mg $MgSO_4.7H_2O$, 1g K_2HPO_4, and 17 g corn meal agar (CMA) (Difco) in 1000 ml sterile distilled water was used (Mircetich, 1971). The *R. solani* medium consisted of 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 10 mg $FeSO_4.7H_2O$, 0.2 g $NaNO_2$, and 20 g agar in 1000 ml sterile distilled water. The following ingredients were added after autoclaving for 20 minutes at 121° C, 0.4 g gallic acid, 90 mg Dexon, 50 mg chloramphenicol and 50mg streptomycin sulphate (Novostrep). For the isolation of *F. oxysporum,* the medium consisted of 10 g glycerine, 1 g urea, 0.5 g L-alanine, 1 g PCNB, 0.5 g rose bengal, 15 g agar in 1000 ml sterilised distilled water to which, 50 mg of streptomycin was added after autoclaving for 20 minutes at 121° C. Control treatments were not treated with plant extracts or dichlorophen. Each treatment was replicated three times. Plates were then incubated for 3 to 4 days at room temperature, and resulting colonies for each respective pathogen were counted. The experiment was conducted twice and an analysis of variance (ANOVA) was performed with the combined data after conducting Bartlett's test for homogeneity of variances. Means were separated using Duncan's multiple comparison tests.

RESULTS

Bioassay of antifungal ability in vitro. All plant extracts exhibited a certain degree of inhibition towards the three pathogens (Figure 1). Three species (*R. prinoides*, *L. sericea* and *A. afra*) showed a broad inhibitory effect on all three fungal pathogens, with *L. sericea* displaying the most fungitoxicity. The extract of *M. azedarach* was less inhibitory towards the three pathogens than the other three plants but nevertheless, displayed a significant level of inhibition ($P < 0.05$) towards mycelial growth of *R. solani* and *P. ultimum*, especially at higher concentrations (Figure 1A & B).

Pythium ultimum was 100 % inhibited by extracts of *L. sericea*, *R. prinoides* and *A. afra* at 0.1g/ml water. A 10 % concentration of these extracts also significantly ($P > 0.05$) inhibited the mycelial growth of *R. solani* (Figure 1B). The same concentration also significantly suppressed mycelial growth of *F. oxysporum* (Figure 1C). All plant extracts and dichlorophen used were highly inhibitory towards mycelial growth of *F. oxysporum* (Figure 1& 2). Extracts generally had a significantly ($P < 0.05$) higher degree of inhibition to *P. ultimum* and *R. solani* than did the dichlorophen. There was however no significant difference ($P < 0.05$) between the level of inhibition shown by extracts and the fungicide towards *F. oxysporum,* at all concentrations (Figure 2).

Bioassay of antifungal ability in vivo. The treatment of pathogen infested soil with plant extracts resulted in a significant ($P < 0.01$) reduction of pathogen inoculum (Table 2). Extracts of *A. afra* and *R. prinoides* significantly reduced the inoculum of *R. solani*, while the effect of *L. sericea* was intermediate (Figure 3). Soils treated with the extract of *M. azedarach*

showed no decrease in *R. solani* inoculum. This plant however had a significant inhibitory effect on the inoculum density of *P. ultimum* (Figure 3). *Pythium ultimum* was highly sensitive to extracts of A. *afra*, *R. prinoides* and *L. sericea*. The recovery of *P. ultimum* from fungicide treated soil was very low compared to soil treated with plant extracts.

A progressive reduction in viable inoculum in relation to the length of the incubation period in plant extract soil was observed. Even though pathogen inoculum began to decline after one day, the most significant reduction was observed after seven days. After 14 days of incubation, the inoculum density of the three pathogens began to increase, but still remained significantly ($P < 0.01$) less than in untreated soil. This effect was more obvious in extracts of *L. sericea*, *R. prinoides* and *A. afra* (Figure 3). Inoculum of *P. ultimum* and *R. solani* in *A. afra* treated soil was significantly ($P < 0.01$) lower than the inoculum of these fungi in untreated soil, 21 days after incubation. The treatment of soil with dichlorophen significantly reduced the inoculum of *R. solani*, *P. ultimum* and *F. oxysporum* although differences in terms of susceptibility were observed between the pathogens.

Phytotoxicity test. Extracts of *R. prinoides*, *A. afra*, *L. sericea* and *M. azedarach* generally did not affect the germination of seeds (Table 1) and there was generally very little difference between treated seeds and control treatments. One exception was the decrease in germination percentage for amaranthus seeds treated with extract of *A. afra* where only 30% of seeds germinated. *Artemisia afra* however, did not suppress the germination of tomato and cabbage seeds where the germination percentage was between 90-100% (Table 1).

Damping-off bioassay. The treatment of pathogen infested soil with the three plant extracts resulted in a significant ($P< 0.05$) difference in the number of diseased plants between treatments (Table 2). The extracts of *R. prinoides*, *A. afra* and *L. sericea* significantly reduced the number of diseased seedlings in soil infested with *R. solani*, *P. ultimum* and *F. oxysporum* compared to untreated, control treatment (Figure 4). There were no differences between plants treated with *R. prinoides* and *A. afra* with regard to the number of diseased plants. The treatment of pathogen-infested soil with the extract of *L. sericea* resulted in the lowest number of diseased seedlings followed by *R. prinoides* and *A. afra*.

The three pathogens also reacted in a significantly ($P < 0.05$) different way to the four tested plant extracts and many interactions were obvious (Table 3). *Pythium ultimum* was highly suppressed by *A. afra* for all three plants (tomato, cabbage and amaranthus), while the extract of *M. azedarach* did not significantly suppress this pathogen. The effect of *M. azedarach* was more suppressive towards *F. oxysporum* in cabbage seedlings. In tomato seedlings, *R. solani* induced damping-off was highly suppressed by the *A. afra* extract, while in amaranthus seedlings it was most suppressed by *R. prinoides*. Significant disease suppression was obtained with dichlorophen in *R. solani* and *F. oxysporum* infested soil. However, this suppression was not significantly different from any plant extract. The treatment of infested soil with dichlorophen did not significantly ($P>0.05$) suppress *P. ultimum* damping-off in all three plants. Suppression of this pathogen by dichlorophen was significantly lower ($P < 0.05$) when compared to its suppression induced by plant extracts.

DISCUSSION

In this investigation, crude methanol extracts of *L. sericea, R. prinoides*, *A. afra* and *M. azedarach* were shown to exhibit strong antifungal activity towards *R. solani, P. ultimum* and *F. oxysporum*. Suppression of disease severity varied according to extracts and the environment in which they were applied. For example, *M. azedarach* did not inhibit the growth of *F. oxysporum in vitro*, but significantly suppressed *Fusarium* root rot. The concentration of plant extracts and dichlorophen played a significant role in the degree of mycelial growth inhibition with the greatest level of inhibition obvious at higher concentrations (Figures 1 & 2).

Botanical extracts can be used successfully to control fungal diseases of crops either in combination or without fungicides (Ushiki *et al.*, 1996; Bowers and Locke, 2000). Understanding the effect of any plant extract on the target pathogen population after a certain time is important for determining whether repeated applications are necessary. In our study, populations of all three pathogens were lowest at 1-7 days, but showed an increase after 14-21 days. This suggests that repeated applications of extracts would be necessary after 21 days. The degree of inhibition of *F. oxysporum*, *R. solani* and *P. ultimum* shown by leaf extracts of *A. afra* and *R. prinoides* makes them highly suitable for controlling diseases of vegetables caused by these pathogens.

Extracts of *R. prinoides*, *A. afra*, *L. sericea* and *M. azedarach* suppressed damping-off of vegetable seedlings caused by *R. solani*, *P. ultimum* and *F. oxysporum* and also reduced pathogen inoculum in treated soil. However, these results suggest that the plant extracts possess a general

biocidal effect, which can also be detrimental to other organisms in the soil. This broad spectrum effect might not be desirable should these extracts kill beneficial organisms in the soil. Other plant extracts, which have been reported to possess general biocidal effect, are those derived from pepper and mustard. According to Bowers and Locke (2000), extracts obtained from these plants were able to kill other soil microorganisms when applied to soil to suppress *F. oxysporum*. In the absence of competitive microorganisms, *F. oxysporum* colonized the soil more readily resulting in a greater disease severity (Bowers and Locke, 2000).

With respect to the results obtained in present study, the four tested plant extracts could readily be used by farmers for the management of vegetable seedling diseases as an alternative to dichlorophen or other fungicides. However, several parameters need to be developed for their effective use in crop production. This includes delivery method, appropriate formulations for delivery and soil type, delivery rate, as well as cultural practices and economic factors involved in a particular cropping situations (Bowers and Locke, 2000). Alternatives such as the use of plant extracts in combination with other crop protection practices should also be investigated. For example, biological agents have effectively been used in combination with extracts of pepper/mustard to suppress *F. oxysporum* (Bowers and Locke, 2000).

Extracts of *L. sericea*, *R. prinoides* and *A. afra* displayed greater suppression of the three pathogens than dichlorophen in laboratory experiments. This phenomenon was also observed by Awuah (1994) on the suppression of *Phytophthora palmivora* with *O. gratissium* and Kocide 101.

Extract of *O. gratissimum* gave better results than copper hydroxide in the control of black pod disease of cocoa caused by this fungus. Although dichlorophen gave better overall suppression of the three pathogens in this study, it is not a viable alternative due to its high cost, broad-spectrum toxicity and phytotoxicity. The possibility of pathogens developing resistance to systemic fungicides are also very high, especially when applied injudiciously.

Most fungicides used in the control of soilborne plant pathogens are non-degradable and produce adverse effect on beneficial soil microorganisms (Kumar and Tripathi, 1991; Ushiki *et al.*, 1996). Amending soil with plant extracts to control soilborne diseases without adverse effects has been successfully demonstrated (Singh and Mehrotta, 1980; Awuah, 1994).

There are no reports on the use of extracts of *R. prinoides*, *L. sericea*, *A. afra* and *M. azedarach* in controlling fungal pathogens. In the present investigation, damping-off of cabbage, tomato and amaranthus seedlings was successfully suppressed by treating infested soil with leaf extracts of these four plants. These plants grow extensively in Lesotho, and crude extracts in water are commonly used by farmers mainly for foliar application and little or no data exists on their activity in soil, especially on pathogens. Our results show that methanol extracts from, *R. prinoides*, *L. sericea* and *A. afra*, are very effective in the control of *R. solani*, *F. oxysporum* and *P. ultimum*. Further investigations are however, necessary to determine the possible interaction of the same extracts with microbial populations in the soil as well as on crops. Many plants extracts have volatile components and essential oils, which are responsible for pathogen growth suppression (Bowers and Locke, 2000). The relative efficiency of crude extracts in water and those

prepared in methanol such as in the present study should also be compared. Research to determine the biologically active chemical ingredients of these plant extracts responsible for pathogen suppression, should also receive more attention.

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Table 1. Effect of plant extracts on seed germination.

Extract	Seed germination (%)		
	Tomato	Cabbage	Amaranthus
L. sericea	100a[a]	97a	90a
R. prinoides	83a	90a	93a
A. afra	93a	80a	33b
M. azedarach	93a	96a	93a
Control	100a	90a	96a

[a] Values followed by different lowercase letters are significantly different ($P < 0.05$) for each main treatment.

Table 2. Analysis of variance for disease severity in soils treated with plant extracts and dichlorophen

Source	*df*	*F-Ratio*	*P>F*
Plant	3	0.88	0.4829
Treatment[a]	5	11.15	0.0008*
Plant x Treatment	15	1.66	0.1594
Pathogen	3	0.36	0.7014
Pathogen x Plant	9	1.33	0.2639
Treatment x Pathogen	15	2.09	0.0316*
Plant x Treatment x Pathogen	45	0.46	0.9744

*Term significant at P = 0.05.

[a]Treatments included extracts of *R. prinoides, L. sericea, A. afra* and *M. azedarach*, and dichlorophen

Table 3. Pathogen populations in soil treated with plant extracts and dichlorophen

Treatment	Pathogen populations[z]		
	P. ultimum	*R. solani*	*F. oxysporum*
L. sericea	7.7a	8.3a	11.5a[w]
R. prinoides	7.7a	5.6b	8.5b
A. afra	6.5b	8.5a	7.1b
M. azedarach	8.5a	8.3a	7.2b
Dichlorophen	7.1a	2.2c	2.0c
Untreated (control)	16.8c	16.8d	20.3d

Source of variation	*df*	*F- Ratio*	*P>F*
Treatment	5	56.03	0.0001
Pathogen	3	1.54	0.2279
Treatment x pathogen	15	2.94	0.0085

[w] Mean values in the same column followed by the same letter are not significantly different at P = 0.05 based on Newmans- Kewls comparison test.

[z] Pathogen populations were determined 14 days after planting.

Figure 1. Growth inhibition *in vitro* of *R. solani* (A), *P. ultimum* (B) and *F. oxysporum* (C) by extracts of *L. sericea*, *R. prinoides*, *A. afra* and *M. azedarach*.

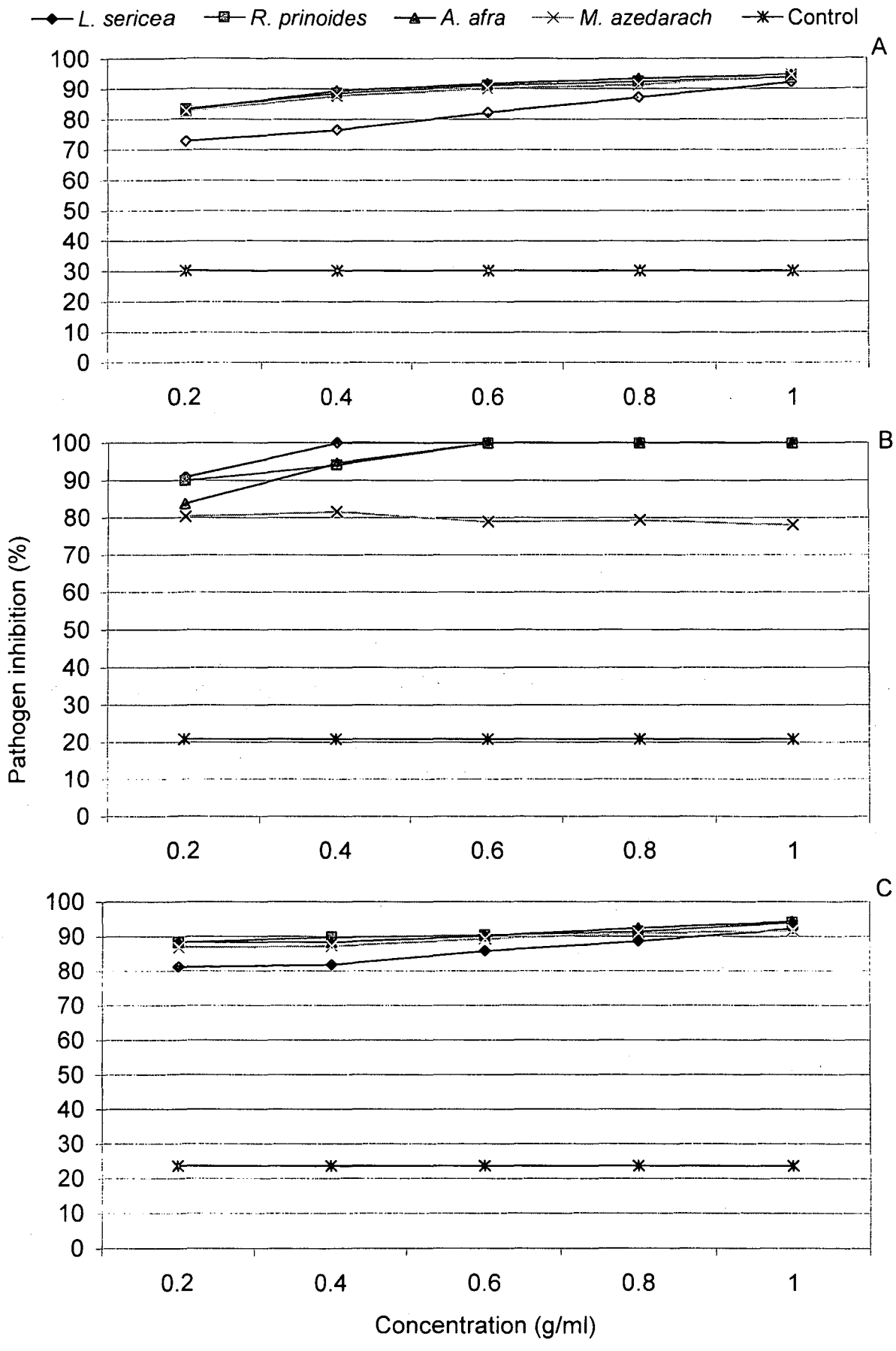

L. sericea
R. prinoides
A. afra
M. azedarach
Control
A
B
C
Pathogen inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
0.2
0.4
0.6
0.8
1
Concentration (g/ml)

Figure 2. Growth inhibition *in vitro* of *R. solani*, *P. ultimum* and *F. oxysporum* with dichlorophen.

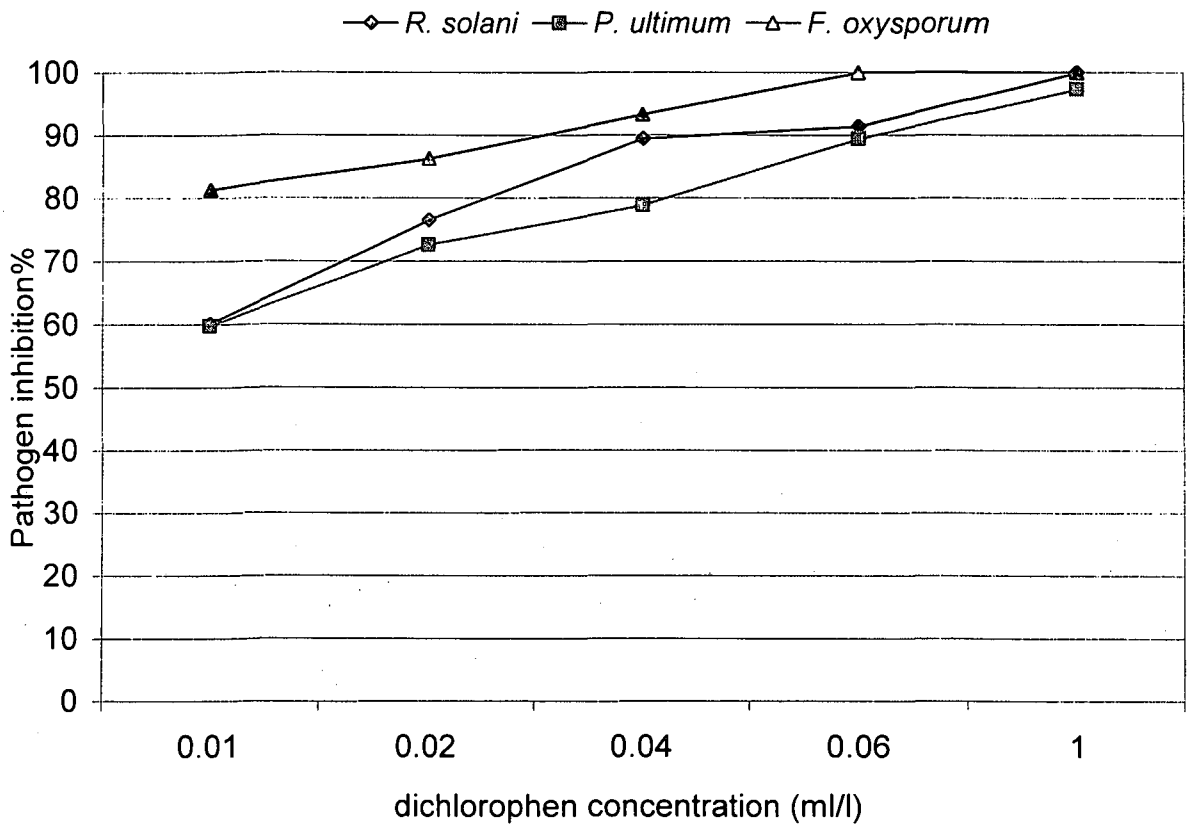

R. solani
P. ultimum
F. oxysporum
100
90
80
70
60
50
40
30
20
10
0
Pathogen inhibition%
0.01
0.02
0.04
0.06
1
dichlorophen concentration (ml/l)

Figure 3. Inhibitory effect of extracts of *L. sericea*, *A. afra*, *R. prinoides* and *M. azedarach*, and dichlorophen on the population densities of *R. solani*, *P. ultimum* and *F. oxysporum* in artificially inoculated soil. Bars denoted by different letters are significantly ($P < 0.05$) different for each treatment.

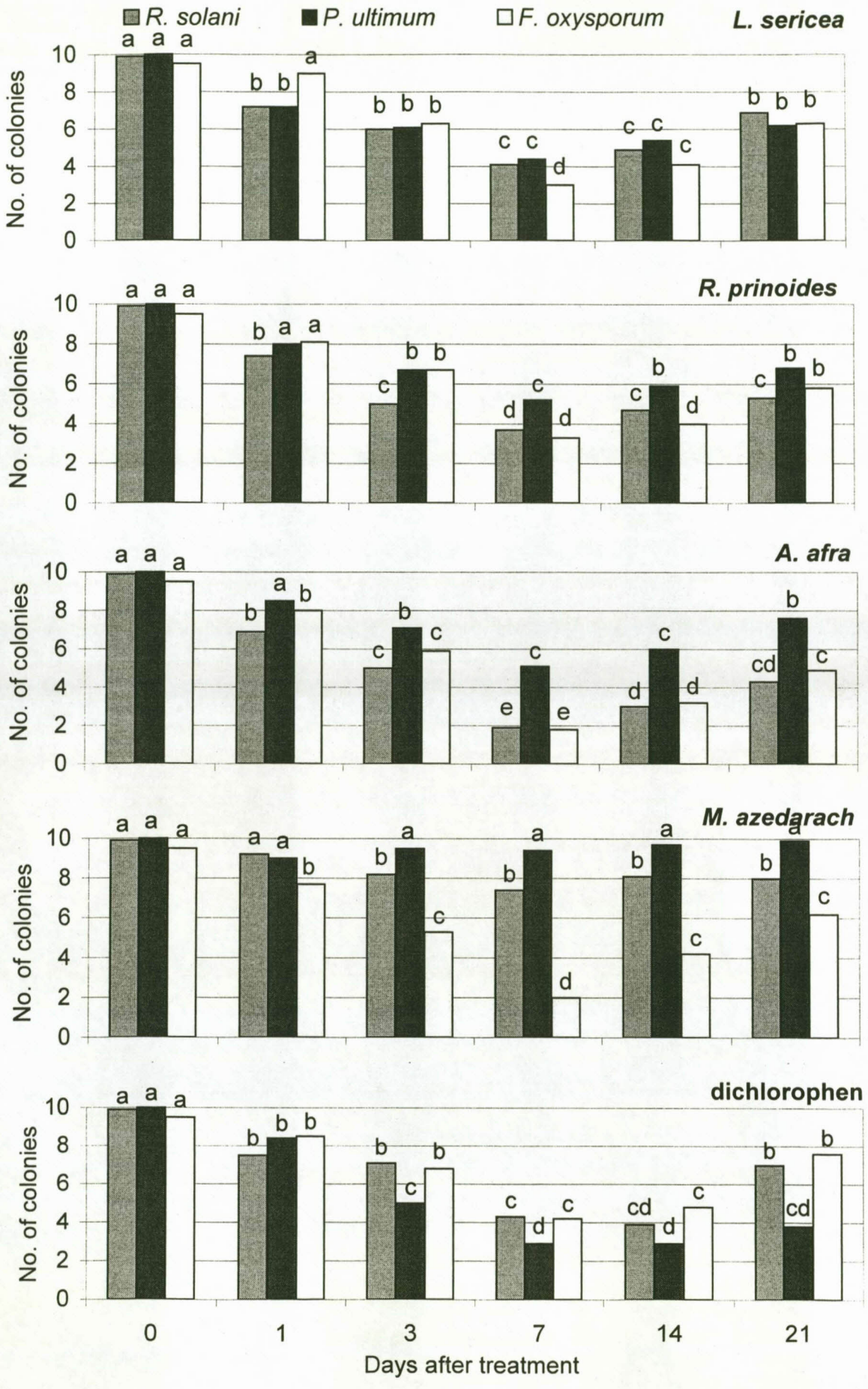
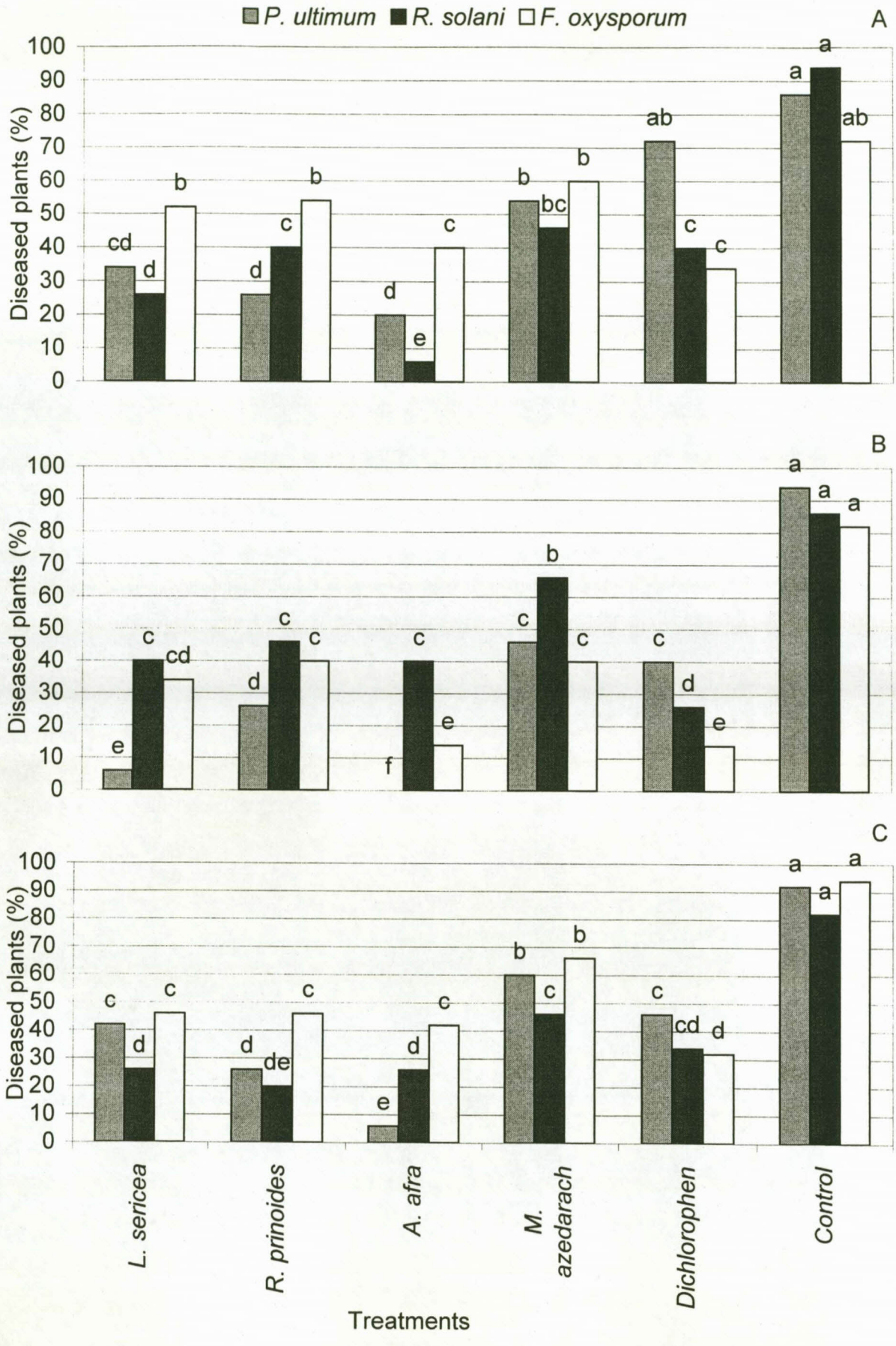

R. solani
P. ultimum
F. oxysporum
L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
No. of colonies
Days after treatment
0
1
3
7
14
21

Figure 4. Incidence of damping-off of tomato (A), cabbage (B) and amaranthus (C) seedlings caused by *R. solani*, *P. ultimum* and *F. oxysporum* in soil treated with extracts of *L. sericea*, *R. prinoides*, *A. afra* and *M. azedarach*, and dichlorophen. Bars denoted by different letters are significantly ($P < 0.05$) different for each treatment.


P. ultimum
R. solani
F. oxysporum
A
B
C
Diseased plants (%)
100
90
80
70
60
50
40
30
20
10
0
cd
d
b
d
c
b
d
e
c
b
bc
b
ab
c
c
a
a
ab
e
c
cd
d
c
c
f
e
c
b
c
c
d
e
a
a
a
c
d
c
d
de
c
e
d
c
b
c
b
c
cd
d
a
a
a
L. sericea
R. prinoides
A. afra
M. azedarach
Dichlorophen
Control
Treatments

CHAPTER FIVE

EFFECT OF PLANT EXTRACTS ON MICROBIAL ACTIVITY AND SEEDLING DAMPING-OFF SEVERITY IN COMPOSTED ANIMAL MANURE

ABSTRACT

Extracts of *Rhamnus prinoides*, *Leucosidea sericea*, *Artemisia afra* and *Melia azedarach* were tested for their effect on microbial activity in composted cattle, pig, poultry and sheep manure. The four plant extracts were also tested for their effect on damping-off of *Amaranthus hybridus* seedlings caused by *Rhizoctonia solani*, *Fusarium oxysporum* and *Pythium ultimum* in composted animal manure. All tested plant extracts significantly reduced the number of fungal colonies in all four composted manures although *M. azedarach* showed less inhibitory effect on fungal populations in composted poultry and pig manure. Reduction was generally more evident after 14 and 21 days of treatment. Microbial activity was suppressed by all four plant extracts in composted cattle, poultry and pig manure. Treatment of the four composted animal manures with dichlorophen also resulted in a significant reduction in the number of fungal colonies and general microbial activity. In a damping-off severity bioassay, all plant extracts suppressed disease and the highest level of disease suppression was obtained in cattle, pig and poultry manure. Composted sheep manure had the highest incidence of damping-off. Treatment of composted animal manure with dichlorophen resulted in the same level of reduction of damping-off as observed for plant extracts.

INTRODUCTION

The application of composted or non-composted animal manure as a soil amendment is a farming practice commonly used by farmers in Lesotho (King, 1989). Numerous reports show that manure has a significant effect on the microbial composition and fertility of soil (Cook and Baker, 1983; Workneh and van Bruggen, 1993; Scholtze and Lootsma, 1998). Compost is an excellent soil conditioner, which can provide nutrients to plants in an easily available form. Composts are known to improve the physical and chemical structure of soil and generally enhance the activity of beneficial soil microorganisms (Inbar *et al.*, 1991). Soilborne diseases can also be suppressed successfully by the addition of composted materials (Hoitink and Fahy, 1986; Alvarez *et al.*, 1995; Klamer, and Søchting, 1998), the effect being mainly due to increased microbial activity, which competes with pathogens (Hoitink and Fahy, 1986; Scholtze and Lootsma, 1998).

The use of natural products for the control of fungal diseases is considered an interesting alternative to synthetic fungicides due to their potentially lower negative impact on the environment (Kaushal and Paul, 1989; Awuah, 1994; Wilson *et al.*, 1997). Numerous plant extracts have been shown to be effective in suppressing soilborne plant pathogens (Dubey and Kishore, 1987; Kishore *et al.*, 1989; Shimoni *et al.*, 1993, Awuah, 1994; Pandey and Bhargava, 1994; Muller-Riebau *et al.*, 1995). The use of plant extracts is a practice used traditionally by farmers in Lesotho to control insect pests (May, 2000). Farmers in Lesotho also report low incidences of plant diseases in fields treated with plant water extracts. *Rhamnus prinoides* L. Herit, *Leucosidea sericea* Eckl. & Zeyh. and *Artemisia afra* Jacg. ex Willd in

particular, are used in Lesotho for medicinal purposes and pest control (May, 2000). *Melia azedarach* L., which is also used in other African countries to combat human ailments, plant pests and diseases, grows extensively as an exotic plant in Lesotho. Crude plant extracts possess a wide variety of antimicrobial compounds which are responsible for the inhibition of plant pathogens in soil (Kumar and Tripathi, 1991; Rao and Alvarez, 1992; Awuah, 1994; Dixon and Walsh, 1998; Bowers and Locke, 2000).

It is commonly acknowledged that the microbial activity of composted organic waste is responsible for the suppression of soilborne pathogens in amended growth media (Hadar *et al.*, 1992; Craft and Nelson, 1996). The presence of fungi such as *Trichoderma* spp. and *Gliocladium* spp. in soil growth media amended with certain composted materials is said to suppress plant diseases caused by *Fusarium* spp, *Rhizoctonia solani* Kuhn and *Pythium* spp. (Alabouvette *et al.*, 1993; Ben-Yephet and Nelson, 1999). Microbial activity in composts can be determined by examining the concentration of soil microorganisms by means of a technique which involves the hydrolysis of fluorescein diactate (FDA). FDA, a non-fluorescent substrate, is hydrolyzed by various enzymes of living organisms and yields fluorescein, which is visible using fluorescence microscopy (Inbar *et al.*, 1991).

The use of plant extracts and composts in combination is a frequent measure employed by farmers in Lesotho and else where in Africa as a means for pest control and soil improvement respectively. At present, there are no reports on the effects of plant extracts on the microbial activity of soil when used in combination with composted animal manure. It is, however,

strongly suspected that populations of beneficial microorganisms in manure can be suppressed by the addition of some plant extracts. Some plant extracts have for example, been reported to possess general biocidal effects when applied to soil as a fumigant to control *Fusarium oxysporum* Schlecht. (Bowers and Locke, 2000).

The objective of this research was firstly, to evaluate the four above mentioned plant extracts for their effect on the total fungal population and general microbial activity in composted animal manure. A second objective was to examine the effect of the same plant extracts on damping-off severity caused by *Pythium ultimum* Trow, *R. solani* and *F. oxysporum* in composted cattle, poultry, pig or sheep manure.

MATERIALS AND METHODS

Preparation of plant extracts. Healthy, fresh leaves of *R. prinoides, L. sericea*, *A. afra,* and *M. azedarach* were collected from different locations in Lesotho during the summer of 1999. The leaves were oven dried at 40° C and then finely ground with a grinder. The resulting powder was stored at 5° C prior to use. Plant material from each plant was placed in a 1 liter bottle and soaked with 500 ml methanol. The bottles were then tightly sealed and placed on a rotary shaker for 12 hours. The methanol extract was evaporated from the plant material in a Rotavapor and the resulting precipitate vacuum dried. The powdery precipitate was stored at 4° C. A plant extract solution of each plant was prepared by dissolving 1 g powdery extract in 100 ml of sterile distilled water.

Fungal bioassay. Fungal populations in composted animal manure and field soil treated with a plant extract or the fungicide (dichlorophen 200 g/l) were enumerated by dilution plating technique (before treatment, and at 1, 3, 7, 14 and 21 days after compost and soil treatment). One gram of composted cattle, poultry, pig and sheep manure, and field soil respectively, was added to test tubes containing 100 ml of either plant extract solution or fungicide and vigorously agitated. The test tubes were closed and stored at 25° C for the duration of the experiment (21 days). Ten milliliters was taken from each soil/ compost suspension and transferred to 90 ml sterile water. The dilution was repeated ten times and one milliliter from the last dilution was aliquoted onto six plates per treatment, containing water agar (WA) amended with 300μg/ml streptomycin sulphate (Novostrep). Plates were then incubated for 48 hours at room temperature, after which developing fungal colonies were counted. Distilled sterile water was used for the control treatment instead of a plant extract or dichlorophen. The experiment was repeated twice. The average population density for the four replicates of each treatment was calculated and data were pooled and subjected to an analysis of variance (ANOVA).

Inhibition of microbial activity. Microbial activity in composted animal manure and field soil treated with plant extracts or fungicide (dichlorophen) was determined by measuring the rate of hydrolysis of fluorescein diacetate (FDA), a technique modified by Schnurer and Rosswall (1982). FDA was dissolved in acetone (2.0 mg/ml) and stored as a stock solution at -20 ° C. One gram of each compost or soil sample was placed in a 250 ml Erlenmeyer flask containing 10 ml of either plant extract solution or

dichlorophen. Twenty milliliters of sodium phosphate buffer (pH 7.6) was added. The FDA stock solution was added (0.2 ml) to the mixture, which was then incubated for 1 hour in a rotary shaker (90 rpm) at 25° C. Each treatment was replicated three times. A control sample containing no FDA solution was used to correct background absorbance for each substrate treated with plant extract or fungicide. Compost residues were removed from the FDA-buffer mixture by means of filtration through filter paper (Whatman No.1). The filtrate was collected in a test tube, covered with Parafilm®, and placed in an ice bath for 30 minutes to reduce volatilization of the acetone. The concentration of free fluorescein after hydrolysis was determined after 30 minutes by reading the optical density at 490 nm using a spectrophotometer.

A standard curve was prepared for all composts treated with plant extracts or dichlorophen by adding various quantities of FDA (0 (control), 100, 200, 300, 400 µl) to 5 ml of phosphate buffer in screw cap bottles. Tubes were tightly closed and placed in a bath of boiling water for 60 min. After removing tubes and cooling for 10 min, quantities of hydrolyzed FDA were added to 250 ml Erlenmeyer flasks containing 0.5 ml of the plant extract and fungicide treated compost or soil. Additional 15 ml of phosphate buffer was used to wash excess fluorescein from the tubes into the samples. The flasks were placed to a rotary shaker for 20 min at 25 ° C after which 20 ml of acetone was added to the sample to stop the reaction. Treated compost and soil samples were then filtered (No 1 Whatman). The filtrate was then collected into test tubes, covered with Parafilm® and placed into an ice bath for 30 min to prevent acetone volatization. Each treatment was replicated four

times and the experiment was conducted twice. The concentration of free fluorescein in the filtrate was then determined as described above.

Damping-off study. Cultures of *R. solani* and *F. oxysporum* were grown in potato dextrose broth and *P. ultimum* in corn meal broth in a vibrating incubator at room temperature for 7 days. Twenty milliliters of pathogen inoculum (*R. solani*, *P. ultimum* and *F. oxysporum*) was added to 200 g of composted cattle, poultry, pig, sheep manure and soil (control) in pots and stored at room temperature for five days. Five seeds of tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* L.) and amaranthus (*Amaranthus hybridus* L.) were planted in pots individually infested with each pathogen. Pots were then treated with extracts of *R. prinoides*, *L. sericea*, *A. afra* and *M. azedarach*, and dichlorophen (200 g/l). Plant extracts were added as a 100 ml water emulsion per pot. Dichlorophen was diluted according to manufacturer's instruction (Plaaschem Pty Ltd). Treatment of the four composts with either plant extracts or dichlorophen was repeated after 1 week. Pots were irrigated regularly and percentage of seedlings showing damping-off symptoms was calculated after three weeks. The experiment was repeated twice and pooled data were subjected to an analysis of variance (ANOVA) after conducting Bartlett's test for homogeneity of variance; means were separated using Duncan's multiple range test. Data from FDA hydrolysis experiments, were analyzed by means of linear regression.

RESULTS

Fungal bioassay. The sensitivity of fungi in composted manure to the four plant extracts was evaluated by counting the number of colonies in plates after treatment of the compost with the respective extract. Poultry, cattle, pig and sheep manure composts treated with the four extracts varied considerably in terms of the number of fungal colonies that developed. A significant reduction in the number of fungal colonies was observed in all four composts treated with *R. prinoides*, *A. afra* and *L. sericea* compared to the control (Figs 1-4). In composted pig manure (Fig. 1) the highest reduction of fungal populations was observed for *A. afra* and *L. sericea* treatments followed by *R. prinoides*. *M. azedarach* was the least inhibitory extract, with very little difference observed between it and the control treatment. The fungicide, dichlorophen, was significantly ($P < 0.05$) more inhibitory than all four extracts and at all four periods after treatment.

In composted cattle manure, all plant extracts significantly reduced the number of fungal colonies compared to the control, after 7 and 21 days of treatment (Fig. 2). Significant interactions were observed. *M. azedarach*, in contrast, was generally the most inhibitory extract. *Artermisia afra* treatment was also, in contrast to pig manure, the least effective extract. Most inhibition was attributed to dichlorophen treatment at all tested times.

The treatment of composted poultry manure with the four plant extracts resulted in most inhibition being observed for *L. sericea* and *R. prinoides,* especially after 7 and 21 days (Fig. 3). In *A. afra* treatments, fungal populations began to increase after 21 days. *Melia azedarach* inhibited fungal colonies far less than the three other plant extracts. Colonies also started to

increase after seven days. The fungicide treatment in this compost again showed most inhibition of fungi especially after 7 and 14 days.

The treatment of composted sheep manure with fungicide or plant extracts resulted in very contrasting observations (Fig. 4). Firstly, there were generally far fewer colonies isolated than for the three other composted manures. The number of fungal colonies observed after treatment with *L. sericea* and *M. azedarach* was in most cases significantly ($P < 0.05$) more than that for the other two extracts. At 14 and 21 days after treatments, these two treatments yielded significantly more colonies than the control treatment.

Microbial activity. General microbial activity in composted manures, as measured by FDA hydrolysis absorbance at 490 nm varied significantly after treatment with all plant extracts and fungicide (Figs 5-8). Microbial activity in composted pig, cattle and poultry manure was significantly inhibited after treatment with plant extracts (Figs 5-7).

Microbial activity in pig and cattle manure composts was significantly inhibited by extracts of *L. sericea*, *R. prinoides* and *A. afra* after 14 days of treatment, followed by a slight increase in activity at 21 days (Figs 5 and 6). Treatment of both pig and cattle manure composts with the extract of *M. azedarach* had no effect on microbial activity (Figs 5 and 6). Fungicide treatment of both composts resulted in a significant ($P < 0.05$) decline in microbial activity.

In poultry manure (Figure 7) microbial activity was significantly ($P < 0.05$) reduced by extracts of *L. sericea* and *A. afra* at 7, 14 and 21 days. A significant reduction of microbial activity in *R. prinoides* was observed after 14 and 21 days of treatment. Microbial activity in this compost was not affected

by the extract of *M. azedarach*. Microbial activity was significantly lower ($P < 0.05$) in fungicide treatment than in the four plant extracts. In sheep manure *R. prinoides* and *A. afra* treatment showed low microbial activity after the first day of treatment and at after 14 days followed by a decline at 21 days.

In composted sheep manure, microbial activity was generally less than for the three other composts (Fig 8). A significant ($P < 0.05$) decline in microbial activity was observed after treatment with *A. afra* and *R. prinoides* at 7 days of treatment. *Leucosidea sericea* extract was more inhibitory after 21 days. Microbial activity in sheep manure was not affected by an extract of *M. azedarach*. The fungicide treatment resulted in significantly less microbial activity compared to all four plant extracts.

Damping-off study. Treatment of composted cattle, pig and poultry manure with extracts of *R. prinoides*, *A. afra* and *L. sericea* resulted in the significant suppression ($P < 0.05$) of damping-off caused by *R. solani*, *P. ultimum* and *F. oxysporum*. *Melia azedarach* gave contradictory results in all four treated composts. In pig manure, damping-off caused by the three pathogens was significantly suppressed by extracts of *L. sericea*, *R. prinoides* and *A. afra* (Fig 9). *Melia azedarach* was also able to reduce disease symptoms caused by all three pathogens to below the level displayed by dichlorophen. Treatment of *R. solani*, *P. ultimum* and *F. oxysporum* with dichlorophen did not reduce damping-off severity more than the control treatment.

In composted cattle manure (Fig 10), damping-off caused by *R. solani* was significantly ($P < 0.05$) suppressed by extracts of *L. sericea*, *A. afra* and *R. prinoides* compared to *P. ultimum* and *F. oxysporum* suppression.

Dichlorophen, however, gave the best suppression of *R. solani* with only 3 % of seedlings showing damping-off symptoms. *Ramnus prinoides* and dichlorophen generally gave the best suppression of disease for all three pathogens. Treatment of composted cattle manure with extract of *M. azedarach* resulted in an increased incidence of damping-off caused by *R. solani*, *P. ultimum* and *F. oxysporum* compared to the control.

Treatment of poultry manure compost with all four extracts and the fungicide resulted in significantly fewer diseased seedlings than the control treatment (Fig 11). The most suppression obtained was observed after treatment by *A. afra,* with *R. solani* being the most significantly ($P < 0.01$) suppressed. *Fusarium oxysporum* in poultry manure compost was suppressed more by extracts of *L. sericea* and *R. prinoides* than the fungicide. *Melia azedarach* also caused disease suppression almost equivalent to that observed for dichlorophen.

In sheep manure, damping-off severity was not at all suppressed by the addition of plant extracts (Fig. 12). There were no differences in damping-off severity between the four plant extracts, dichlorophen and the control. The highest incidence of damping-off was recorded where *P. ultimum* and *F. oxysporum* infested compost was treated with an extract of *R. prinoides*.

DISCUSSION

The presence of antifungal compounds in certain higher plants has long been recognized (Shimoni *et al.*, 1993, Awuah, 1994; Pandey and Bhargava, 1994). The amendment of container media with extracts from these plants in order to control soilborne plant diseases has also often been

demonstrated (Singh and Mehrotta, 1980; Kumar and Tripathi, 1991; Bowers and Locke, 2000). Plant extracts have also been used as seed treatments to prevent disease (Kumar and Tripathi, 1991).

In the present study, extracts of *L. sericea*, *R. prinoides*, *A. afra* and *M. azedarach* were tested for their inhibitory effects on the microbial activity of four composted animal manures. Damping-off severity induced by three fungal pathogens in the composts was also evaluated. The microbial activity of composted cattle, pig and poultry manure was significantly reduced after treatment with the four plant extracts. By comparing the number of fungal colonies isolated from different composts treated with each of the four plant extracts, it was possible to make some generalization concerning the biocidal effect of the extracts.

Although the absolute level and consistency with which the tested extracts reduced fungal populations varied, extracts of *A. afra*, *R. prinoides* and *L. sericea* were generally the most inhibitory to fungi. Amendment of growth media with certain plant extracts has previously also been shown to have a negative effect on soil microorganisms (Gruzdyev, 1983; Bowers and Locke, 2000). Bowers and Locke (2000) reported a general biocidal effect incited by extracts of pepper and mustard in *Fusarium* infested soil. *Reinoutria* spp. is reported to induce a rapid and abundant accumulation of glycosidically bound phenolics, which in turn, suppresses the growth of *R. solani* and *F. oxysporum* and other soil microorganisms (Daayf *et al.*, 1995). As expected, treatment of the four composted animal manures with dichlorophen resulted in a significant decrease of fungal propagules and microbial activity. Fungicides such as dichlorophen used to control soilborne

pathogens are generally nonselective and have a broad-spectrum activity (Awuah, 1994; Wilson *et al.*, 1997).

All tested plant extracts significantly suppressed disease development in composted animal manures compared to control treatments that did not contain extracts. Various factors have been proposed as being responsible for the suppression of plant pathogens in growth media treated with plant extracts (El-Gammal and Mansour, 1986; Kumar and Tripathi, 1991; Bowers and Locke, 2000). Disease suppression has been attributed to the volatile compounds that plant extracts contain (Kaushal and Paul, 1989; Awuah, 1994; Wilson *et al.*, 1997). For example, leaves of *Geranium* spp are reported to have flavonoids, which exhibit an inhibitory effect on the growth of *R. solani*, and *F. oxysporum* (El-Gammal and Mansour, 1986). The root extract of *S. officinalis,* which inhibits the growth of *F. oxysporum* and *R. solani in vitro* is reported to contain high amounts of tannin (Nonaka *et al.*, 1982), which is the source of pathogen inhibition and disease suppression (Ushiki *et al.*, 1996).

The treatment of composted cattle, poultry, pig and sheep manures with extracts of *M. azedarach* gave contradictory results. *Melia azedarach* was able to significantly reduce the number of fungal colonies in cattle manure but had no effect on fungal populations in pig, poultry and sheep manures. *Melia azedarach* was also able to suppress damping-off caused by *R. solani*, *P. ultimum* and *F. oxysporum* in poultry and pig manure but failed to incite the same suppression in cattle and sheep manure composts. In composted sheep manure, which initially displayed the lowest number of colonies before treatment with either plant extract or dichlorophen, fungal

colonies began to increase significantly after 14 and 21 days of treatment with *M. azedarach*. This suggests that the extract of *M. azedarach* had some stimulatory effect on fungi in sheep manure. Addition of some botanical extracts, e.g., seaweed to soil are reported to result in increased microbial activity and populations of some fungal species (Dixon and Walsh, 1998).

Plant extracts, when applied to soil or growth media, may alter the mode of activity of microorganisms in terms of whether they act as parasites, mutualists or as saprophytes. This may result in plant extracts when applied to soil altering, directly or indirectly, characteristics such as root penetration and colonization, competition, microbiostasis and antibiosis (Nonaka *et al.*, 1982; El-Gammal and Mansour, 1986; Ushiki *et al.*, 1996; Dixon and Walsh, 1998). Treatment of composts with plant extracts has resulted in increased activities of enzymessuch as, amylase, cellulase and glucanase (Dixon and Walsh, 1998). These enzymes induce systemic resistance in plant and promote rhizosphere bacteria (Linthorst, 1991).

The use of plant extracts and composted animal manure in combination is effective in controlling soilborne pathogens. However, general biocidal effects displayed by the four tested plant extracts especially of *R. prinoides*, *A. afra* and *L. sericea* can also have negative effects. Low suppression of damping-off in composted sheep manure even after treatment with plant extracts suggests that phytotoxicity was responsible for less seed germination and seedling death. It is therefore important to first determine what causes phytotoxicity of this compost before it can be used to suppress plant diseases either alone or in combination with plant extracts. In further studies, it is also important to test the inhibitory effects of plant extracts used

in this study on some biological control agents. This information is important to further assess the compatability of composted animal manures and plant extracts. Though the botanical extracts reduced fungal populations as well as suppressing microbial activity, thus creating a vaccum for pathogens to colonize, they are probably still better alternatives than fungicides to farmers in Lesotho due to their affordability.

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Figure 1. Number of fungal colonies isolated from composted pig manure after treatment with four plant extracts and dichlorophen. Each datum point is the mean of eight replications. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

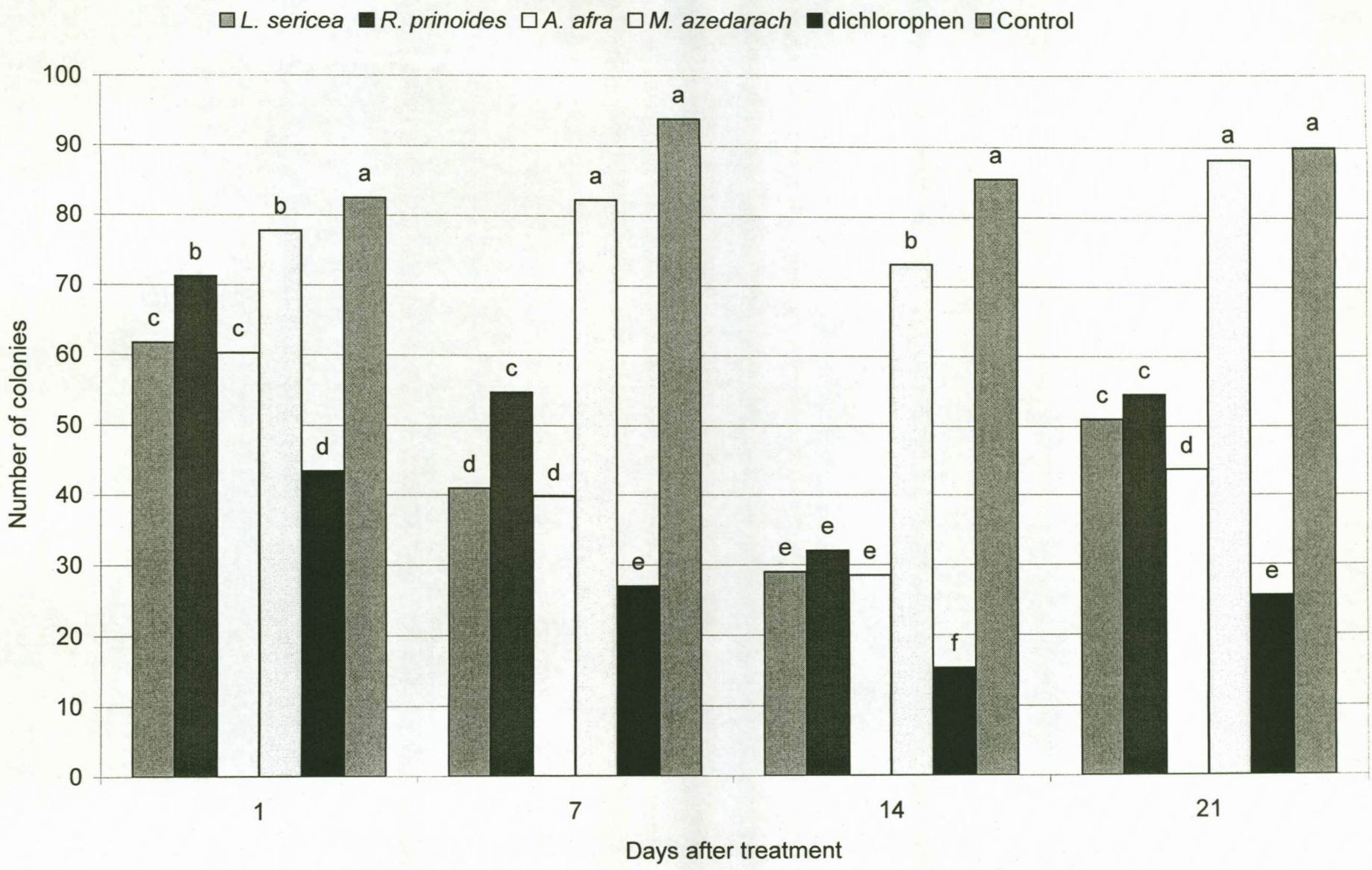

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Number of colonies
100
90
80
70
60
50
40
30
20
10
0
1
7
14
21
Days after treatment

Figure 2. Number of fungal colonies isolated from composted cattle manure after treatment with four plant extracts and dichlorophen. Each datum point is the mean of eight replications. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

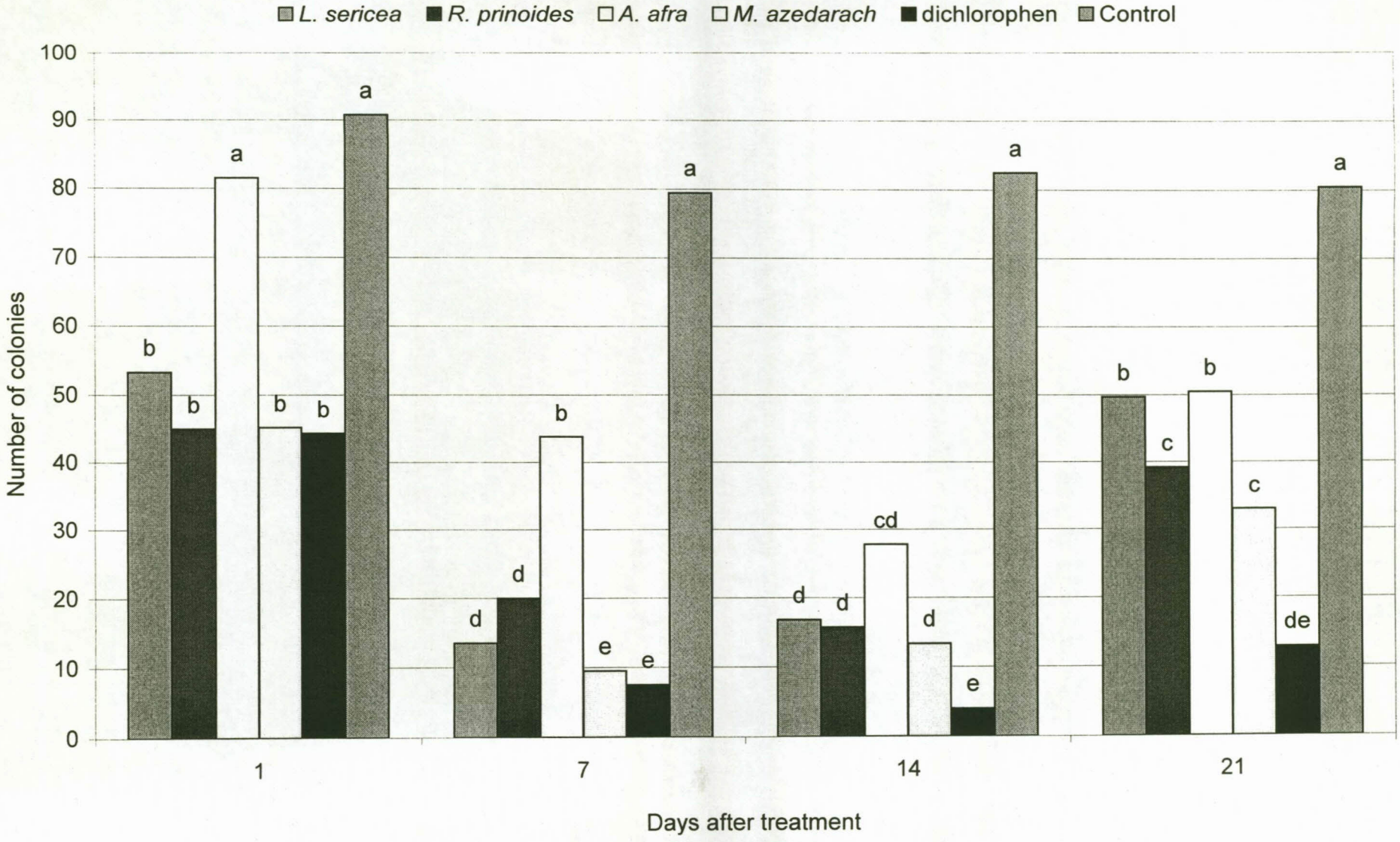

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Number of colonies
100
90
80
70
60
50
40
30
20
10
0
1
7
14
21
Days after treatment
b
b
a
b
b
a
d
d
b
e
e
a
d
d
cd
d
e
a
b
c
b
c
de
a

Figure 3. Number of fungal colonies isolated from composted poultry manure after treatment with four plant extracts and dichlorophen. Each datum point is the mean of eight replications. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

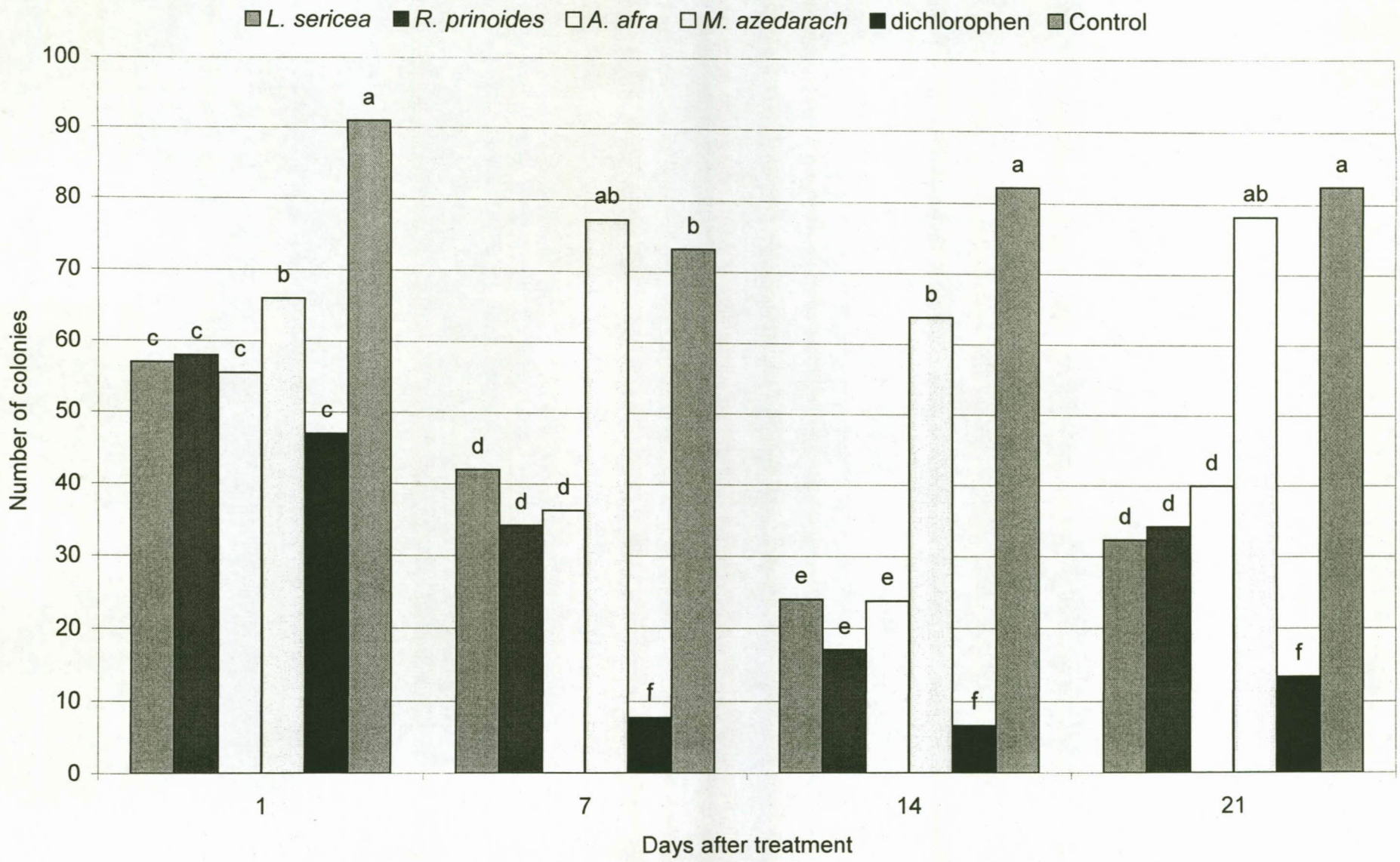

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Number of colonies
0
10
20
30
40
50
60
70
80
90
100
1
7
14
21
Days after treatment
c c c b c a
d d d ab f b
e e e b f a
d d d ab f a

Figure 4. Number of fungal colonies isolated from composted sheep manure after treatment with four plant extracts and dichlorophen. Each datum point is the mean of eight replications. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

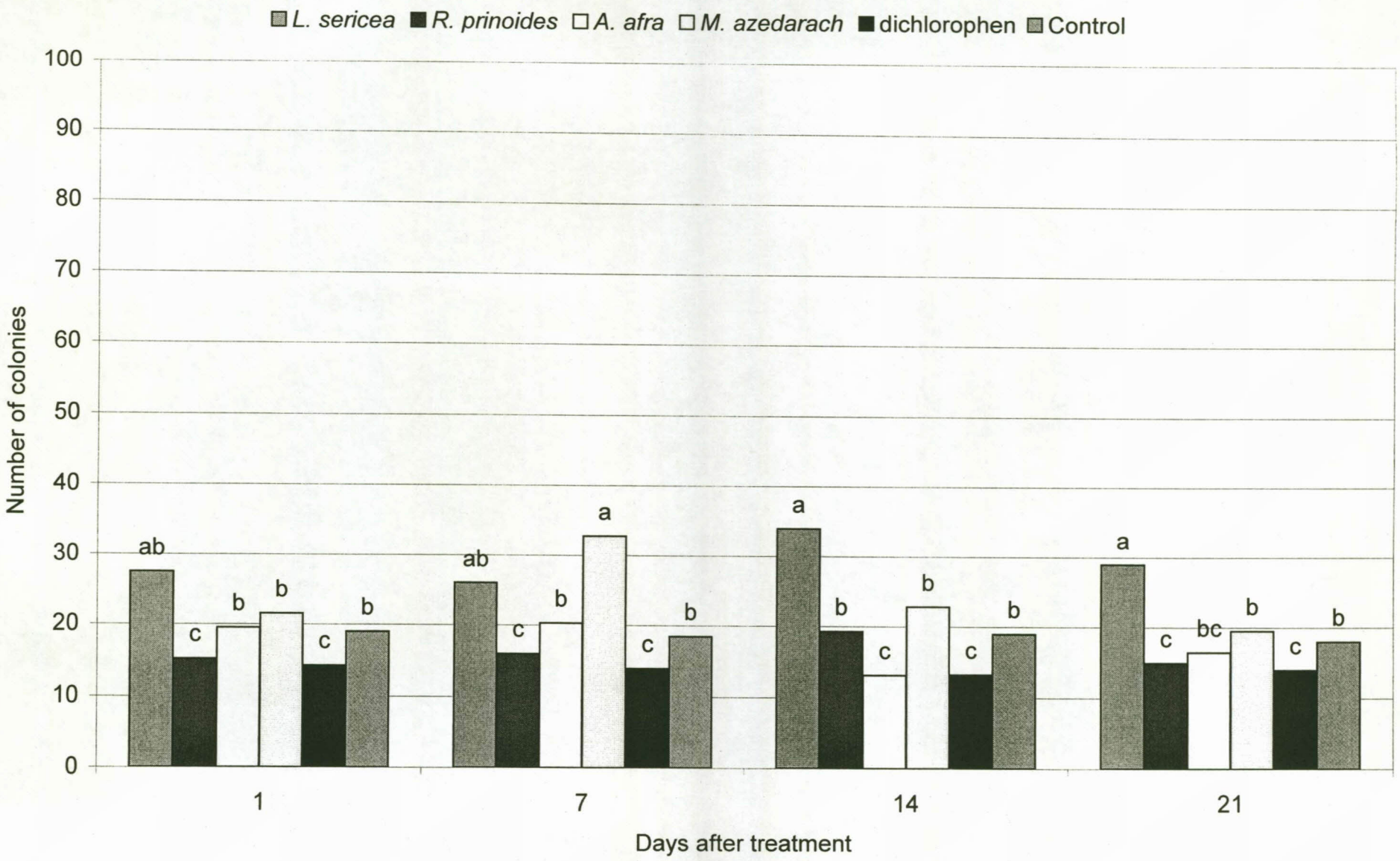

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Number of colonies
100
90
80
70
60
50
40
30
20
10
0
ab
c
b
b
c
b
ab
c
b
a
c
b
a
b
c
b
c
b
a
c
bc
b
c
b
1
7
14
21
Days after treatment

Figure 5. Microbial activity in composted pig manure treated with plant extracts or dichlorophen expressed as a percentage of initial microbial activity before addition of extract or fungicide. Each datum point represents a mean of eight replications per treatment.

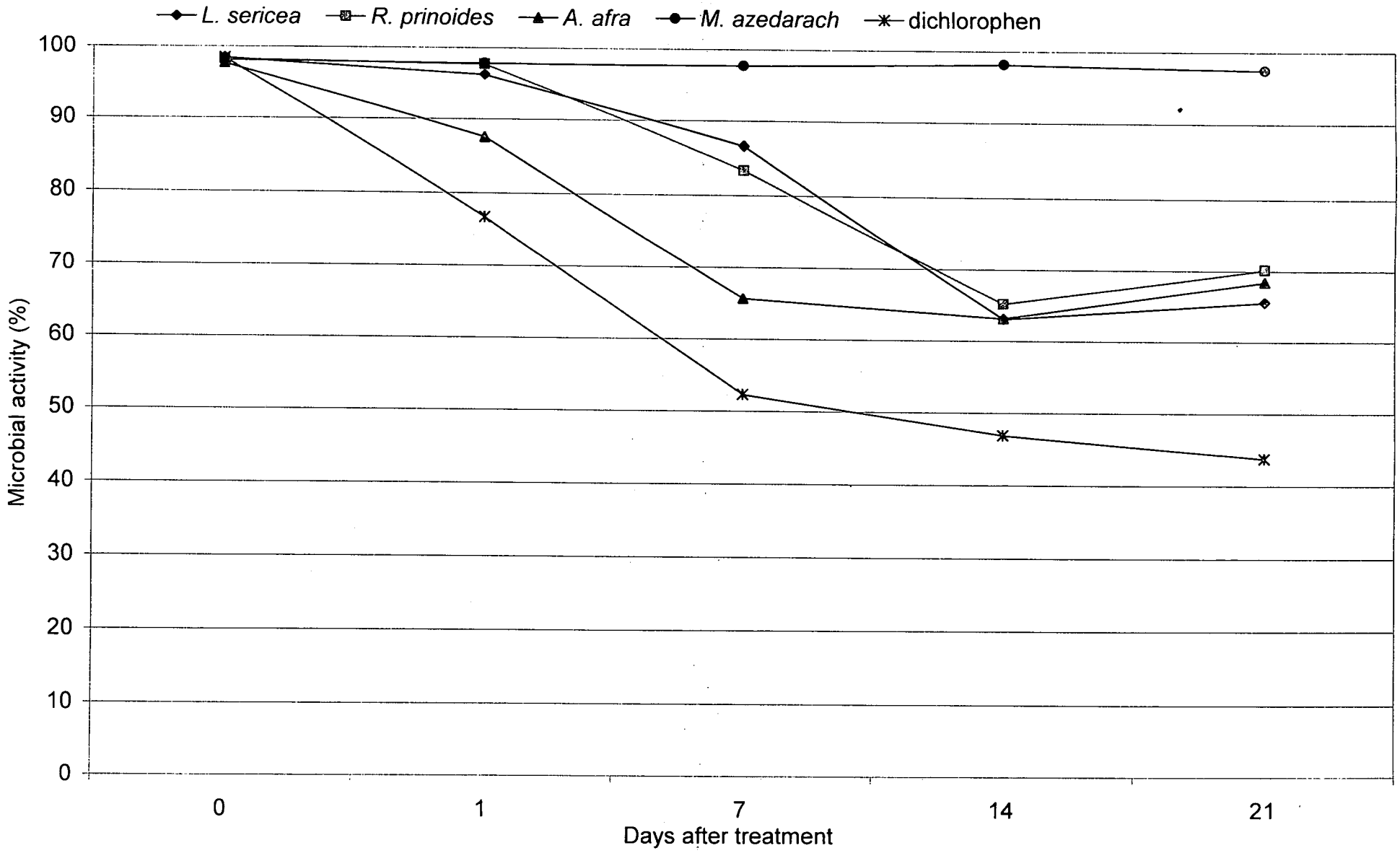

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
100
90
80
70
60
50
40
30
20
10
0
Microbial activity (%)
0
1
7
14
21
Days after treatment

Figure 6. Microbial activity in composted cattle manure treated with plant extracts or dichlorophen expressed as a percentage of initial microbial activity before addition of extract or fungicide. Each datum point represents a mean of eight replications per treatment.

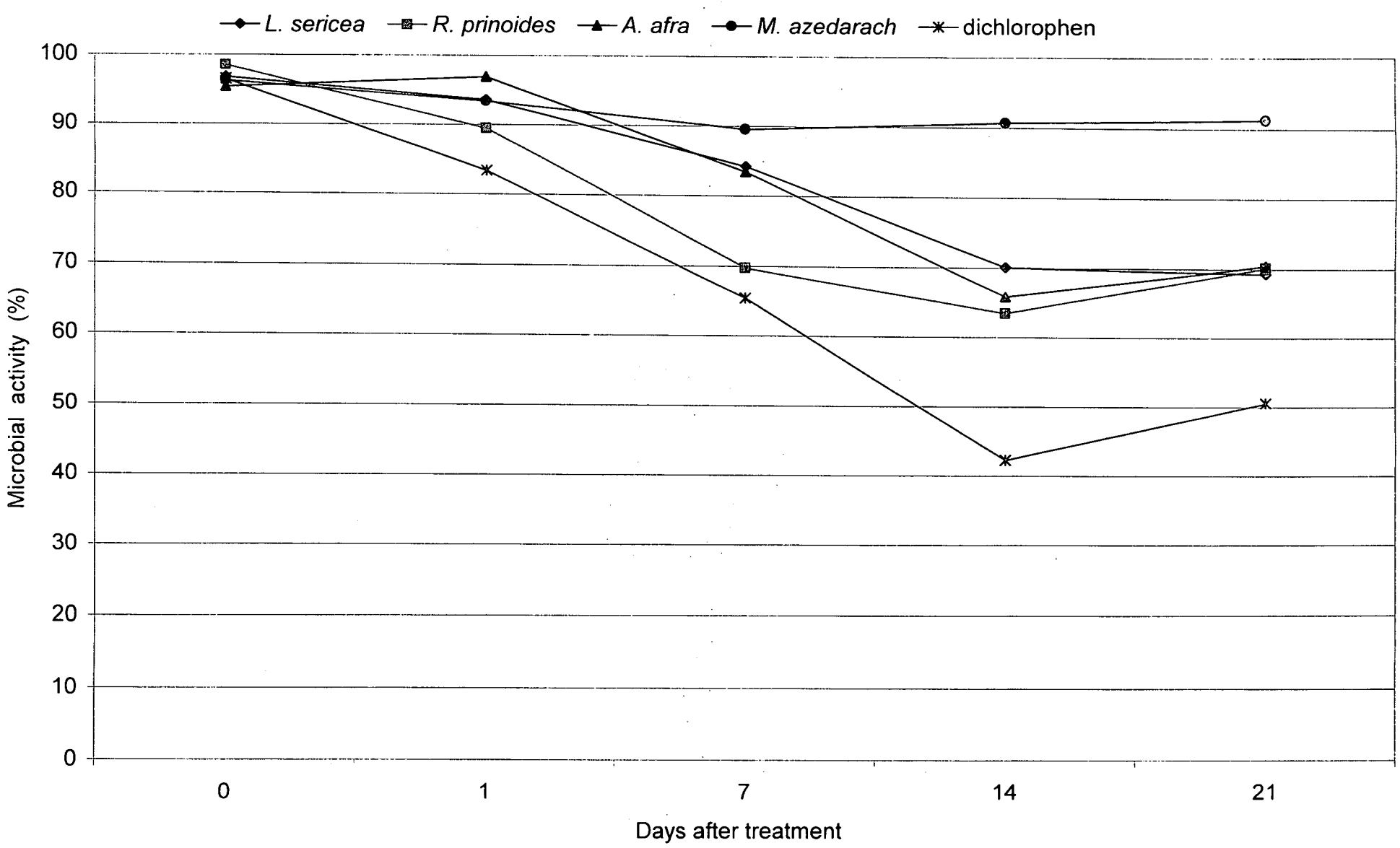

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Microbial activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
7
14
21
Days after treatment

Figure 7. Microbial activity in composted poultry manure treated with plant extracts or dichlorophen expressed as a percentage of initial microbial activity before addition of extract or fungicide. Each datum point represents a mean of eight replications per treatment.

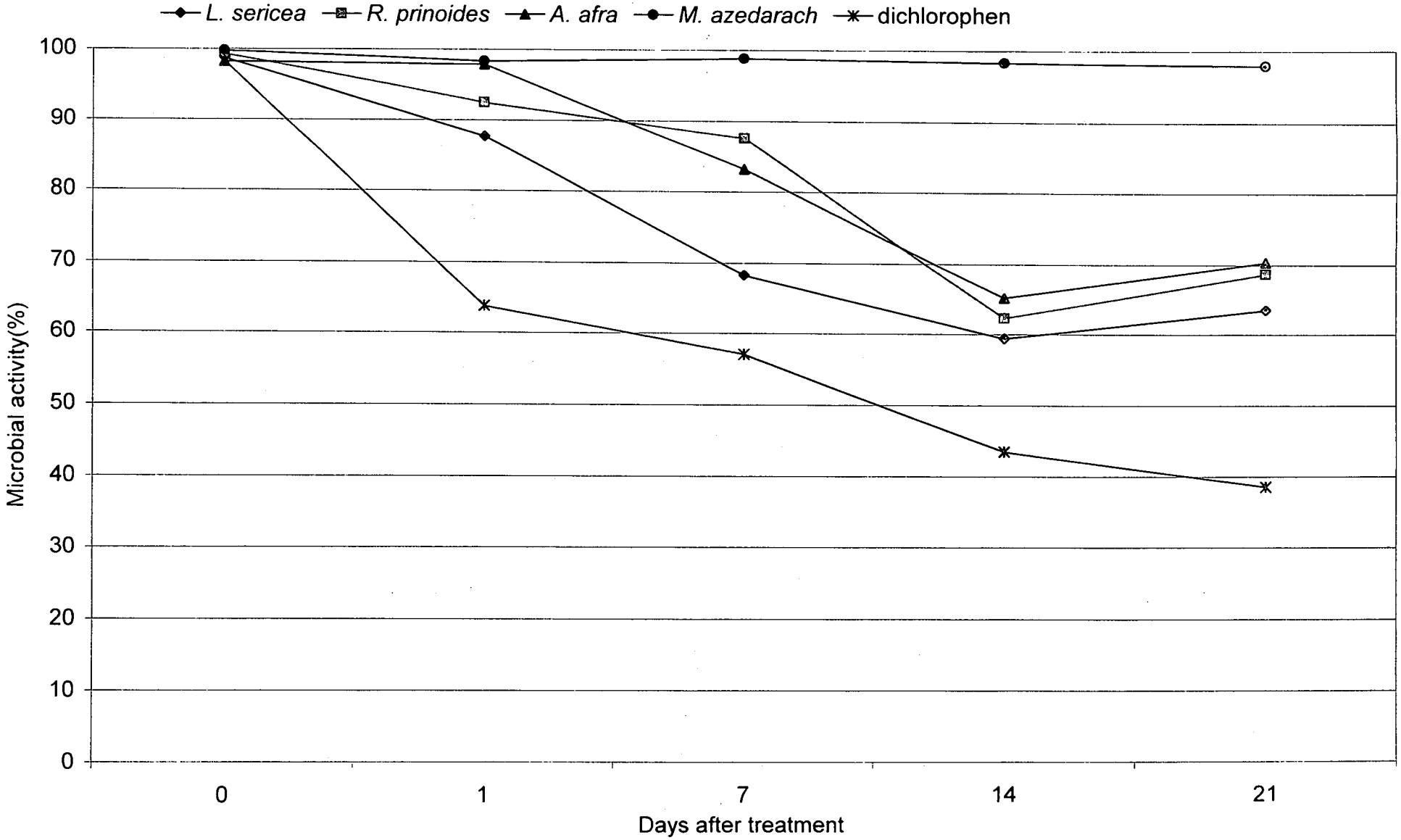

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Microbial activity(%)
100
90
80
70
60
50
40
30
20
10
0
0
1
7
14
21
Days after treatment

Figure 8. Microbial activity in composted sheep manure treated with plant extracts or dichlorophen expressed as a percentage of initial microbial activity before addition of extract or fungicide. Each datum point represents a mean of eight replications per treatment.

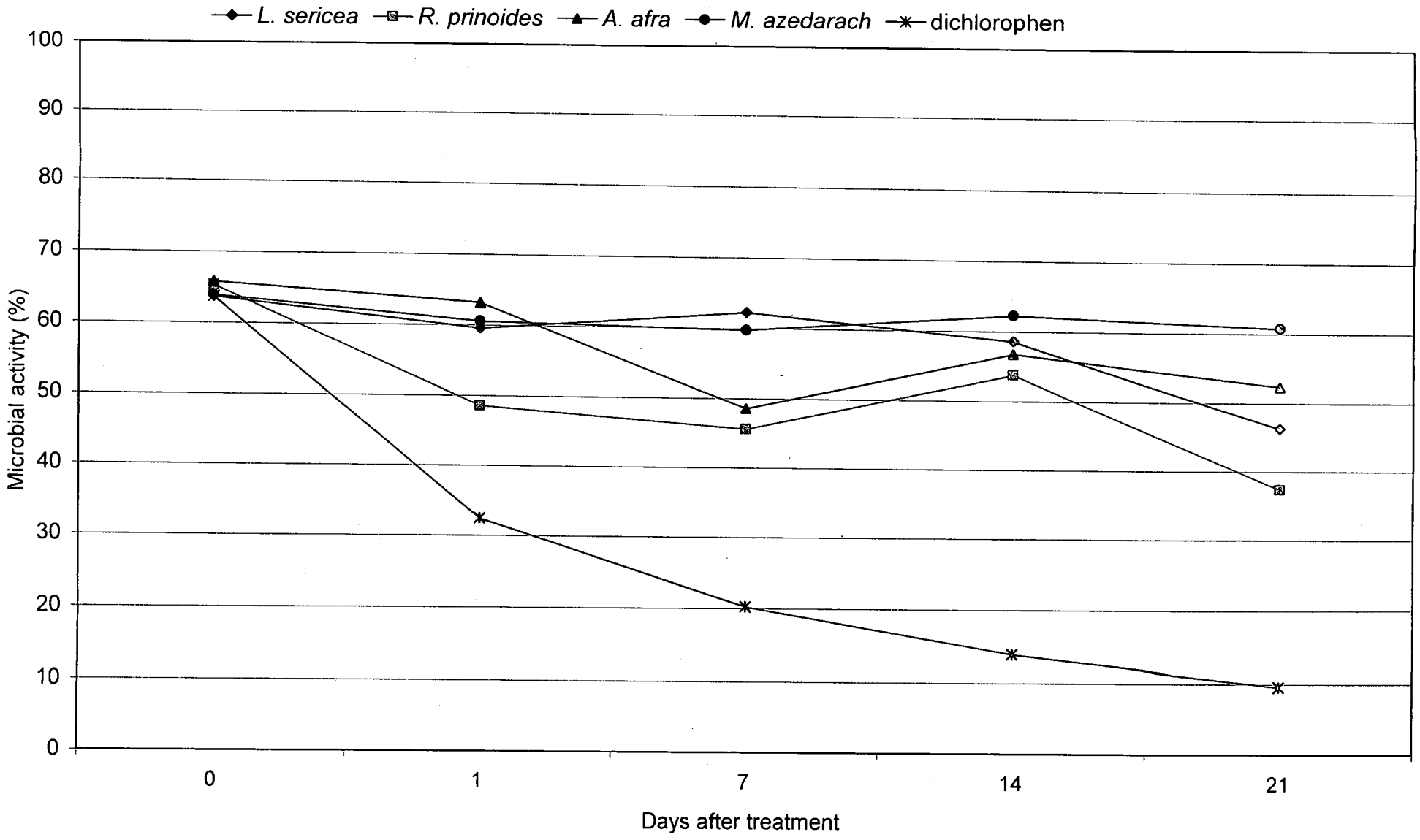

L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
100
90
80
70
60
50
40
30
20
10
0
Microbial activity (%)
0
1
7
14
21
Days after treatment

Figure 9. Damping-off severity of seedlings in composted pig manure treated with four plant extracts and dichlorophen. Each datum point represents a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

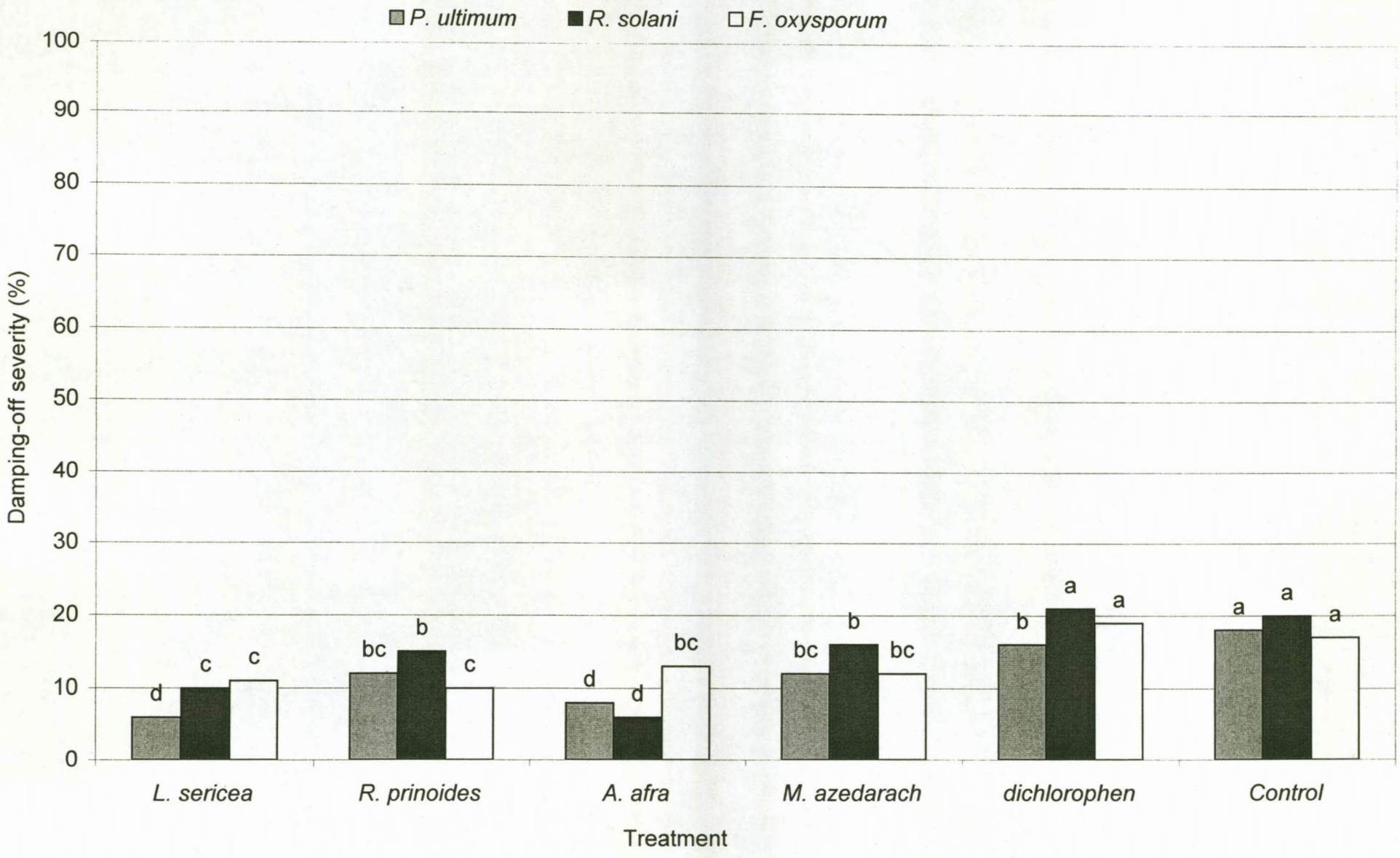

P. ultimum
R. solani
F. oxysporum
Damping-off severity (%)
100
90
80
70
60
50
40
30
20
10
0
d
c
c
bc
b
c
d
d
bc
bc
b
bc
b
a
a
a
a
a
L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Treatment

Figure 10. Damping-off severity of seedlings in composted cattle manure treated with four plant extracts and dichlorophen. Each datum point represents a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

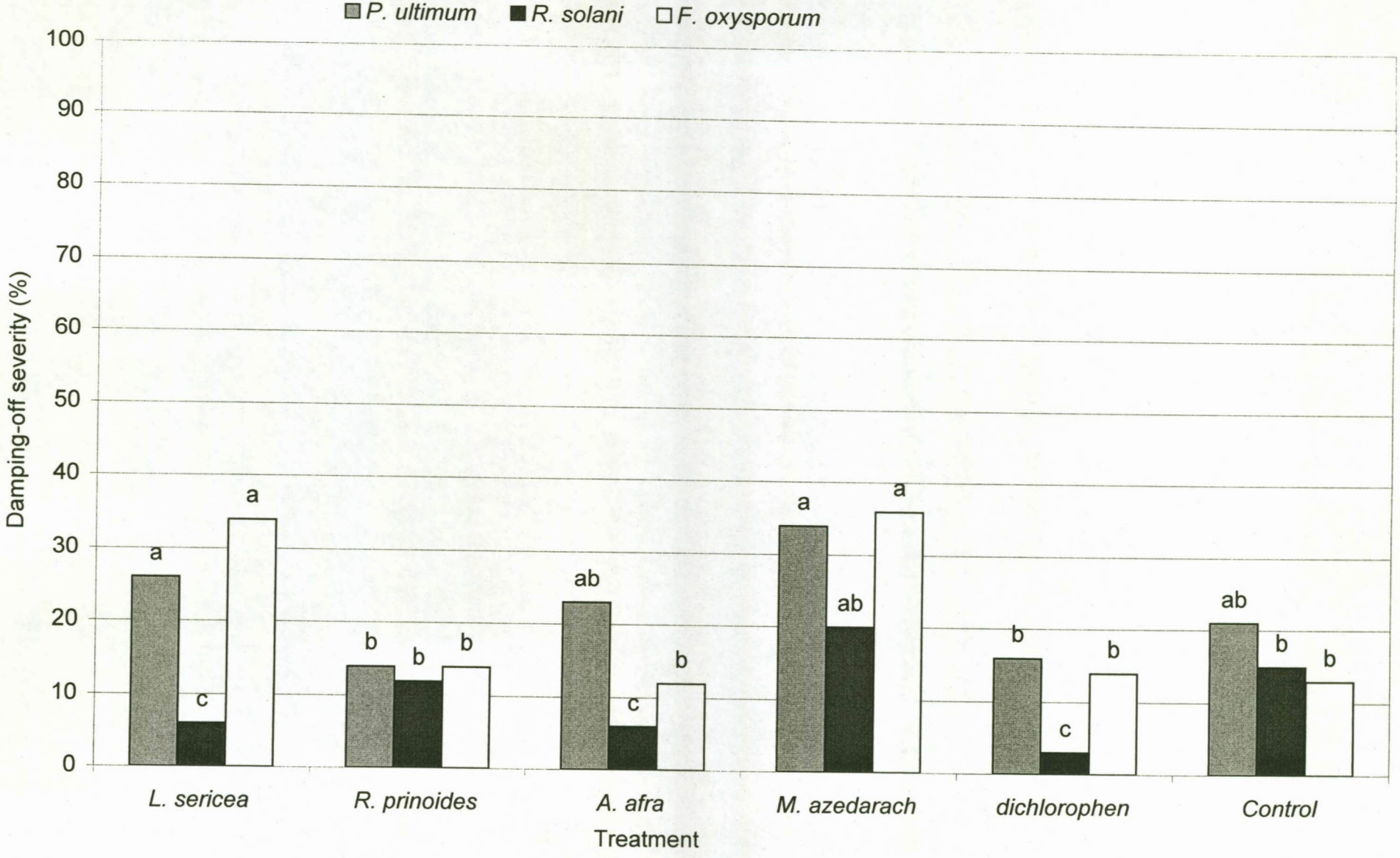

P. ultimum
R. solani
F. oxysporum
Damping-off severity (%)
100
90
80
70
60
50
40
30
20
10
0
a
c
a
b
b
b
ab
c
b
a
ab
a
b
c
b
ab
b
b
L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Treatment

Figure 11. Damping-off severity of seedlings in composted poultry manure treated with four plant extracts and dichlorophen. Each datum point represents a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

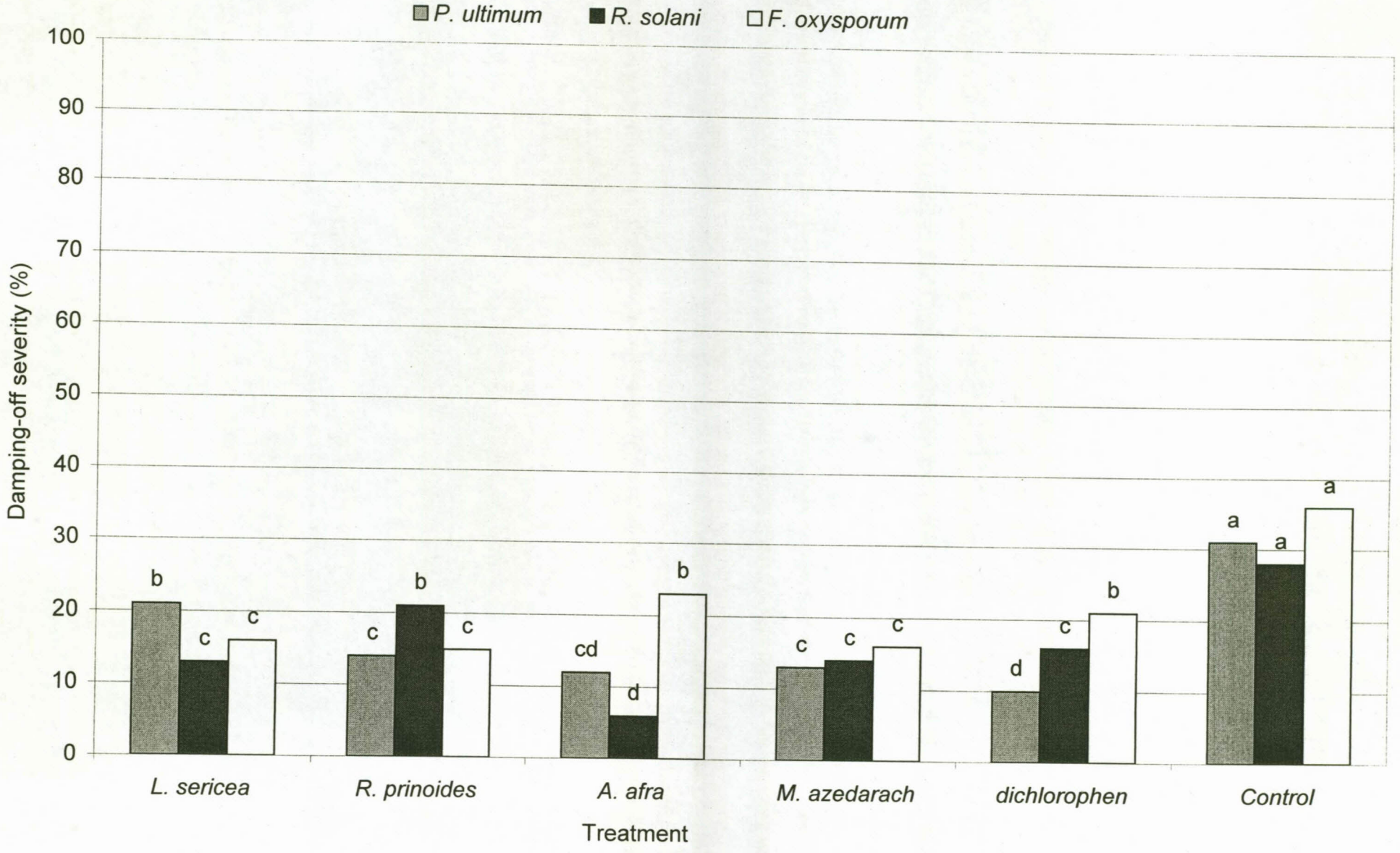
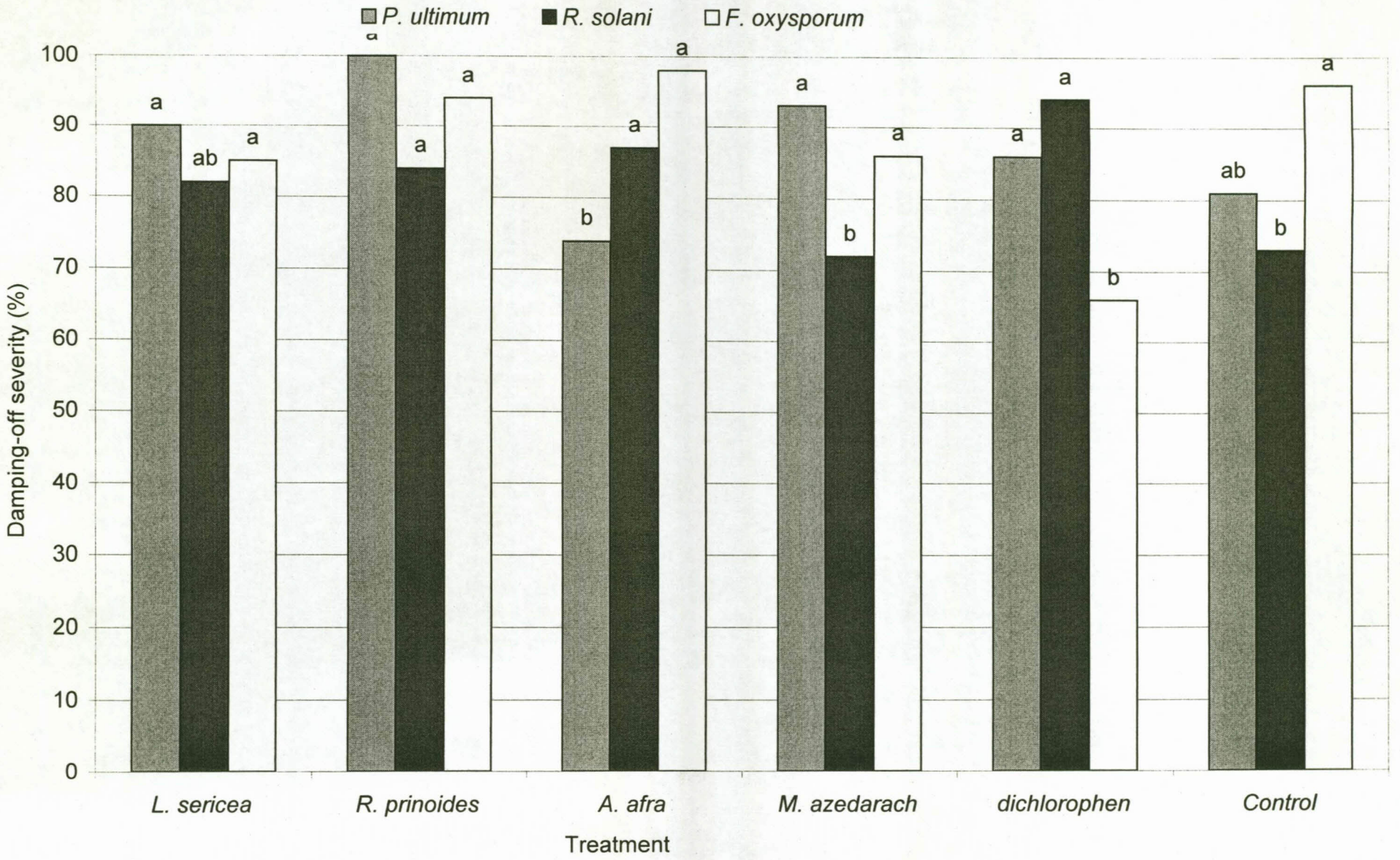

P. ultimum
R. solani
F. oxysporum
Damping-off severity (%)
100
90
80
70
60
50
40
30
20
10
0
b
c
c
c
b
c
cd
d
b
c
c
c
d
c
b
a
a
a
L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Treatment

Figure 12. Damping-off severity of seedlings in composted sheep manure treated with four plant extracts and dichlorophen. Each datum point represents a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).


P. ultimum
R. solani
F. oxysporum
Damping-off severity (%)
100
90
80
70
60
50
40
30
20
10
0
a
ab
a
a
a
a
b
a
a
a
b
a
a
b
a
ab
b
a
L. sericea
R. prinoides
A. afra
M. azedarach
dichlorophen
Control
Treatment

SUMMARY

Damping-off of vegetable seedlings caused by *Rhizoctonia solani* Kuhn., *Pythium ultimum* Trow. and *Fusarium oxysporum* Schlecht. cause serious losses in seedbeds in Lesotho. Composts prepared from poultry, cattle, pig and sheep manure and commonly used by Lesotho farmers were shown to decrease disease severity and increase plant biomass. Cattle, pig and poultry manure composts gave the best disease suppression and stimulated the growth of vegetable seedlings. Composted sheep manure however, did not suppress damping-off nor increase seedling biomass. Seed germination was also significantly suppressed in this compost. High concentrations of phytotoxic elements were possibly responsible for this phenomenon. Disease suppression was also investigated in field studies. Composted cattle, pig and poultry manures significantly suppressed seedling damping-off. Sheep manure compost was also able to reduce damping-off severity under field conditions but to a lesser extent than the other three composts.

General microbial activity in field soil including fungal populations present in the four tested composts was evaluated in the laboratory using FDA. Composted cattle, pig and poultry manure displayed a significantly higher level of microbial activity and consequently yielded more fungal colonies. The lowest microbial activity was observed in composted sheep manure.

Research conducted on the suppression of *R. solani*, *P. ultimum* and *F. oxysporum* with crude extracts of *Rhamnus prinoides* L. Herit, *Artemisia afra* Jacg. ex Willd., *Leucosidea sericea* Eckl. & Zeyh and *Melia azedarach* L,

confirmed their ability to suppress these pathogens. Mycelial growth of *R. solani*, *F. oxysporum* and *P. ultimum* was inhibited *in vitro* by extracts of *R. prinoides*, *L. sericea* and *A. afra*. The growth of these pathogens was however not significantly suppressed by extract of *M. azedarach*. The plant extracts were also able to suppress damping-off of seedlings when added to soil. Populations of *R. solani*, *F. oxysporum* and *P. ultimum* in soils treated with these extracts were also significantly reduced. Disease reduction was highest 7 and 14 days after treatment with the extracts.

The combination of composted animal manures with plant extracts resulted in a reduction of general soil microbial activity, especially fungi. Microbial activity was most reduced in cattle, pig and poultry manure composts while the microbial activity in composted sheep manure was not affected by plant extracts. In fact in some cases, the application of plant extracts resulted in an increased microbial activity in sheep manure. Disease suppression in greenhouse studies was not negatively or positively affected by the addition of plant extracts.

The present study confirms that composted manure of cattle, poultry and pig as used by Lesotho farmers, can be effectively used to control damping-off of vegetable seedlings in seedbeds. The use of sheep manure is however still questionable due to its negative effect on seed germination and general plant growth. Extracts of *L. sericea*, *A. afra*, *R. prinoides* and *M. azedarach* as used by Lesotho farmers, also have the potential to suppress soilborne pathogens. They can be used to control soilborne diseases either individually or in combination with animal manure composts.

OPSOMMING

Vooropkoms-afsterwing van groentesaailinge wat deur *Rhizoctonia solani* Kuhn., *Pythium ultimum* Trow. en *Fusarium oxysporum* Schlect. veroorsaak word, lei soms tot ernstige verliese in saadbeddings in Lesotho. Kompos wat met bees-, vark-, skaap- en hoenderbemesting voorberei is, en algameen deur selfversorgende boere in Lesotho benut word, is in die glashuis- en veldproewe met betrekking tot hul siekteonderdrukkende vermoeëns ondersoek. Met die uitsondering van skaapbemesting, was die intensiteit van saailingsiektes wat deur bogenoemde patogene veroorsaak word beduidend minder terwyl die biomassa van saailinge ook aansienlik toegeneem het. Kompos wat met skaapbemesting voorberei is het ook saadontkieming onderdruk, 'n verskynsel waarvoor hoë konsentrasies fitotoksiese verbindings moontlik verantwoordelik was.

Die algemene mikrobiese aktiwiteit van gewone leemgrond asook die van die vier tipes kompos hierbo genoem, was in die laboratorium met behulp van fluorosien-diasetaat (FDA) geëvalueer. Kompos wat met bees-, vark-, en hoenderbemesting voorberei is het 'n beduidende hoër vlak van mikrobiese aktiwiteit getoon en dienooreenkomstiog ook meer swamkolonies in kultuur opgelewer. Skaapbemesting het 'n beduidend laër mikrobiese aktiwiteit as grond en die ander bemestingstowwe getoon.

Navorsing is op die siekte-onderukkende vermoë van ru-plantekstrakte van *Rhamnus prinoides* L. Herit, *Leucosidea sericea* Eckl. & Zeyh., *Artemisia afra* Jack. ex Willd en *Melia azedarach* L. uitgevoer. Al vier plantsoorte het vooropkomsafsterwing van groentesaailinge, wat deur bogenoemde patogene veroorsaak word, aansienlik verminder wanneer dit tot grond-groeimedia

toegvoeg was. Miseliumgroei van die drie patogene was ook *in vitro* deur ekstrakte van *R. prinoides*, *L. sericea*, en *A. afra* geïnhibeer maar nie deur dié van *M. azedarach* nie. Siekte-onderdrukking was mees beduidend tussen sewe en veertien dae na behandeling van die grond met die onderskeie ekstrakte.

'n Kombinasie van gekomposteerde dierebemestingstowwe met plantekstrakte het tot 'n afname in die algehele mikrobiese aktiwiteit daarvan gelei. Die grootste afname het in bees-, vark-, en hoenderbemesting plaasgevind terwyl kompos met skaapbemesting nie beïnvloed was nie. In sommige gevalle het daar met skaapbemesting selfs 'n toename in mikrobiese aktiwiteit plaasgevind. Die siekte-onderdrukkingsvermoë van die groeimedia het stabiel gebly.

Die studie bevestig dat die gekomposteerde dierebemestings wat selfversorgende boere in Lesotho gebruik effektief is in die bekamping van saailingsiektes van groentegewasse. Voorts, het ru-plantekstrakte van *R. prinoides*, *L. sericea*, *M. azedarach* en *A. afra* ook potensiaal, alleen of in kombinasie met dierebemestingstowwe, om grondgedraagde plantpatogene te onderdruk.