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Cape Peninsula University of Technology

Effects of *Clonostachys rosea* f. *catenula* inoculum on the composting of cabbage wastes

by

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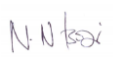
DECLARATION

I, Nomfusi Ntsobi, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



24 December 2020

Signed **Date**

DEDICATION

This thesis is dedicated to my parents.

My mother Nomthandazo Florida Ntsobi; you will always be a pillar, usisikhukukazi with so much love, strength, wisdom, fearlessness and compassion. You have never thrown a towel through your life experiences kodwa wayiMbokodo eyakhetha ukutyala imbewu usazi uyakuze uvune imizamo yemisebenzi yakho. Ndinje-nje kungenxa yemithandazo yakho kwaye uyinto yonke kum MaNdungwana, Bhejula, Diya, Gungu, Mageza, Qhwesha.

My late father Vuyile Michael Ntsobi; although he is not here to give me strength and support, I always feel his presence that urges me to strive to achieve my goals in life.

Vale! Nzotho, Nyelenzi, Maphango, Magatyana, Gxugxa, Wele.

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CONTENT TABLE

LIST OF ACRONYMS

CPUT – Cape Peninsula University of Technology

ANOVA – Analysis of variance

N – Nitrogen

P – Phosphorus

K – Potassium

Ca – Calcium

Mg – Magnesium

Na – Sodium

Mn – Manganese

Fe – Iron

Cu – Copper

Zn – Zinc

B – Boron

C – Carbon

C/N ratio – Carbon to Nitrogen ratio (proportion of organic carbon to total nitrogen of organic material or the mass of carbon to the mass of nitrogen in a substance)

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ABSTRACT

Globally, fungal inocula are being explored as agents for the optimization of composting process. This research primarily evaluates the efficacy of inoculating organic vegetable heaps with the entomopathogenic fungus, *Clonostachys rosea* f. *catenula* (Hypocreales), on the biophysicochemical properties of the end-product of composting. Six heaps of fresh cabbage wastes were inoculated with conidia of *C. rosea* f. *catenula* conidia and another six were not exposed to the fungus. The composted materials from fungus- and control-treated heaps were subsequently used as a medium to cultivate tomatoes (*Solanum lycopersicum*). The heaps were allowed to undergo composting. Heap temperature and moisture content were recorded weekly, and the chemical characteristics of the heaps were assessed after twelve weeks of composting. In addition, we evaluated the protective effect of the fungal inoculum against red spider mite (*Tetranychus urticae*) infestations of the tomatoes - conidial colonization of the plant tissue and the number of plants infested by the pathogen were recorded. Additionally, phytotoxicity tests, seed germination, and seedling toxicity on tomatoes were carried out.

There were few significant variations ($p < 0.05$) in heap temperature or moisture level between treatments based on the weekly data; they ranged from 23.778 – 39.35 °C and 0.00 – 52.92% heap moisture readings from weeks 1-12, respectively. We found no significant differences in the levels of compost macronutrient N, P, K, Ca, Mg, C and Na) and micronutrient (Mn, Fe, Zn, Cu) constituents. Remarkably, the composted materials, when incorporated into a growth medium, from fungus-treated heaps induced a 100% endophytic tissue colonization in cultivated tomato plants. It was observed that the tomato plants grown in composted materials from heaps inoculated with *C. rosea* f. *catenula* had fewer red spider mite (*Tetranychus urticae*) infestations; however, the difference was not significant ($\chi^2 = 0.96$ and $p = 0.32$). Remarkably, the fungus was re-isolated from leaves of all the plants that were cultivated on the composted materials from the fungus-treated heaps. Moreover, the fungus treatment yielded composted

materials that significantly ($p < 0.05$) enhanced tomato seed germination. The phytotoxicity tests revealed that the composted samples from the heaps exposed to the *C. rosea* f. *catenula* inoculum was not toxic to tomato seeds and seedlings when the end-product of composting was used at a concentration of 25% v/v in a soil mix with 75% coarse sand.

In conclusion, this study showed that the quality of composted materials containing *C. rosea* f. *catenula* was improved in terms of fungal endophytism, seed germination. and induced 100% endophytic tissue colonisation of tomatoes. However, *C. rosea* f. *catenula* did not confer protection against red spider mite infestations to tomatoes. *C. rosea* f. *catenula* inoculation did not significantly affect the composted materials' chemical constituents nor led to phytotoxicity of composted materials. Overall, the use of the entomopathogenic fungus, *C. rosea* f. *catenula* inoculum as a bio-enhancer in composting organic waste is beneficial to the organic composting process.

***Keywords*:** Composting, Organic vegetable wastes, Inoculation, *Clonostachys rosea* f. *catenula*, Tissue nutrient content, Toxicity

CHAPTER ONE

General Introduction and Literature Review

1.1 Introduction

Food wastage and spoilage are increasing worldwide. This problem is relatively high in developing countries, where farming practices, transportation, and storage of perishable food crops are inefficient (Aschemann-Witzel et al., 2015). Organic wastes have the potential to cause environmental contamination (Pan et al., 2012). These materials are often dumped in landfills, which eventually leads to increase in methane production. Globally, approximately 1.3 billion tonnes of food wastes are generated annually (Oelofse, 2019). A study by Nahman & De Lange (2013) revealed that agricultural production produces the bulk of organic wastes. Wastes are produced during the production, harvesting, storage, and processing systems. About 4.3% of South Africa's greenhouse gas emissions are produced from organic waste disposal.

The good news is that the government, consumers, and businesses are increasingly becoming aware of the menace posed by poor management of these wastes. The increased demand for sustainable waste management and organic farming has incentivised the quest for better ways of managing and beneficiating from vegetable wastes. Composting is seen as an environmentally benign way of treating and managing vegetable wastes. Composting recovers nutrients and mitigates carbon release through the generation of humic substances (Girotto et al., 2015). In South Africa, 2.4 million tonnes of the 10.2 million tonnes of generated wastes destined for disposal were recycled (Oelofse, 2019).

Generally, many countries are experiencing problems caused by organic wastes due to inefficient waste management strategies. Jara-Samaniego et al. (2017) argued that a quick and efficient management of these wastes is possible because of their higher degradability. Recycling of food and organic wastes containing high concentrations and nutrient contents can

be achieved through composting. According to Varma & Kalamdhad (2014), there is a rising interest in treating organic wastes by composting. Composting, as a waste remedy has many environmental and economic benefits. For example, Zhang et al. (2013) showed that composted organic waste was utilised in the state nurseries in Beijing (China) to substitute peat in growing media. Despite the types, nature, and composition of organic waste, composting remains the most economical and efficient management method amongst others in developing countries (Taiwo, 2011).

At an environmental level, composting aids in obtaining an end-product that can enhance the soil and conserves water (USEPA, 1998). From an economic point of view, Lim et al. (2015) revealed that composts could be distributed to various markets as agricultural fertilisers (Jara-Samaniego et al., 2017). Taiwo (2011) also recommends composting organic waste in countries to promote jobs and higher yield of organically produced food crops.

While composting of food and green wastes is a potential solution to solid waste management, there are still operational challenges that hinder its global adoption. These include the lack of suitable composting location, financial support for the installation and maintenance of such facilities, and technical knowledge, especially in developing nations. Failure to maintain the biological conditions during the decomposition process is another issue (Hournweg et al., 1999). Other challenges associated with composting of food and organic wastes are odours, maintaining physical and chemical consistencies, including porosity, C: N ratio, biological and biochemical parameters of composts heaps (Cerda et al., 2018).

Various studies have investigated the use of mechanical and physical interventions, including turning frequency of compost heaps, shredding of raw materials, and using ground raw materials to optimise composting process (Mthimkulu et al., 2016; Kalamdhad & Kazmi, 2008;

Ogunwande et al., 2008; Getahun et al., 2012; Petric et al., 2015). However, fewer studies have reported on the use of microorganisms for the purpose of enhancing the composting process (Gaur et al., 1982; Chaturvedi et al., 2010; Pan et al., 2012; Irawan et al., 2014; Sarkar et al., 2016; Nakasaki & Hirai, 2017; Tsai et al., 2007; Fan et al., 2017; Ke et al., 2010; Zhao et al., 2016; Manu et al., 2017). Karchanawong & Nissaikla (2014) and Onwosi et al. (2017) utilised inoculants to enhance the organic matter degradation rate. Jurado et al. (2014), Nair & Okamitsu (2010),), and Zhao et al. (2016) used lignocellulosic microorganisms to improve the lignocellulose degradation.

Ascomycetes are one of the most versatile classes of fungi. Many ascomycetes are soil-borne saprophytes that are capable of breaking down lignocellulose of plant-based organic materials (Ferreira et al., 2016). Other ascomycetes like entomopathogenic fungi can infect and regulate insect populations naturally (Singh et al., 2017). The current study seeks to investigate the enhancement effects of entomopathogenic fungal inoculum on composting process and the end-product of composting. Entomopathogenic fungi are used as biocontrol agents against agricultural arthropod pests (Noble et al., 2018). Amendment of soil with entomopathogenic fungi has been shown to enhance plant growth and plant resistance to phytopathogens and herbivores (Saikkonen et al., 2004). Furthermore, some entomopathogenic fungi are rhizopheric and endophytic. These fungi can persist in soils while offering protection to plants and improving plant nutrition (Pieterse et al., 2014; Chandler, 2016).

C. rosea belongs to the division Ascomycota. It occurs in soil and can suppress soil-borne pathogens and nematodes (Toledo et al., 2006; Sutton et al., 1997; Knudsen et al., 1995; Stewart & Harrison, 1989; Jensen, 2000). It is rhizospheric and enhances plant growth through plant hormones, signalling factors (Lahoz et al. 2004), and nutrient utilisation (Sutton et al., 2006). This entomopathogen exerts influence on the initiation of roots and shoots, leaf physiology,

flowers and fruits, and plants' productivity (Sutton et al., 2006). A plethora of studies have utilised fungal inocula as biofertilizers in field trials and/or greenhouses (Grigera et al., 2007; Rahi et al., 2009; Groetten et al., 2016; Zhang et al., 2016a; Wang et al., 2018c). However, no information provided on the use of *Clonostachys rosea* f. *catenula* as a bioinoculant in composting organic wastes. It has also been reported to be endophytic in many plants (Sutton et al. 1997) and pathogenic against many arthropod pests (Chatterton & Punja, 2009; Nordström, 2014).

Also, the indiscriminate and prolonged use of chemical fertilisers has had adverse effects on soil fertility, crop productivity, produce quality, and the environment. It is worth noting that currently, most of the inorganic fertilisers are costly and harmful for the soil microorganisms. Hence, it is necessary to develop environmentally friendlier fertilisers. The optimisation of organic composting can improve the value and uptake of composts. Bacteria and fungi are essential sources of composting bio-enhancers that can be used to optimise the composting process and quality of end-product of composting.

A preliminary investigation of the effects of *Beauveria bassiana* (Hypocreales) inoculum on the composting of vegetable wastes found that heap temperature and moisture content from the treated mixed vegetable waste materials were not significantly affected by fungal inoculation. In the same study, the quantities of micronutrients (Fe, Zn, Mn and Cu) in the composted materials from the fungus treated heaps were higher than the control. These results agree with those of Jusoh et al., (2013); Parė et al. (1999). Previously, Ribeiro et al. (2017) found increased temperature throughout the process of composting in treatment piles inoculated with microbes; and inoculation of beneficial microorganisms accelerated the process of composting and improved the final quality of the product (Gaur et al., 1982).

This study intended to establish the effect of fungal inoculation on the physicochemical properties, endophytic activity, phytotoxicity of composted materials and protective effect against insect infestations of *Tetranychus urticae* (Acari) on potted tomatoes. This study will advance knowledge on the role of bio-enhancers and help in developing a pest-free and quality fertiliser with low phytotoxicity.

Scientific Rationale

Worldwide, agriculture and the food industry are generating staggering amounts of solid organic wastes (fruit and vegetables, peels, roots and tubers) driven mainly by extreme demand of production and inefficient operation processes (Chang et al., 2006). In South Africa, nearly 10.2 million tonnes of edible food wastes are generated annually, throughout the supply chain, most of which end up in the landfill and/or incinerated (Wani et al., 1995; Gautam et al., 2010). These treatment methods are harmful to human health and cause environmental hazards. The rate of landfilling is outrageously high and not sustainable (Taiwo, 2011). Landfilling has many apparent challenges such as a prolonged period of waste stabilisation in landfill sites, constant production of hazardous gases and leachate over time, and demanding rehabilitation, monitoring and control. Moreover, incineration have similar negative impacts such as air emission, ash and liquid discharge and some of these, particularly air emission, causes diseases to human health, and toxins in ash eventually leach into the soil and water from landfill ash deposits (Narayana, 2009).

Interestingly, globally, there is an urgent need to mitigate waste management hazards and to develop a sustainable waste economy that is based on innovative, efficient and environmentally benign waste management technologies. And composting is considered as one of the most effective approaches of managing organic wastes and adding value to organic wastes. Composting recycles the wastes to organic fertilisers for enriching soils and reducing the need

for synthetic fertilisers (Seufert et al., 2012). Recycling of waste could reduce pressures on landfills and improve management of wastes. The drivers of composting are microorganisms and play a crucial role in biodegradation and bioconversion of organic matter during composting (Baldrian et al., 2008; Hou et al., 2012). Many studies have demonstrated that several beneficial microbes are capable of degrading recalcitrant organic wastes into high-quality compost end products with improved nutrient properties to support soil productivity. These microorganisms excrete lignocellulose enzymes such as hydrolase, proteinase, cellulase and laccase (Irawan et al., 2014).

Fungi, especially fungal saprophytes, contribute majorly to composting and can biodegrade recalcitrant lignocellulose materials (Janusz et al., 2017). Some fungal species in the classes Deuteromycetes and Ascomycetes can provide other benefits to composts, besides the breaking down of organic materials, such as endophytic conidial colonisation and protection of plants, and plant growth enhancement (Mengistu, 2020). For example, some soil-borne entomopathogenic fungi are simultaneously saprophytic, endophytic, and pathogenic against arthropod pests (Barra-Bucarei et al., 2019). However, few past studies have investigated bio-enhancing effects of fungi, especially entomopathogenic fungi, on composting of organic wastes.

Therefore, it was hypothesised that inoculating organic wastes with entomopathogenic fungus could enhance the decomposition organic waste heaps that are treated with conidia of *Clonostachys rosea* f. *catenula* (Hypocreales) and improve the nutrient contents, and endophytic and anti-insect properties of the composted materials. It was expected that the finished end-product from the fungus-inoculated heaps will continue to harbour conidia as well as have improved physical stability and quality, and macronutrient and micronutrient richness that will promote plant vigour and growth (Sebastian & Christopher, 2007; Vega et al., 2009; Pathak & Ram, 2012). This type of compost end-product has the potential to substitute chemical

fertilisers, synthetic pesticides and be recommendable in agro-production because of plant growth-promoting and plant protection properties (Pathak & Ram, 2012).

Statement of the research problem

The increased demand for organic compost and the need to sustainably manage organic wastes have created the need to optimise composting protocols. Microbes such as fungi and bacteria can breakdown organic materials; hence, they might be used as bio-enhancers during composting to improve the quality of the composted materials. This study seeks to determine the effects of the fungus (*Clonostachys rosea* f. *catenula*) inoculant on biophysochemical properties of the composting of cabbage wastes. The goal was to establish whether fungal inoculation improves the composting process and quality of composted materials.

1.2 Hypotheses of the study

- *C. rosea* f. *catenula* inoculum will enhance the rate of breakdown of vegetable wastes during composting.
- *C. rosea* f. *catenula* inoculum will affect physical properties (heap temperature and humidity) of vegetable wastes during composting.
- *C. rosea* f. *catenula* inoculum will increase the concentrations of macro-and micro-nutrients in compost heaps.
- Compost materials from heaps that were pre-treated with conidial suspension of *C. rosea* f. *catenula* will retain the conidia. They will change the colour of compost during composting in order to determine the maturity of compost at the end of composting.
- The *C. rosea* f. *catenula* conidia in composted materials, obtained from heaps that were pre-treated with the same fungus, will colonise exposed tomato endophytically.
- Compost from heaps that were pre-treated with *C. rosea* f. *catenula* will improve the germination of seeds and growth of tomato plants.

- Tomatoes (*Solanum lycopersicum*) grown in a growth medium containing composted materials from heaps that were pre-exposed to *C. rosea* f. *catenula* inoculum will have fewer cases of *T. urticae* infestation compared to control treated heaps.

1.3 Overall aim of the study

The main goal of the study was to determine the beneficial effects of inoculating organic compost heaps with the entomopathogenic fungus *C. rosea* f. *catenula* during composting on the biophysicochemical properties of the end-product of composted cabbage wastes.

Specific objectives of the study were to:

- Determine the effect of *C. rosea* f. *catenula* inoculation during composting of vegetable waste.
- Determine the effect of *C. rosea* f. *catenula* inoculation during composting on physical parameters (heap temperature and humidity).
- Assess the relationship between *C. rosea* f. *catenula* inoculation and nutrient contents of the compost end-product.
- Assess the effect of *C. rosea* f. *catenula* inoculum from pre-treated compost materials on colonising composted materials and the colour of compost during composting in order to determine maturity of compost at the end of composting.
- Assess effect of *C. rosea* f. *catenula* inoculation of compost heaps on endophytic colonisation of seedlings of *Solanum lycopersicum* grown on the composted materials.
- Assess effect of *C. rosea* f. *catenula* inoculation of compost heaps on germination and survival of seedlings of *Solanum lycopersicum* on the composted materials.
- Assess the effect of *C. rosea* f. *catenula* inoculation of compost heaps against insect infestation on seedlings of *Solanum lycopersicum* grown on the composted materials.

1.4 Structure of the research

This thesis contains five chapters, which are briefly described.

Chapter One: Introduction and Literature review

The chapter presents the structure of the research, provides scientific justification of the study, hypotheses, aim and specific objectives of the study.

Chapter Two: Materials and methods

The chapter includes information on the experimental design, detailed descriptions of the materials and methods, and the parameters that are assessed.

Chapter Three: Results

In this chapter the results are concisely described and presented in tables and graphs.

Chapter Four: Discussion

This chapter contains detailed interpretations of the results. Arguments and inferences are made on the results in relation to other studies.

Chapter Five: Conclusion and recommendation of the study.

1.5 Literature review

1.5.1 Waste generation in South Africa

South Africa is confronted by waste generation challenges impelled by the increasing population, income level (where the amount of waste generation rate varies between income groups i.e., low, medium and high-income groups) and increasing standards of living, urbanisation and economic growth (World Bank, 2012; Stats SA, 2017). All waste produced in South Africa were estimated at 108 million tonnes (DEA, 2011), of which 98 million tonnes were landfilled, with only 10% recycled. In South Africa, wastes are classified into three categories: general waste, hazardous waste and unclassified waste.

General waste consists of paper, plastic, glass and metal. The waste (only recyclable waste stream) is estimated at 4.9 million tonnes, accounting to 11% that was recycled and 34% was an estimate of its recycling rate. This type of waste is the largest contributor (35%) mainly from sugar mills, sawmills, and paper and pulp industry (Department of Environmental Affairs, 2018). Approximately 38 million tonnes of hazardous wastes are generated in South Africa, but only 7% of the hazardous wastes are recycled, and the remainder was treated or landfilled (DEA, 2018). These wastes include by-products of domestic, industrial, commercial, and health care activities. Many of these wastes are produced in the manufacturing of products or further industrial application. Sources of hazardous waste include mining sites, agricultural facilities and natural environment, research laboratories, industry and institutional establishments. Based on the DEA (2018) report, fly ash and dust (81.7%)[1] and bottom ash (14.4%)[2] were the most dominant portions of hazardous waste generated, at 96.1% mainly from coal-fired power stations.

1 Fly ash is any industrial by-product or discarded commercial product that poses a threat to the environment or people and other living organisms; mainly from coal-fired power stations because it is explosive, flammable liquid/solids, poisonous, toxic, ecotoxic and infectious substances.

2 Bottom ash is the residue that is fused into heavy particles that drops out of the furnace gas streams (air and combustion gases) after the combustible matter in coal has been burned off.

Unclassified waste includes slag (52%) predominantly from mills and foundries, brine (42%) and mineral wastes (3%). Approximately 8-11% of the recycled unclassified waste stream was Waste Electronic and Electrical Equipment (WEEE) (Lydall et al. 2017).

1.5.2 Waste management in South Africa (SA)

South Africa is faced with waste management challenges which are mainly caused by urbanisation and population growth, as well as inefficient, non-compactable and non-biodegradable practices (Nkosi et al., 2013). A waste management hierarchy was introduced into SA's management policy through the White Paper on Integrated Pollution and Waste Management, 1999 (National Waste Management Strategy) NWMS (DEA, 1999). The goal of the White Paper (later passed in the 2008 Act) was to ensure an integrated strategy for management of pollution and waste. Currently, all spheres of government and provincial environmental authorities (municipalities) are legally responsible for the management of wastes in South Africa, which is done in partnership with the private sector. However, the private sector (commercial and industrial) is responsible for the managing their wastes.

1.5.3 Vegetable Wastes in South Africa

The production of vegetable wastes in South Africa is increasing and estimated at 45% (including fruits) of the 31 million tonnes of food waste, annually (Notten et al., 2014)

The most types of vegetables wasted are potatoes, tomatoes and cabbage (Cronjé et al. 2018), but vegetables such as broccoli, cauliflower, butternut, sweet potatoes and spinach also form part of these wastes. Fei et al. (2014) reported that these wastes possess high potential for methane generation through anaerobic conversion, but they are mostly landfilled. Previous research showed that large quantities of fruit and vegetables are produced by food industries and agricultural sectors globally (Ayeleru et al., 2016). Plenty of these products disintegrate during harvesting, sorting, packing and storage, as they are all subjected to putrefaction with

short shelf life (Ayeleru et al., 2016). This agrees with an earlier report by Nahman & De Lange, (2013) that such food wastes escalate throughout the food supply chain, including during production, storage, transportation, processing, at retailers and in the kitchens of households and restaurants. Sometimes, these materials are easily damaged due to the tendency of farmers to 'leave food in the field' in response to either weather or pest-related damages or market forces (Gunders, 2012) and might have encountered a delay in shipment to the end consumer (Hawkins, 2013; Boyer & McKinney, 2013; Roberts and Graham, 2004; Rosa, 2006; Acedo & Weinberger, 2010).

Fruit and vegetable wastes are easily biodegradable; thus, become very challenging to manage due to its high-water content as these wastes wilt rapidly, causing microbiological instability, leachates and formation of odours. Landfilling of these materials causes more significant threat to the public health and the environment (Scano et al., 2014; Asankulova & Obozov, 2007; Zuru et al., 2004; Zhao et al., 2010; IBISWorld, 2016) considering that they are permanently causing damage, requires a lot of time to rehabilitate and emit a large amount of greenhouse gases into the atmosphere. According to Terry et al. (2011); Negi & Anand (2015) environmental changes such as unfavourable weather, pest attack and diseases, damages during harvesting and low consumer product demand contribute substantially to fruit and vegetable wastes. However, factors like temperature, gaseous composition with storage, relative humidity, from harvesting through handling, packing, storage and transportation to final delivery of the fresh produce to the consumer, influence the post-harvest life. Additionally, other main causes of wastage are general senescence, water loss, diseases and pests, mechanical injury and chilling injury.

1.5.4 What are the challenges of managing wastes in South Africa?

As a developing economy, South Africa is confronted by numerous challenges that influence the efficient and appropriate management of wastes. There are three categories of drivers namely: socio-economic, environmental- and institutional, that guide the direction of waste management laws and policies in South Africa. Challenges hindering waste management in South Africa include growing population and economy; urbanisation and industrialisation; historical backlog of waste services for informal urban settlements; limited understanding of the waste flows and national waste balance.

According to the DEA (2012), SA has a total of 826 landfill sites. According to Jan Palm, president of the institute of Waste Management of southern Africa (IWMSA), SA's landfills are not adequate enough, as a result, new regulations have been implemented due to poorly located, designed and operated landfill sites. Consequently, landfill operators can only acquire a license by implementing adequate waste sorting, and classification, better landfill design, improving operation and monitoring the surrounding environment for contamination.

The Department of Environmental Affairs conducted a study to identify and determine the number of unlicensed waste disposal facilities in SA. Of 581 identified sites, 431 waste disposal facilities, needed to be licensed (Pienaar & Howard, 2014). It was evident from the study that the municipalities needed to encourage and support separation of waste from source, provide clear guidelines to food production companies and households regarding the types and sorting of the waste and encourage community involvement in recycling. Moreover, provide execute environmental compliance and enforcement functions and monitoring inspections, to determine whether, facilities are compliant with the conditions of their licence or the management thereof. Later, a backlog of 122 municipal waste disposal facilities was identified and still needed to be approved. The DEA appointed numerous consulting companies to undertake the licensing process including Architecture, Engineering, Construction, Operations and Management

(AECOM) Company, to prepare the license application for 50 illegal operating communal landfill sites in the Western Cape Province (Pienaar & Howard, 2014).

South Africa is classified as a developing country (Rodseth et al., 2020). While SA is considered as a substantial economy of the southern African region, it is also reflected by a few characteristics of a developing country such as increased level of urbanisation, wealth and infrastructure. Nonetheless, it is noted that this development has been unequally distributed among the population and exists from both the economic and social approach. Stats SA (2016) also reported on disparity differences among municipalities. Rodseth et al. (2020) demonstrated that waste management encapsulate this inequality. In SA, the local government is responsible for the management of wastes. Nonetheless, immense inconsistencies exist in the services provided by different municipalities. For instances, many smaller cities have insufficient capacity for any form of waste service delivery (Friedrich & Trois, 2010). In urban communities, informal settlements often lack basic refuse removal capacity, while relatively affluent urban areas are normally provided with satisfactory waste service (Stats SA, 2016). This inequality in waste management is leading to the establishment of many illegal waste dumping sites in more impoverished communities.

1.5.5 Waste treatment strategies

The most used methods to manage wastes are incineration, landfilling and composting. These three methods are described below.

1.5.5.1 Incineration

Incineration is a process of burning waste materials that result in ash residues, liquid discharge and air emission (Narayana, 2009). This process is mostly used for materials with high calorific value, such as paper, cardboard, and plastics, to sustain combustion levels (Davis, 1994; Murray, 1999). During incineration, waste is not eliminated but transformed into several new

forms, which become more difficult to deal with since physical matter cannot be destroyed (Narayana, 2009). Heavy metals, dioxins, and other volatile organic compounds are produced from incinerated solid wastes. Numerous substances, like dioxins, can be carried over long distances from their emission sources, last for years in the environment without breaking down into less harmful compounds, and increase in soil, water, and food sources (Narayana, 2009).

1.5.5.2 Landfilling

A landfill is an area of land onto which waste is deposited. The intention is to avoid any contact between the waste and the immediate environment, particularly the groundwater. Landfills are classified into three categories, which are: 1) Open landfills, which are common in all developing countries, involve the waste directly being dumped haphazardly into low lying areas of open land. 2) Semi-controlled landfills are designated locations where the dumped waste is compacted, and a topsoil cover is prepared daily to prevent pests. This type of landfill is not planned to manage the leachate discharge or emissions of landfill gases. 3) Sanitary landfills are used in industrialised countries and have facilities for the prevention and treatment of the leachate utilising a sequence of ponds. According to Tchobanoglous et al. (1993), this type of landfill also has arrangements for the control of gases from waste decomposition.

1.5.5.3 Composting

Composting is a friendly way of breaking down and converting organic materials utilising oxygen and microorganisms through the biological process into soil conditioner and organic fertiliser. Composting has various methods used to manage waste such as traditional, wind-row, in-vessel and vermicomposting. The traditional method is based on anaerobic decomposition or one based on aerobic decomposition using passive aeration through measures such as little and infrequent turnings or static aeration provisions such as perforated poles/pipes. These processes take several months but mostly include a high-temperature period, and this adds further value to the product by eliminating pathogens and weed seeds. Traditional methods are

passive and involve stacking of materials in piles to decompose over a long period with little agitation and management. The process is mostly used in developing countries to treat urban wastes. They do not require sophisticated infrastructure and machinery and are not capital intensive but requires high labour. These could be ideal for small-scale farming as they are easy to practice, especially where physical labour is not a constraint. However, the low income and prolonged processing time are the major drawbacks of traditional composting methods (FAO, 1980).

Wind-row composting is the mixture of materials placed in long piles that are turned on a regular basis, which mixes the composting materials and enhance passive aeration (NRAES, 1992). The group of methods confined with this process are turned wind-rows, passively aerated wind-rows and aerated static pile. In-vessel composting is dependent on different forced aeration and mechanical turning techniques to stimulate the decomposition process. This process includes a group of methods that confines the composting materials within a container or vessel (NRAES, 1992). In-vessel methods are a variety of different combination of vessels, aeration devices and turning mechanisms such as: bin composting, rectangular agitated beds, silos, rotating drums and transportable containers. Vermicomposting uses earthworms for composting of organic residues, and they feed on living or dead materials. Earthworms play an essential role in shaping soil structure and cycling nutrients such as P, K, Ca and Mg, nitrogen (N) mineralisation and water filtration (Mengistu et al., 2017). The turning of compost heaps is not necessary in the presence of earthworms.

1.5.6 Aerobic and anaerobic composting

Composting is the disintegration of organic matter through biological processes, resulting in nutrient-rich humus (Narayana, 2009; Gautam et al., 2010). This method is dependent on the raw materials used and the conditions maintained during the process (Insam & Bertoldi, 2007;

Souza et al., 2014; Ribeiro et al., 2017). By nature of the decomposition process, composting is divided into two methods, namely aerobic and anaerobic composting.

1.5.6.1 Aerobic composting

Aerobic composting takes place in the presence of adequate oxygen. During this process, the production of carbon dioxide, ammonia, water, heat and humus, occurs due to break down of organic matter by the existing microorganisms, resulting to a stable, sanitised, high-quality organic end-product compost (Sayara et al., 2020). Additionally, this process involves three different stages which are mesophilic, thermophilic and maturation to divert the organic waste to humic end-product. The breakdown of proteins and fats, complex carbohydrates such as cellulose and hemicellulose is accelerated by the heat generated, consequently, the short processing time (Williamas, 2005). Furthermore, many microorganisms that are human and plant pathogens, are destroyed in this process provided it encounter sufficient high temperature.

1.5.6.2 Anaerobic composting

Anaerobic composting is a process of breaking down of organic matter by a microbial consortium in an oxygen-free environment (Kiyasudeen et al., 2016). This process is mainly found in anoxic environments including water-logged soils, watercourses, and sediments. The biogas compounds such as methane, organic acids, hydrogen sulphide, are composed during this method (Rasi et al., 2007). Moreover, these substances accumulate and are not metabolised further, with limited oxygen. Hence the process is longer than aerobic composting. During this process, fewer nutrients are lost (Williams, 2005). Additionally, the resultant compost becomes more effective in bioremediation, re-vegetation and erosion control due to its high organic matter content and biological activity (Alexander, 1999).

1.5.7 Advantages of composting

Composting has a distinct advantage of recycling waste compared to incineration and landfilling. Composting has several environmental problems, such as the emission of greenhouse gases (methane, ammonia emissions, and nitrous oxide) that contribute to eutrophication and acidification. However, it can transform complex organic substances, which are potentially hazardous to the environment, to mature and stable compost, with good hygiene and physical characteristics humic matter (Ranalli et al., 2001). Composting improves soil organic matter and mineralisation, and is also responsible for various soil properties, including soil productivity, water-holding capacity, soil aeration, medium biological activity and soil structure (Adugna, 2016). It also provides microorganisms capable of transforming insoluble matter into plant nutrients, degrading harmful substances and providing carbon to sustain the biodiversity of micro- and macro-fauna (Azim et al., 2018). The literature has also highlighted that composting enhances biological properties, such as basal respiration, microbial biomass C and some enzyme activities (Sayara et al. 2020); regulates moisture and improves drainage (Roman et al., 2015; Adugna, 2016; Scotti et al., 2015). This report can be explained by the slow release of nutrients during organic matter decomposition.

1.5.8 Composting of organic wastes

Compost is an environmentally sound natural material obtained when organic waste is reduced to organic fertiliser and soil conditioners through biological processes known as composting (Alexander, 1999; Gautam et al., 2010; Pan et al., 2012). While the potential benefit of compost is unquestionable, ultimately however, the quality of compost depends on the raw materials and biological processes and environmental factors. Hence, there is justifiable interest in finding ways to optimise the quality and yield of end-products of composting.

The composting process for converting organic wastes to humified compost end-product includes three phases (Pedro et al., 2003; Schloss et al., 2003; Pan et al., 2012). During the first

phase, a preliminary mesophilic stage (20 – 40 ^{o}C) temperature increases with carbon dioxide. Hellmann et al. (1997), Schloss et al. (2003), Novinsak et al. (2008) confirmed that the activities of mesophilic organisms reduced the compost substrate due to degradation of sugar and proteins. In phase two, thermophilic stage (35 – 65 ^{o}C) the mesophilic organisms are substituted by thermophiles causing temperature increase in the compost heaps from 45 ^{o}C to approximately 70 ^{o}C (Pedro et al., 2003; Schloss et al., 2003). The raised temperature during the thermophilic phase is responsible for eliminating pathogens and weed seeds (Jadia & Fulekar, 2008). Thus, it plays a vital sanitation role from an agricultural perspective (Mehta et al., 2014). Novinsak et al. (2008) also observed a rapid degradation of the vast number of pathogenic individuals during this time.

The third phase is the cooling stage, where the temperature of compost piles begins to decrease (Pan et al., 2012). The last stage, maturation is characterised by reduced microbial activity (Pan et al., 2012). Danon et al. (2008) and Bernal et al. (2009) demonstrated that during this stage, the removal of environmentally harmful products from the composted materials and phytotoxins occurs. However, the stability and quality of the compost end-product is determined by the raw composting materials (Ranalli et al., 2001; Benito et al., 2003; Wang et al., 2004). Several parameters including composting temperature, presence of potential pathogens, moisture content, C: N ratio and pH are used to assess the compost end-product quality and stability (Steger et al., 2007; Erickson et al., 2009; Fourti et al., 2011, Pan et al., 2012).

1.5.9 Biophysicochemical changes

The composting process also involves different biophysicochemical changes including pH, temperature, nitrogen and C: N ratio, moisture content, water holding capacity, etc. A previous study by Jadia & Fulekar (2008) reported a pH variation during the composting process. During

the first phase, a drop of 5.9 in pH was observed compared to 6.5 of the composting material in the ambient stage. However, a rapid increase of the pH value 8.6 occurred during the thermophilic stage. In the cooling phase, the pH shown a drop of 7.2 nearer to alkalinity. Nakasaki & Ohtaki (2002) reported that vegetable wastes contain acidic pH, and its material is further affected by the metabolic process during composting.

A rapid increase of temperature is normally observed during mesophilic and thermophilic stage, during organic decomposition (Jadia & Fulekar, 2008). The reason for temperature rise has been associated with the increased number of mesophiles. During the thermophilic stage, lignin degradation occurs (Fang & Wong, 2000). The temperature slowly decreases during the cooling stage. The temperature and the action of microorganisms decreases in the cooling phase. Zibilske (1999) confirmed that the maturation of compost starts when the temperature drops to that of the ambient phase.

The moisture content of 65.52% was maintained at the ambient phase and slowly dropped to 50% at the cooling stage (Jadia & Fulekar, 2008). Crawford (1985) recorded a variation of 50 to 70% of optimum moisture, in composting. Nakasaki & Ohtaki, (2002) for example, showed that the use of bulking agents made up of sawdust, tree bark, straw, and dry leaves maintained optimum moisture content. Guest et al. (2001) confirmed that the moisture content decreases as the organic matter decomposes. Moreover, Larney & Blackshow (2003) recorded a decline in the moisture content percentage due to high evaporating rates during the thermophilic phase of composting. During the composting process, the water holding capacity and electrical conductivity of organic matter was found to increase (Jadia & Fulekar, 2008). Guoxue et al. (2001) observed a slight increase in electrical conductivity (EC) of compost and degradation of organic matter to release cations might be the result.

Composting influences the chemical composition of organic matter. Decomposition of organic wastes by microbial populations leads to a decline in the concentration of organic carbon (Guest et al., 2001), nitrogen also affected, for example Jadia & Fulekar (2008) observed a speedy decline in the nitrogen content throughout composting in the thermophilic stage than that of the initial material. Crawford (1985) reported that the results of nitrogen loss might be because the organic material was in the form of recalcitrant cellulose and the unavailability of nitrogen present in "lignin humus complexes" formed by microbial activity in the composting process. Guest et al. (2001) concluded that the decrease in percentage of nitrogen concentration is due to the release of ammonia during the composting process. Guoxue et al. (2001) also recorded a reduction in C/N ratio during composting.

Jadia & Fulekar (2008) recorded high concentrations of nutrients, such as Ca, Zn, Cu, Mg, Fe, and Mn in the vegetable wastes. These observations in the status of nutrients are in conformity with those obtained by Dickerson (1999). Gaur et al. (1982) observed an increase in the total phosphorus content from the first up to the second month of Jowar stalk plus wheat straw compost. On the other hand, Gaur et al. (1982) observed a greater increase in the available phosphorus in the inoculated series of Jamun leaf compost during the first and second month by 15% and 65.6%, respectively.

1.5.10 Variation in microbial populations

Jadia & Fulekar (2008) showed a variation of microbes, such as *Pseudomonas* sp., *Streptomyces* sp. and *Bacillus* sp., at the ambient stage and *Pseudomonas* sp., *Bacillus sp*., *Flavobacterium* sp., *Closteridium* sp. and *Streptomyces* sp. were found during the mesophilic. Several thermophiles, such as *Bacillus* sp., *Streptomyces* sp., *Thermoactinomyces* sp., *Thermonospora* sp. and *Micropolyspora* sp. were found dominant during the thermophilic phase. During the entire process of composting, *Bacillus* species, were found to be dominant. Similar observations

were obtained by other authors (Fang & Wong, 2000; Gestel et al., 2003; Pedro et al., 2003). Fang & Wong (2000) reported that high temperature in composting mass might decrease the diversity of microbes. During the thermophilic phase of composting, the thermophiles play an important role in organic matter degradation (Zibilske, 1999). Jiarg et al. (2003) reported that the actinomycetes and fungi become dominant as the temperature decreases during the composting.

1.5.11 Enhancement of the composting process

While numerous studies have reported on the use of mechanical and physical interventions, including increasing turning frequencies of compost heaps, shredding of raw materials, and using ground raw materials to optimise composting process (Ogunwande et al. 2008 ; Kalamhad & Kazmi, 2009; Getahun et al. 2012; Petric et al. 2015; Mtimkulu et al. 2016), fewer studies have explored the use or manipulation of microorganisms for the purpose of enhancing the composting process (Gaur et al., 1982; Chaturvedi et al., 2010; Pan et al., 2012; Irawan et al., 2014; Sakar et al., 2016). Similarly, García et al. (2006) investigated the use of bacterial inoculants (Bacillus and actinobacteria) during composting of vegetable products. Zeng et al. (2009) and Figueiredo et al. (2013) also conducted a study using microbial inoculants to accelerate and improve the outcome of composting.

1.5.12 Ascomycota

Ascomycota is one of many other phyla that plays a significant role in the biotransformation of organic materials during composting (Meng et al., 2019). According to Singh et al. (2010), members of Ascomycota can secrete a variety of degrading enzymes and efficiently use nutrients in the compost. An increased rate of degradation of organic waste and decreased composting period has been reported by Zhang et al. (2016) as a result of Ascomycota dominance in lignocellulose-based compost. Wang et al. (2018) also reported the abundance of Ascomycota in composting of cow manure, food and garden waste and sewage sludge. Similar

findings were demonstrated by Meng et al., (2019). A recent study by Cai et al. (2018) showed relative dominance of these fungi during aerobic composting and vermicompost of green waste. The predominance of phylum Ascomycota was also reported (de Oliveira et al. 2016) throughout the composting process of press mud. Jurado et al. (2014) established similar results from lignocellulose-based composting.

Microorganisms, such as *Aspergillus niger*, *Aspergillus* sp. (R), *Trichoderma viride* and *Penicillium* sp. have been successfully used in the past to break down organic composts constituting varying waste materials (Gaur et al., 1982). These microorganisms have also been effectively utilized as an inoculant to treat seeds for germination and seedling vigor (Murali et al., 2012). Therefore, employing such microorganisms can play a vital role in recycling agro-industrial waste as they are known to have different characteristics such as phosphorus-solubilization and particularly, lignocellulolytic activities (Hyde et al., 2019).

1.5.13 The use of fungal inoculant

Fungal species are numerous and diverse during both mesophilic and thermophilic phases of composting. Their activities release important plant macro-and micro-nutrients to plants. The composting process depends on the activities of various groups of microorganisms, such as bacteria and fungi during the breakdown of raw compost materials to humus (Pan et al., 2012; Chatterjee et al., 2013). The beneficial effects of bacterial and fungal inoculants have been demonstrated by a few researchers. Gaur et al. (1982), for example, demonstrated that inoculating compost materials made up of jowar stalk, wheat straw (5:3) and jamun leaves with four mesophillic fungi, viz., *Aspergillus niger*, *Aspergillus* sp. (R.), *Thrichoderma viride* and *Penicillium* sp. reduced the composting period by one month and improved the quality of the composted end-product. In a more recent study by Pan et al. (2012), three bacterial isolates improved the chemical composition of the end-product of composting. Saprophytic fungi are

among the many groups of microorganisms that breakdown or mineralise organic materials. Some saprophytic fungi use lignocellulosic polymers as the primary source of carbon (Miller, 1996). Lignocellulose is generally considered to be recalcitrant and difficult to decompose and is typically biodegraded by Actinomycetes, Streptomycetes and some saprophytic fungi. Hence, certain saprophytic fungi play a vital role in the biodegradation and conversion process during composting.

Interestingly, many entomopathogenic fungi are saprophytes (Fernandes et al., 2012). Saprophytes live on dead or dying material and obtain energy by breaking down organic matter in dead plants and animals. Entomopathogenic fungi are better known for their ability to cause infection to insects and acarines (Fernandes et al. 2012; Clifton et al. 2018) than their bio-enhancing effects on composting. It has been reported that *Metarhizium anisopliae* var. *anisopliae*, *Beauveria bassiana* and *Verticilium lecanii* (Hypocreales) can produce extracellular enzymes, such as endoproteases, aminopeptidases, lipases, esterases and chitinases (St Leger et al. 1986) all of which have pertinent roles during composting. A few researchers, Stewart & Brown (2008) for examples, showed that the endophytic fungus *Clonostachys rosea* f. *catenula* can be combined with other beneficial fungi or bacteria.

1.5.14 *Clonostachys rosea* f. *catenula*

Clonostachys rosea f. *catenula* belongs to the order Hypocreales. It is endophytic, saprophytic and capable of parasitising insects, nematodes and other microorganisms. As a saprophyte, *C. rosea* is expected to be capable of degrading organic waste and lignocellulose as other Ascomycota fungi. Saprophytes can break down and transform organic residues into a humus-like quality end-product (Bustamante et al., 2013). These fungal microorganisms are known to produce kinds of enzymes involved in the decomposition of recalcitrant plant residual matter. For instance, a study by Osono (2006) and Osono et al. (2009) recommended utilisation of

lignocellulosic fungi during composting of leaf litter. Nonetheless, the fungal abundance in the process of composting may be influenced by the natural or initial quality of crop residues and ambient environment (Dilly & Munch, 2004; Johnson et al., 2007; Betrand et al., 2009; Wu et al., 2011; Zhao et al., 2011).

Because *C. rosea* f. *catenula* has endophytic and a mycoparasitic activities against pathogenic fungi, it is currently used as a biocontrol agent for management of insect pests and stem and root rots (Chatterton & Punja, 2010; Moloinyane, 2018). It exudes mycoparastic enzymes, antibiotics or metabolites, which are potentially responsible for the bioactivities of this fungus. *C. rosea* f. *catenula* reduces the independency on chemical pesticides for pest control, for examples, control of *Pythium*, *Botrytis,* and *Fusarium* spp. This fungus can stimulate plant health, root growth, size or increases yields (Stewart & Brown, 2008). However, little or no information is available on the use of *C. rosea* f. *catenula* for enhancement of the composting of organic wastes. The current study investigated the prospects of using *C. rosea* f. *catenula* as an inoculant for enhancing the composting of organic wastes.

CHAPTER TWO

2.1 Materials and Methods

2.1.1 Collection of organic wastes

Disposed fresh cabbage wastes (Figure 2.1) were collected from two farms (Groente Verpakkers farm and Schultz Vars Produkte Mark) situated in the Philippi horticultural area, Cape Town, South Africa. The collection was done in the mornings (between 08H:00 and 12:00 noon), and the wastes were collected using sanitary hand gloves to avoid contamination. The collected wastes were placed in black polythene bags. The wastes were chopped separately (approx. size 2–4 cm^2), and then mixed well to form a single big heap. Subsequently, the big heap was sub-divided equally into 12 smaller heaps of 1 m diameter and 50 cm height.

Figure 2.1: Collection of vegetable waste from the farms (A), preparation of heap treatments and arrangement of compost piles (B).

2.1.2 Fungal isolate

An indigenous *C. rosea* f. *catenula* (Hypocreales) fungal isolate (SM4A) (Figure 2.2) was used in this study. The fungal isolate was isolated from a soil sample originating from a vineyard in the Western Cape Province by baiting of the soil with fifth instar larvae of *Cydia pomonella* (codling moth) (Insecta) as described in Moloinyane (2018). Pure cultures of the isolates were maintained in the Biology Research Laboratory of the Department of Horticultural Sciences, Cape Peninsula University of Technology.

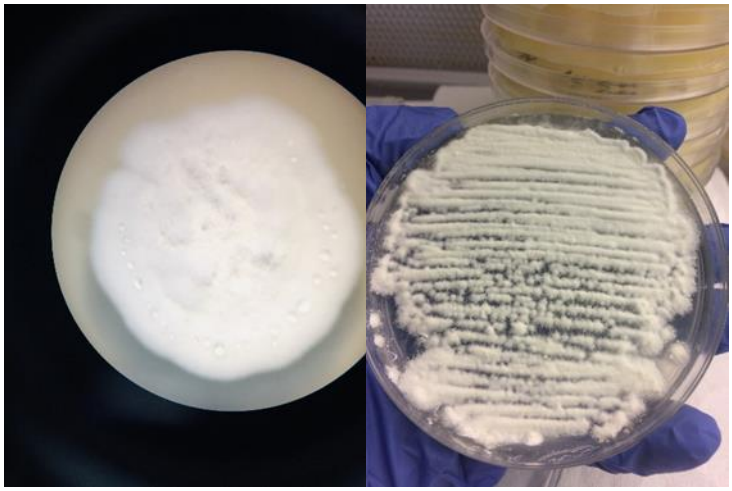

Figure 2.2: Cultures of *Clonostachys rosea* f. *catenula* fungal isolate.

2.1.3 Culturing of fungi and preparation of fungal treatment

Clonostachys rosea f. *catenula* was cultured in Petri dishes (Figure 2.3) containing a selective medium, made up of half-strength Potato Dextrose Agar (PDA), 0.02 g/L of ampicillin (Sigma-Aldrich (Pty) Ltd, Kempton Park), and 0.04 g/L streptomycin (Sigma-Aldrich (Pty) Ltd, Kempton Park) to prevent bacterial growth. Fungal cultures were incubated at 25 °C in the dark for four weeks. Mature conidia were obtained by gently scraping the conidia off from the surface of the culture. Sub-samples of the harvested conidia were used in a germination test to determine conidial viability according to the method described by Goetel & Inglis (1997). Briefly, 0.1 ml of conidial suspension prepared at ($1 \text{ X } 10^4$ conidia mL^{-1}) and containing 0.1%

(v/v) Tween 80 was spread-plated on sterile PDA plates and incubated for 24 hours in the dark, and then examined under a light microscope.

Conidial germination was determined from 100-conidium counts of four replicates. The remaining conidia were used to prepare conidial suspension for the fungal treatment. Matured four weeks old *C. rosea* f. *catenula* conidia obtained from PDA plates were transferred to 2 L glass bottles containing sterile 0.1% (v/v) Tween 80 in distilled water, capped, mixed by agitating the bottle for 5 minutes by shaking and using a magnetic stirrer (at 20 °C, 300 rpm for 30 minutes) to form a homogenous conidial suspension. The conidia concentration was enumerated using a haemocytometer (Neubauer, Merck, South Africa) and observed with a light microscope at 40X magnification. In order to obtain the desired **1** X 10^4 conidia mL $^{-1}$mL, the volume of sterile 0.1% (v/v) Tween was increased, or conidia added to the glass bottle.

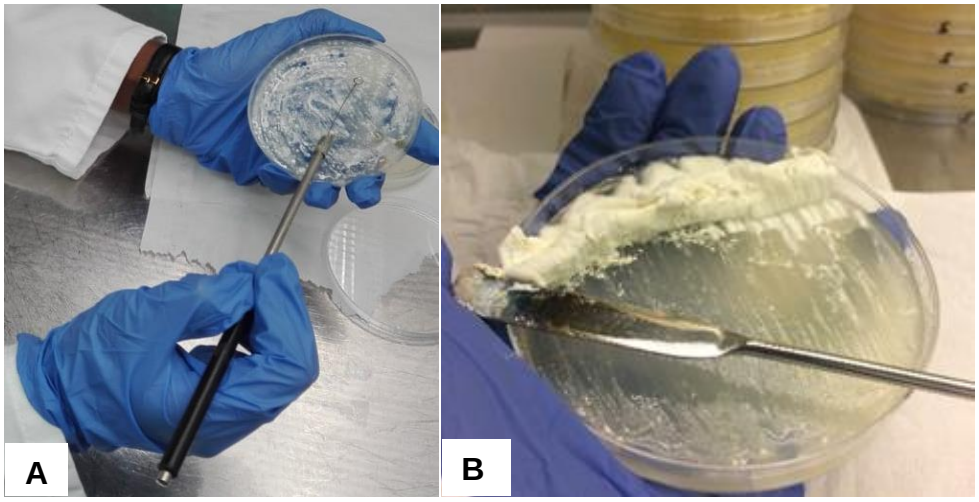

Figure 2.3: Culturing *C. rosea* f. *catenula* on petri-dishes containing PDA medium (A). Scrapping of fungus from the surface of the selective medium (B).

2.1.4 Experimental design and treatments

A single factor experiment design was used to test the main effect of one factor (fungus inoculum of *Clonostachys rosea* f. *catenula*) with one conidial concentration on composting materials consisting of solid raw organic cabbage wastes. This experiment was conducted in

open-air conditions at the Department of Horticultural Sciences, Cape Peninsula University of Technology (Bellville Campus) South Africa. Figure 2.4). Twelve heaps (dimension: 1 m diameter x 50 cm height) of compost materials consisting of chopped cabbage wastes were each placed two meters apart. Six compost heaps were inoculated with a 1000 ml suspension of *C. rosea* f. *catenula* at a concentration of 1 X 10^4 conidia mL^{-1}. Each of six control heaps received 1000 ml of sterile distilled water containing 0.1% (v/v) Tween 80 without the fungal conidia. The heaps were turned twice a week using clean and sterile spades to increase aeration and the oxygen supply to microorganisms. The used spades were dipped into 1% sodium hypochlorite solution, and then rinsed with sterile water before reuse. Each heap was randomly allocated to the test or control treatments. All heaps were uncovered and exposed to environmental conditions throughout the composting process. The composting process was conducted for three months (from December 2019 to February 2020). Water was equally supplied to the compost heaps using a watering can and each of the composting mixture received 1000 ml of sterile water. Heaps were only watered once every two weeks and watering was stopped from nine to 12 weeks, and only 9 L of sterile distilled water added in total.

Figure 2.4: Treatment heaps; Fungus treated cabbage waste materials (A). Composting materials with no fungus treatment (B).

2.1.5 Capturing heap temperature, pH and humidity data

During the composting period, heap temperature was monitored weekly, and twice each day in the mornings and afternoons until the end of the composting process, the average daily data was subsequently pooled to give mean weekly data, which was eventually analysed statistically and presented as results. A 1 m long thermometer probe (Major Tech). A 2-in-1 soil pH-Moisture Meter (Major Tech [Cape Agricultural Products] Cape Town, South Africa) with a humidity reference scale of 0-10 (equivalent to 0-100% humidity) was used to monitor pH and moisture content. The humidity reference scale was further classified as follows: 0-3 (low humidity), 4-7 (moderate humidity), and 8-10 (high humidity) was used to monitor pH and moisture content. All heap measurements were taken in the center, avoiding the side walls as these could be influenced by wind and direct sunlight. The ambient environmental conditions such as temperature and humidity data were also recorded.

Figure 2.5: Measuring compost heap temperature data (A) and recording pH & moisture content of the compost heaps (B).

2.1.6 Heap colour assessment

The colour of the compost was observed and classified using the Munsell Book of colour (Munsell, 1976) as a reference at the end of the experiment. The colour of heaps is an important physical characteristic that can be used to assess the rate of composting and the maturing of the compost.

2.1.7 Chemical analysis of composted materials and plant tissue nutrient content

Chemical analyses of the composted materials were carried out by Bemlab Pty Ltd, Somerset West, South Africa. The micro-nutrients (B, Fe, Zn, Cu, and Mn) and macro-nutrient (N, P, K, Ca, Mg and C) contents in one kilogram were determined at the end of the trial using the methods described by Campbell & Plank (1998), Miller (1996); Walkley & Black (1934) and the Non-affiliated Soil Analyses Work Committee (1990) as described in Mtimkulu et al. (2016).

Plant Tissue/Nutrient Analysis of Leaf samples of tomato plants that were cultivated in substrate mix from pre-treated heaps with *C. rosea* inocula were analysed for macro- and micro-nutrient contents at Bemlab (Pty) Ltd, Somerset West, South Africa. The K, P, C, Ca, Mg, Na, Mn, Fe, Cu, Zn, and B and B contents of the extracts were measured using ICP-AES analysis method (Campbell & Plank, 1998). The total nitrogen (N) content of the leaves was determined through combustion in a Leco N-analyser (Leco Corporation, St Joseph, MI, USA).

2.1.8 End product toxicity assessment - germination and seedling tests

Germination and seedling toxicity tests were conducted to assess composting end-product (Figure 2.6) toxicity on tomato Composted materials obtained from the control and test treatments following three months of composting were mixed with coarse river sand obtained from Stanler Farms Nursery Pty. Ltd., Cape Town, as follows: 25% fungus treated compost end-product and 75**% (**by volume) course river sand as test sets and 25% untreated compost

end-product and 75% (by volume) course river sand as control sets.The samples were each placed in a 15 cm plastic potting cups. Each of the 15 cm empty plastic pots were placed on a scale and weighed out at “TARE” 0.00 g. The pots were separately filled with coarse river sand approximately at 11.25 cm of the pot and weighed 1443.9 g and represented 75% proportion of coarse river sand and the remaining part in the pot was topped with composting end-product approximately at 3.75 cm up to the rim of the pot and weighed 263.8 g and represented 25% proportion of the composting end-product, for the test and control treatments. The total weight of each filled pot was scaled at 1707.7 g, equivalent to 100% compost-river sand growth media. Sixty Oxheart heirloom tomato seeds were sown in separate pots for germination test. For the seedling toxicity test, sixty Oxheart heirloom tomato seedlings were also transplanted into separate pots. Composted samples were obtained from three randomly selected heaps belonging to control and test treatments. The samples were watered once each day and maintained for eight weeks in a greenhouse under the following conditions: an average day temperature of 25 ± 5 °C and average relative humidity (RH) of 65 ± 5%.

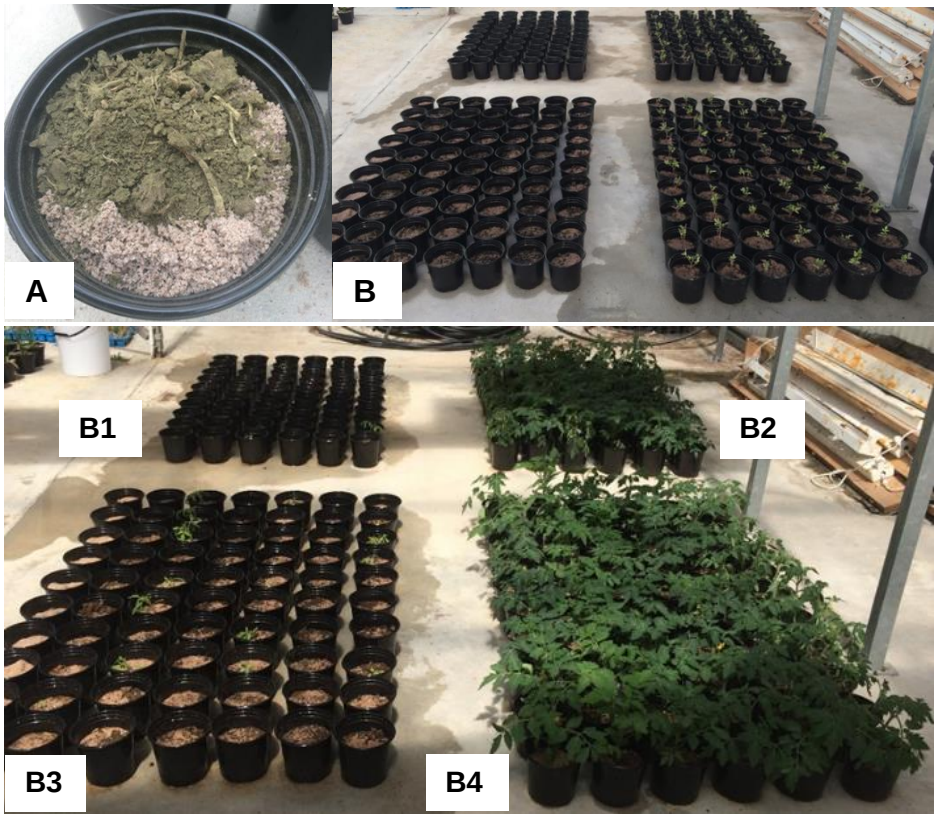

Figure 2.6: Composted end-product materials (grounded & sieved) mixed with coarse river sand (A). Pots containing media concentration ratio of 25% compost end-product and 75% coarse river sand (B), Fungus-treated potted *S. lycopersicum* seeds (B1) & seedlings (B2) and control-treated potted *S. lycopersicum* seeds (B3) & seedlings (B4).

2.1.9 Evaluating endophytic activity and red spider mite infestations on tomato plants following treatment of raw compost materials with *C. rosea* f. *catenula*

Twenty potted plants were randomly allocated to substrate mix containing composted materials from heaps that were pre-treated with *C. rosea* f. *catenula* or composted materials from heaps that were not pre-treated with the fungus. The soil mix consisted of 263.8 g composting end-product and 1443.9 g coarse river sand by weight, representing 25%:75% ratio by volume. The plants were irrigated with sterile distilled water and 250 ml was drenched into each plant and were maintained in a greenhouse that had residual red spider mite infestations, and which were allowed to infest the plant naturally. The experiment was conducted in an environmentally controlled greenhouse (an average day temperature of 25 ± 5 ˚C and relative humidity (RH) of 65 ± 5%) located at the Cape Peninsula University of Technology, Bellville, Western Cape, South Africa, from 11 November to 10 January 2020 (2 months).

The effect of tissue colonization of *S. lycopersicum* by the fungus was assessed two months after the cultivation of tomato plants in the greenhouse. The samples of substrate mix from pre-treated compost heaps with *C. rosea* f. *catenula* inoculum was used to grow tomato plants in pots. A total of 9 plants were randomly selected from the fungus-treatment and the control-treatment, making up to 18 potted replicates and examined. Endophytic colonization of *C. rosea* f. *catenula* of tomato plant leaf was assessed at 21 days by re-isolation. One leaf was carefully excised from individual plant materials and transferred to sterile laminar flow cabinet. Rectangular leaf sections of 1-2 mm^2 were cut. These sections were separately surface-sterilised with 1% sodium hypochlorite for 1 min, followed by 1 min in 70% (v/v) ethanol and rinsed

twice in sterile distilled water, then transferred on to the selective medium [half-strength (PDA at 19.5 g/L containing 0.02 g/L ampicillin (Sigma-Aldrich), and 0.04 g/L streptomycin (Sigma-Aldrich, Johannesburg, South Africa)], and incubated at 25 ˚C. Three leaf parts per plant were plated on PDA, equating to 54 leaf sections. The leaf sections were visually examined daily, up to 10 days, for the presence of fungal outgrowth. The presence of *C. rosea* outgrowth in at least one of the leaf sections was considered as an indication of successful plant colonisation (Figure 2.7). The data was expressed in percentage colonisation (number of plant replicates colonized / number of plants replicates excised X 100).

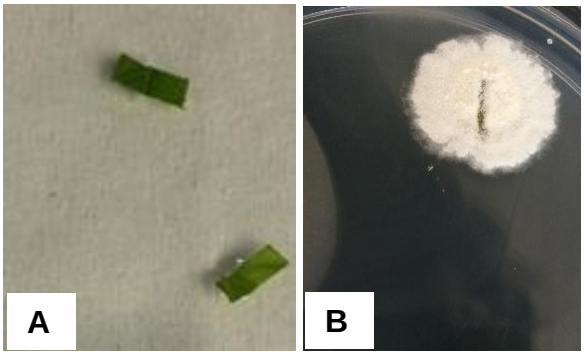

Figure 2.7: Leaf sections of tomato plants that were grown in composted materials obtained from compost materials inoculated with *C. rosea* f. *catenula* and control treatment were plated on PDA selective media. These samples were surface sterilised: (a) The petri dish correspond to control samples; (b) *C. rosea* treated samples.

2.1.10 Insect infestation of tomato plants

The effect of treating compost heaps with *C. rosea* f. *catenula* inocula on the red spider mite (*T. urticae*) infestations of tomatoes was assessed by enumerating the number of plants that were infested by the red spider mites at the end of the experiment (2 months' post-treatment). The average temperature in the environmentally controlled greenhouse ranged from 25 ± 5 °C, and the average relative humidity of 65 ± 5%.

2.1.11 Statistical analyses

For comparison of the control and treatment groups, the experiment data collected, viz., heap temperature, pH, moisture, compost nutrient contents, number of seeds germinated, number of live seedlings and endophytic test were analysed using One-Way ANOVA. The post hoc Tukey test was used to separate the means. Statistical significance was performed at $p = 0.05$ level. The data for environmental conditions was collected in the morning and afternoon and was statistically analysed using One-Way ANOVA. The significant difference of end-product toxicity on tomatoes were determined using Pearson Chi-square (χ^2) test on the seed germination and seedling growth in the test and control and the number of plants that were infested with the red spider mite infestations in fungus and control composted materials. All statistical analyses were performed using PAST version 3.20 (Øyvind Hammer, Oslo, Norway) (Hammer et al., 2001). The values are reported as mean ± SE (standard error).

CHAPTER THREE

3 Results

3.1 The effect of fungal treatment on compost heap temperature and heap humidity

Heap temperature showed significant differences between treatments at weeks one, seven, eight, and nine in the morning (DF (degree of freedom) = 1, 10; $p = 0.05$), and weeks two, and nine in the afternoon (Table 3.1).

Table 3.1: Variation in heap temperature (mean ± SE) following exposure to *Clonostachys rosea* f. *catenula* inoculum and control treatment (non-fungus) during the composting of raw cabbage waste over a 12-week period of composting

	Fungal treatment		Treatment Control	
Weeks	Morning	Afternoon	Morning	Afternoon
1	35.12 ± 0.6 b	36.58 ± 0.9 a	39.35 ± 0.2 a	36.28 ± 0.3 a
2	26.92 ± 1.5 a	25.88 ± 0.2 b	28.68 ± 2 a	26.83 ± 0.3 a
3	23.77 ± 0.5 a	25.62 ± 0.5 a	24.08 ± 0.1 a	29.53 ± 2.0 a
4	28.98 ± 1.7 a	30.53 ± 0.6 a	27.9 ± 0.2a	31.98 ± 0.6 a
5	29.12 ± 1.6 a	31.62 ± 0.7 a	29.63 ± 1.5 a	33.2 ± 0.4 a
6	28.53 ± 0.4 a	34.48 ± 0.9 a	28.72 ± 0.3 a	34.17 ± 0.3 a
7	24.37 ± 0.3b	27.76 ± 0.4 b	26.8 ± 0.3 a	31.22 ± 0.8 a
8	26.9 ± 0.8 b	29.62 ± 2.3 a	29.15 ± 0.5 a	29.55 ± 0.8 a
9	27.37 ± 0.3 b	31.05 ± 0.4 b	29.45 ± 0.2 a	34.23 ± 0.8 a
10	28.82 ± 10 a	32.58 ± 0.9 a	30.68 ± 1.0 a	33 ± 0.8 a
11	27.23 ± 0.9 a	31.62 ± 0.9 a	28.65 ± 0.6 a	34.33.00 ± 0.9 a
12	29.03 ± 2.0 a	33.22 ± 0.9 a	33.37 ± 1.0 a	34.55 ± 0.9 a

Values are means ± SE. (standard error). Values followed by the same lowercase letters in the same row do not show s significant difference at $p > 0.05$ following a comparison by using the Tukey test.

3.2 Variation in environmental conditions (relative humidity and ambient temperature)

The data for environmental conditions was collected in the morning and afternoon and is shown below (Table 3.2).

Table 3.2: Changes in relative humidity (mean ± SE %) and ambient temperature readings (mean ± SE ˚C) during the composting of raw cabbage waste over a 12-week period of composting

	Relative humidity		Ambient temperature	
Weeks	Morning	Afternoon	Morning	Afternoon
1	50.2 ± 3.5	52.84 ± 5.1	30.52 ± 3.6	31.16 ± 4.7
2	54.42 ±1	57.1 ± 1.7	25.04 ± 1.0	24.18 ± 1.9
3	55.54 ± 4.9	53.96 ± 2.7	24.66 ± 0.9	23.12 ± 0.4
4	60.76 ± 2.7	56.16 ± 3.0	26.26 ± 1	26.32 ± 1.3
5	59.4 ± 3.1	56.32 ± 5.7	25.68 ± 0.7	24.78 ± 0.6
6	63.3 ± 2.6	66.04 ± 3.4	28.08 ± 1.4	26.34 ±1.2
7	53.36 ± 1.5	51.6 ± 2.4	28.1 ± 0.6	29.42 ±1.4
8	55.36 ± 2.0	53.74 ± 2.7	27.68 ± 0.6	26.62 ± 0.8
9	59.18 ± 3.6	60.02 ± 2.4	28.08 ± 1.6	27.68 ± 0.3
10	59.64 ± 0.3	57.06 ± 2	26.34 ± 0.6	27.76 ± 0.5
11	60.76 ± 2.7	56.06 ± 1.8	27.2 ± 1.0	28.26 ± 0.6
12	51.5 ± 1.4	47.74 ± 2.6	28.66 ± 0.6	27.18 ± 0.6

Values are means ± SE. (standard error). Values followed by the same lowercase letters in the same row in either relative humidity or ambient temperature do not show a significant difference at $p > 0.05$ following a comparison by using the Tukey test.

3.3 Heap moisture

The fungal inoculum did not have any significant effect ($p > 0.05$) on heap moisture compared to the control at week one, three to four, six to seven, and ten to 12 weeks in both morning and afternoon. However, there were significant differences recorded in heap moisture in the morning at week five and nine, and afternoon at week two and eight post-treatment (Table 3.3).

Table 3.3: Changes in heap moisture reading (mean ± SE) following exposure to *Clonostachys rosea* f. *catenula* inoculum and control treatment (no fungus) during composting of raw cabbage waste

	Fungal Treatment		Control Treatment	
Weeks	Morning	Afternoon	Morning	Afternoon
1	52.97 ± 22.2 a	46.65 ± 1.8 a	51.92 ± 1.7 a	40.98 ± 7.5 a
2	43.53 ± 3.3 a	48.5 ± 2.6 a	39.92 ± 1.9 b	40.96 ± 1.5 b
3	44.23 ± 4.9 a	46.97 ± 3.9 a	39.33 ± 2.7 a	40.3 ± 3.9 a
4	44.87 ± 2.9 a	40.58 ± 4.9 a	42.23 ± 3.0 a	47.23 ± 3.4 a
5	43.2 ± 0.8 a	49.23 ± 2.6 a	52.1 ± 3.7 b	52.23 ± 1.2 a
6	44.45 ± 1.7 a	43.42 ± 1.5 a	40.1 ± 15.5 a	45.45 ± 1.0 a
7	37.9 ± 1.2 a	38.42 ± 3.1 a	42.52 ± 2.2 a	37.85 ± 1.6 a
8	44.72 ± 1.1 a	49.47 ± 1.2 a	38.87 ± 3.9 b	44.45 ± 2.1 b
9	22.15 ± 1.6 a	19.48 ± 0.9 a	17.9 ± 0.7 b	21.07 ± 1.3 a
10	0.0 ± 0.00 a	0.00 ± 0.0 a	0.00 ± 0.0 a	0.00 ± 0.0 a
11	12.07 ± 1.4 a	0.00 ± 0.0 a	11.37 ± 0.9 a	0.00 ± 0.0 a
12	0.0 ± 0.00 a	0.000 ± 0.0 a	0.00 ± 0.0 a	0.00 ± 0.0 a

Values are means ± SE. (standard error). Values followed by the same lowercase letters in the same row do not show a significant difference at $p > 0.05$ following a comparison by using the Tukey test.

3.4 pH variations

There were no significant differences ($p > 0.05$) of compost heaps with fungal treated materials and the control at week one to 12- weeks, for both the morning and afternoon readings including, the final end-product after composting (Table 3.4). An interesting similarity was observed in the end-product of composting; the pH values were alkaline for both treatments, ranging from 7.5 to 7.6 (Table 3.5).

Table 3.4: pH variation (mean ± SE) following exposure to *Clonostachys rosea* f. *catenula* inoculum and control treatment (no fungus) during the composting of raw cabbage waste over a 12-weeks period of composting

	Fungal treatment		Treatment Control	
Weeks	Morning	Afternoon	Morning	Afternoon
1	4.50 ± 0.1 a	4.70 ± 0.1 a	4.60 ± 0.1 a	4.80 ± 0.1 a
2	5.30 ± 0.2 a	5.00 ± 0.2 a	5.20 ± 0.0 a	5.60 ± 0.4 a
3	5.20 ± 0.2 a	4.90 ± 0.2 a	5.40 ± 0.2 a	5.30± 0.1 a
4	5.00 ± 0.1 a	5.20 ± 0.3 a	5.00 ± 0.1 a	4.80 ± 0.2 a
5	5.10 ± 0.0 a	4.60 ± 0.1 a	4.50 ± 0.2 a	4.50 ± 0.1 a
6	5.40 ± 0.3 a	5.00 ± 0.1 a	5.20 ± 0.1 a	4.90 ± 0.0 a
7	5.30 ± 0.1 a	5.30 ± 0.2 a	5.00 ± 0.5 b	5.30 ± 0.1 a
8	5.00 ± 0.1 a	4.70 ± 0.1 a	5.30 ± 0.3 a	5.00 ± 0.1 a
9	6.10 ± 0.1 a	6.20± 0.0 a	6.30 ± 0.0 a	6.1 ± 0.1 a
10	7.00 ± 0.0 a	7.00 ± 0.0 a	7.00 ± 0.0 a	7.00 ± 0.0 a
11	6.40 ± 0.1 a	7.00 ± 0.1 a	6.50 ± 0.0 a	7.00 ± 0.0 a
12	7.00 ± 0.0 a	7.00 ± 0.0 a	7.00 ± 0.0 a	7.00 ± 0.0 a

Values are mean ± SE. (standard error). Values followed by the same lowercase letters in the same row do not show a significant difference at $p > 0.05$ following a comparison by using the Turkey test.

3.5 Changes in colour of the compost during composting

Variations in colour were observed between treatments of compost heaps, green raw materials at the beginning of the experiment to brown (control) and dark brown (fungal treated heaps) colour at 12 weeks post treatment. The Munsell book of colours (Munsell, 1976) was used, where compost heaps with no fungal inoculum was 5 YR 4/4 (brown in colour) and fungal treated heaps was 5 YR 3/3 (dark-brown colour).

3.6 Chemical analysis of compost

Generally, the macro- and micro-nutrient levels in composted materials were not significantly different ($p > 0.05$) between control and fungus treatments (Table 3.5).

Table 3.5: The effect of treatment with *Clonostachys rosea* f. *catenula* on the chemical composition of the end-product of the composting of solid cabbage waste compared to the control treatments (no fungus) over a 12-weeks period of composting

Parameters	Fungus treatment	Control treatment
pH	7.60 ± 0.0 a	7.50 ± 0.0 a
N mg/kg	22540.00 ± 1111.1 a	24660.00 ± 2182.1 a
P mg/kg	6400.00 ± 158.1 a	5860.00 ± 254.2 a
K mg/kg	6740.00 ± 958.4 a	7400.00 ± 931.1 a
Ca mg/kg	39880.00 ± 446.5 a	38220.00 ± 950.5 a
Mg mg/kg	2960.00 ± 67.8 a	3180.00 ± 345.5 a
Na mg/kg	2099.60 ± 571 a	1680.90 ± 217.6 a
Mn mg/kg	34.90 ± 1.5 a	36.20 ± 0.9 a
Fe mg/kg	199.92 ± 14.7 a	210.30 ± 6.6 a
Cu mg/kg	6.90 ± 1.1 a	8.32 ± 1.3 a
Zn mg/kg	75.04 ± 6.8 a	68.538.00 ± 8.6 a
B mg/kg	26.83 ± 1.9 a	27.9 ± 2.7 a
C mg/kg	221.34 ± 23.3 a	182.56 ± 12.8 a
C/N ratio	8:1 ± 0.4 a	8:1 ± 0.2 a

Values are mean% ± SE. (standard error). Values followed by the same lowercase letters in the same row do not show a significant difference at $p > 0.05$ following a comparison by using the Tukey test.

3.7 Re-isolation of the fungus from the compost end-product

Clonostachys rosea f. *catenula* was successfully re-isolated from the composted materials from the fungus-treated heaps (Figure 3.8).

Figure 3.8: Reisolation of *C. rosea* f. *catenula* isolate from the composted end-product materials obtained from fungus-treated heaps.

3.8 End product toxicity -Germination and seedling test

At the 25%:75% compost: sand ratio mix, composted materials from fungus inoculated heaps were more favourable to germination of tomato seeds, where a significant difference (p <0.05) in seed germination (χ^2 = 12.102; p = 0.0005) was obtained. We obtained 93% successful germination in the fungus inoculated compost compared with 68% in the control treatment. In the seedling test, no seedling was affected by the fungus or control treatment, 100% of seedings were alive. Furthermore, there were no symptoms of yellowing and burning of leaf edges of plants were observed on tested seedlings (Table 3.6).

Table 3.6: Seed germination and seedling growth of *Solanum lycopersicum* plants cultivated in growth medium made up of the end-product of composting that were obtained from *Clonostachys rosea* f. *catenula*-containing compost heaps and control- compost heaps (no fungus). The growth medium was composed of 25% composted materials: 75% sand

Percentage Seed germination		**Percentage Seedling growth**	
Treatment	Control	Treatment	Control
93 a	68 b	100 a	100 a

Means with same lowercase letters in the same row in either seed germination or seedling growth are not significantly different following Pearson Chi-square test (χ^2) at $p = 0.05$ level of significance for seed germination and seedling growth.

3.9 Re-isolation of fungus from tomato tissues

After 21 days of inoculation, *C. rosea* f. *catenula* was successfully re-isolated from leaf sections placed onto PDA plates from all nine plants (100%) grown on composted end-product materials obtained from fungus-treated raw compost heaps (Figure 2.7). No fungal outgrowth of *C. rosea* f. *catenula* was observed in the plants grown in the control treatment compost and sand substrate.

3.10 Protection of Tomato Plants Against Red Spider Mite Infestation

A lower *T. urticae* infestation level was observed among plants grown on composted materials from fungus treatment. However, overall, the *Clonostachys rosea* f. *catenula* isolate had a limited effect on *T. urticae* infestation occurrence on tomato plants cultivated in compost end-product substrate originating from control and fungus-treated heaps (χ^2= 0.96; $p = 0.32$). Of the 20 potted plants, cases of red spider mite infestations ranged between 6 and 9 for fungus and control treatments, respectively (Table 3.7).

Table 3.7 Infestation occurrence of *Tetranychus urticae* on tomato seedlings cultivated in substrate mix containing composted materials from either organic waste heaps that were inoculated with *Clonostachys rosea* f. *catenula* or control treated heaps

	Plants cultivated on fungus-treated heaps	Plants cultivated on control-treated heaps
Number of plants infested by *T. urticae*	6/ 20	9/ 20

Samples of plants that were infested with red spider mites from the total number of planted tomato seedlings.

3.11 Effect of fungus on plant tissue nutrient content

Generally, the compost from heaps exposed to fungus did not significantly increase the tissue macronutrient and micronutrient levels of plants compared with the control composted samples. However, K (32750 mg/kg), Ca (19250) and Na (2887.5 mg/kg), were higher in the leaf tissues of fungus-treated plants (DF = 1, 6; $p < 0.05$) compared to the control plants, which were 26000 mg/kg K, 17425 mg/kg Ca and 1950.5 mg/kg Na (Table 3.8). However, the Fe concentration in plants exposed to fungus-treated materials was in general lower compared to the control treatment compost ($p > 0.05$).

Table 3.8: Tissue nutrient contents (mean ± SE) of leaves of *Solanum lycopersicum* plants grown for eight weeks under greenhouse conditions that were exposed to composts originating from control compost heaps and *Clonostachys rosea* f. *catenula* inocula exposed

Parameters	Treatment	Control
Macronutrients		
N mg/kg	16575.00 ± 1527.2 a	17600.00 ± 1987.0 a
P mg/kg	1500.00 ± 91.3 a	2200.00 ± 385.1 a
K mg/kg	32750.00 ± 3637.2 a	26000.00 ± 4830.5 a
Ca mg/kg	19250.00 ± 1600.8 a	17425.00 ± 2917.9 a
Mg mg/kg	5000.00 ± 430.1 a	5650.00 ± 1278.3 a
Na mg/kg	2887.50 ± 617.2 a	1950.50 ± 355 a
Micronutrients		
Mn mg/kg	59.10 ± 9.1 a	49.70 ± 11.9 a
Fe mg/kg	120.50 ± 14.9 a	153.85 ± 65.4 a
Cu mg/kg	4.20 ± 0.5 a	4.35 ± 0.9 a
Zn mg/kg	50.10 ± 1.5 a	53.80 ± 10.6 a
B mg/kg	56.37 ± 6.9 a	46.58 ± 4.8 a
C mg/kg	413.30 ± 1.3 a	414.10 ± 6.5 a
C/N ratio	25:1 ± 2.6 a	24:1 ± 2.6 a

Values are mean ± SE. (standard error). Values followed by the same letter in a row do not show a significant difference at $p > 0.05$ following a comparison by using the Tukey test.

CHAPTER FOUR

4 Discussion

Besides colour change, generally, *Clonostachys rosea* f. *catenula* inoculum on composting heaps did not showed varied effects on the physical characteristic (heap temperature) over time in the current study. Similarly, there were no significant differences in the chemical constituents of composted materials in this study. However, *C. rosea* offered some benefits to the composted materials and was generally not toxic to plants. The fungus persisted in the composted materials and was successfully re-isolated from the composted end-product of fungus treated heaps and from plants' leaves that were cultivated on the composted material from fungus pre-treated heaps. Furthermore, lower number of plants cultivated on the composted materials from the fungus treatment were infested by red spider mites. These findings are discussed below.

4.1 Heap temperature and humidity

The highest weekly average temperature recorded in this study was 39.35 °C, and this was relatively lower when compared to other similar studies (Ribeiro et al., 2017; Sundberg & Jonsson, 2005); however, the heaps in this study were small and in open air conditions. Gaur et al. (1982) observed maximum temperatures of 45 °C and 51 °C during composting of the mixture of jowar stalk and wheat straw, and jamun leaves inoculated with *Aspergillus niger* and *Trichoderma viride*, respectively. However, the study by Sakar et al. (2016) differed from the present study. For example, the study by Sakar et al. (2016) did not show an increase in temperature in pits and earthen pots of compost wastes containing dung and market waste and vegetable waste and, dung but when the ratio of 1: 5 chopped rice straw was added to the waste, heap temperature of 65.9 °C was observed after 24 hours. Nevertheless, the influence of the experimental conditions, such as heap size, ambient temperature and humidity and turning frequencies on the composting process and the outcomes cannot be underestimated (Mtimkhulu et al., 2017).

Possible reasons for the low temperature of composting in the present study could have been due to high water content of the solid vegetable wastes, evaporation, frequent turning of the heaps, compost heaps exposure to the environment and a low volume of heap material (Mtimkulu, 2016). Temperature is a key parameter in composting, as it indicates the state of decomposition and guarantees sanitation of the end-product (Paradelo et al., 2013). Generally, low temperatures were recorded in the current study, but there are subtle differences noted in heap temperatures among the different treatments. Higher temperatures were recorded in the morning and afternoon during the first week in both treatments before dropping in the second and third week, although the experiment ran for 12 weeks.

It was also observed that treatments showed higher values in the afternoon, from the 4th to the 6th week while temperature drops in the morning, respectively. The rise and drop in temperature suggest that the composting process might have gone through the three phases: the mesophilic, thermophilic and maturation phases. In this study, the data shows that the effect of fungus on moisture content was not significant. However, the heap moisture contents decreased in both treatments over time, from weeks 1 to week 12.

Moisture is considered as one of the important parameters affecting the composting process (Anastasi et al., 2005; Pan et al., 2012). However moderate moisture content of 40-60% obtained in this study is the range of optimum moisture content for composting. This agrees with those of Bernal et al. (2009). Ribeiro et al. (2017) also expressed that values of 50 to 60% of moisture content are ideal for efficient composting. Higher moisture content during composting causes waterlogging that might delay the composting process (Saidi et al., 2008; Chen et al., 2012; Makan et al., 2013, Sarkar et al., 2016). It is worth noting that the inoculation of composting materials with beneficial microorganisms has a potential to sustain the water content of heaps.

4.2 Chemical properties

Nutrient contents of the cabbage wastes were assessed in this study and found that P, K, Na, C and B were not significantly influenced by exposing composting materials to a *C. rosea* inoculum. These findings contradict those of Jusoh et al. (2013), who reported higher levels of P and K contents ($p < 0.05$) in a mixture of rice straw with a solution of effective microorganisms in comparison to those without microorganisms. This study recorded the same C/N ratio of 8:1 for both treatments of the end-product of composting (Table 3.5). Nevertheless, Kumar et al. (2010) and Petric et al. (2015) reported results with essential C/N values between 20-50. Previously, Mtimkulu (2016) reported improved C/N ratio in treatments with spent wine filter material content (10:1; 13:1 and 10:1). A low C/N ratio result in higher available nitrogen per carbon, and inert nitrogen, probably to be lost as ammonia (Zhang et al., 2016). Yang et al. (2015); Wang et al. (2015) and Rastogi et al. (2019) observed decreased C/N ratio in the process of composting attributed to an elevated waste biodegradation (carbon) to mineralisation (nitrogen) ratio.

Traditionally, C/N ratio is used to indicate maturity degree of compost (Zeng et al., 2009; Guoxue et al., 2001). Poincelot (1974) suggested that a C/N ratio below 20 is indicative of mature compost. Therefore, based on our results, fungal-treated compost heaps and control-treated heaps were mature and complete at the end of the composting period. Similarly, N and C in plant tissue nutrients did not show a significant difference ($p > 0.05$) in both treatments; a higher C/N ratio (25:1) was obtained (Table 3.8). Fungus treated heaps appeared to have a dark brown colour compared to the control heaps. This effect demonstrates that perhaps the fungal inocula enhanced the decomposition of organic wastes. This affirmation correlates with the findings of Sakar et al. (2016).

The existence of heavy metals in compost were determined at the end of the composting period, and interestingly, no effect on micro-nutrients was observed in the fungus-treated heaps compared with the control (Table 3.5). Overall, these results suggest that inoculation of cabbage wastes with the *C. rosea* f. *catenula* strain did not change the chemical characteristics of the final product of composting. Previously, a decrease in the status of nutrients had been obtained by Chaturvedi et al., (2010). While the findings of Jusoh et al. (2013) indicated that the composting process of rice straw containing effective microorganisms tended to increase heavy metals (Cu and Zn). Previous research by Paré et al. (1999) also explored increased accumulation of heavy metals during the composting of biosolids. These trace metals are required in small quantities by plants as they could be phytotoxic at high concentration (Petric et al., 2015). Paré et al. (1999) confirmed that extractability and exchangeability of some of these heavy metals could be reduced during the composting of biosolids and municipal solid wastes.

No differences shown in pH values of treated heaps and the control heaps over the period of composting were recorded. The pH in this study ranged from 4.5 – 7.0. Previous studies showed pH values ranging from 5.5 to 9.0 to be suitable for the composting process, while values between 6.5 to 8.0 are thought to be most effective (Christian et al., 1997). According to Chen et al. (2012) pH ranges from 6.8 – 7.3 is optimum for composting. Zhang & Sun (2016) suggested that pH values from 5.5 – 8.0 are ideal for composting; whereas Bernal et al. (2009) suggest that a value of pH from 6.7 – 9.0 is optimum to promote good microbial activities.

The pH of the compost has a noticeable effect on the microbial populace, and it increases as a result of the decomposition of acids, which releases ammonium (Pan et al., 2012). The pH values in the reports by Miller (1992); Christian et al. (1997); Sarkar et al. (2016); Ribeiro et al. (2017) were closer to those observed in the present study (pH ranging between 7.0 – 7.6).

This pH range of 7 to 7.6 is within the optimum range from 5.5 to 8.0 for compost pile materials with fungi (Chen et al. 2012) thereby, suggesting that the compost in the current study was subjected to good oxidation.

In the treated heaps and heaps with no fungal inoculum pH levels were acidic (4.5 – 6.3) from the first week to the 9th week, both morning and afternoon, and during the 11th week only in the morning (6.4 – 6.5). A similar pH drop was reported in earlier studies (Poincelot, 1974; Chaturvedi et al., 2010; Pan et al., 2012 and Ribeiro et al., 2017). According to Kiehl (2002) acidic pH could result from the formation of organic acids during the thermophilic phase of active degradation, at the initial stage of composting. Alternatively, the pH reduction during composting could be the result of acids and microbial nitrification (Wang et al., 2016). Pan et al. (2012) indicates that pH levels of 6.0 or below can interrupt the decomposition process of organic matter.

4.3 Re-isolation of fungus

In this study, *Clonostachys rosea* f. *catenula* conidia were successfully re-isolated from the composted materials originating from fungus pre-treated and all tomato plants that were cultivated on the composted materials obtained from the fungus pre-treated heaps. These results suggest that the fungus is certainly persistent in the composted materials; thereby, enhancing the endophytic quality of the composted materials. *C. rosea* f. *catenula* are endophytic and pathogenic to insects and antagonistic against phytopathogen. Hence, its presence should add value to organic composts. The re-isolation of *C. rosea* f. *catenula* from leaf sections of the cultivated tomato plants clearly demonstrated the *C. rosea* f. *catenula* strain used in this study was an efficient endophyte.

The colonization of potted tomato plants by endophytic fungi have been reported previously (Pappas et al., 2018). Xia et al. (2019) isolated six endophytic fungal isolates belonging to very diverse orders such as Eurotiales, Pleosporales, Hypocreales, and Saccharomycetales. The recovery of *C. rosea* f. *catenula from* the leaves of tomato plants indicates the potential of these strains to become successful endophytic agents. Andrade-Linares et al. (2011) also reported endophytic colonisation by species belonging to the genera *Verticillium, Penicillium, Cladosporium, Fusarium* and *Trichoderma*, which can also be isolated from stems. It is worth mentioning that successful colonisation of fungal endophytes across different types of tissues is influenced by factors such as fungal species, fungal strain and host, etc. (Tefera & Vidal, 2009: Arnold & Herre, 2003; Gurulingappa et al. 2010) as well as plant species, growth stages, soil types, and environmental conditions (Yang et al., 2018). Variability in colonisation efficiency of strawberry leaves (Cota et al. 2008) and potato tubers (Jima, 2013) with a different strain of *C. rosea* was previously reported. Nordström (2014) reported variable colonization of tomato and *Arabidospis thaliana* by a *C. rosea* f. *catenula* strain. Sutton et al. (1997) also reported that *C. rosea* is a plant endophyte on different plant species and was isolated from roots and stems of soya beans (*Glycine max*) - that were either inoculated with or were grown in soil infested with *C. rosea* (Mueller & Sinclair, 1986). Nordström (2014) suggested that the rhizosphere can affect the establishment of *C. rosea*. In another study, it was reported that growth media has a greater influence on the level of plant endophytic colonisation than the inoculation method (Bamisile et al., 2018).

4.4 Phytotoxicity assessment

Results of this study showed a better germination of tomato seeds sown in composted materials from heaps that were inoculated with *C. rosea* f. *catenula* during composting and no phytotoxicity was observed on percentage seedling growth in both treatments. The increase in

the number of seeds that germinated in treated compost as well as seedling growth may be due to the presence of the fungus. A recent study showed that endophytic fungi can produce phytohormones, particularly gibberellins that can alleviate the harmful effects of abiotic stresses and enhance crop growth (Bilal et al., 2018). According to Davies (2010) these phytohormones can regulate various developmental and physiological processes in plants such as seed germination, seedling development, stem and leaf growth, and flower and fruit growth. Previous studies reported on plant-growth promoting fungi (PGPF) from various genera including, *Thrichoderma*, *Fusarium*, and *Penicillium*, which can be beneficial in several crop plants (Nagaraju et al., 2012; Chowdappa et al., 2013). A similar study has been reported, where *Penicillium oxalicum* (Pennisetum glaucum (L.) R. Br.) enhanced seed germination and seedling vigour of Peal millet (Murali & Amruthesh, 2015). However, the authors have indicated that the efficacy of the endophytic fungus may vary significantly among the treatments. Similarly, Nagaraju et al. (2012) observed enhanced seed germination and seedling vigour where seed priming with conidial suspension of PGPF *T. harzianum* was used compared to a non-primed control in sunflower (*Helianthus annus* L.). Moreover, Jogaiah et al. (2013) demonstrated enhanced seed germination and seedling vigour in tomato of seeds of tomato treated with a conidial suspension of different PGPF's. Furthermore, Murali et al. (2012) has also demonstrated the use of these microbes as inoculants to treat seeds for germination and seedling growth. Herrera et al. (2008) found that seeds sown on a media composition of white peat mixed with municipal solid waste compost had better quality seedlings than those grown with standard peat mixtures. The compost-river sand ratio (25% treated compost and 75% course river sand) was a suitable treatment for the vegetative growth of seedlings in both treatments (Table 3.6).

4.5 Insect infestation

C. rosea was isolated and examined for pathogenicity against a tomato plant red spider mite infestation and the results showed that the fungus did not significantly reduce the insect infestation levels despite the high endophytism observed in this study, suggesting that many factors influence the efficacies of endophytic entomopathogens *in vivo*. Moloinyane & Nchu (2019) did not observe reduction of grapevine mealy bug infestations by the endophytic *Beauveria bassiana* and they argued that factors fungal strain, and insect species may influence efficacy of endophytic entomopathogens on both the plant and the insect pest. However, inoculation method and effect of endophytic colonisation can be influenced by biotic and abiotic factors, such as growth substrate, plant species and age, fungal species and inoculum density (Posada et al. 2007; Tefera & Vidal, 2009; Parsa et al. 2013). For instance, Tefera & Vidal (2009) demonstrated that despite the inoculation method used, *B. bassiana* colonised the entire parts of sorghum plants. However, a study by Posada et al. (2007) reported that plants species, fungal isolate and inoculation methods used influenced the endophytic colonisation by *B. bassiana*. Some studies have demonstrated fungal endophytes reduces insect infestations (Arnold & Lewis, 2005; Jallow et al., 2008; Saikonnen et al., 2004; Reddy et al., 2009; Vega et al., 2009). For instance, the effect of environmental changes on colonisation of corn by the fungal isolate *B. bassiana* was reported by Bing & Lewis (1991) and its effect on targeted pests (European corn borer larvae) *Ostrinia nubilalis* (Hübner). However, others did not find any benefit of endophytic fungal colonisation on insect infestations (Moloinyane & Nchu, 2019). It appears many other factors moderated the fungus-plant-herbivore relationship, which research has shown is quite complex.

It is worth noting that this fungus is known as an antagonist which is widely used as a biocontrol agent for divergent plant pathogens including plant pathogenic fungi such as *Alternaria* and Fusarium species (Nygren et al. 2018).

This study suggests that inoculating organic waste materials with endophytic entomopathogenic *Clonostachys rosea* f. *catenula* conidia could enhance the rate of decomposition process of organic waste heaps, help to produce high quality composted materials rich in endophytes, and composting materials could serve as a substrate for storing entomopathogenic fungi for long periods and for fungal application in the field. The finished end-product from the fungus-inoculated heaps could improve physical stability, chemical constituent richness and promote plant vigour and growth enhancement. This compost end-product contains plant-growth promoting and protection properties, could substitute chemical fertilizers and synthetic pesticides, inhibit toxic chemicals to crop plants and be recommendable in agricultural application. Future evaluation of enzymes could help better understand the mechanism through which the fungus influence composting, changes in microbial species and population over time could help identify the specific microbes involved and the relationship with *C. rosea*. Assessment of phytotoxic chemicals of these fungi in composted materials could help understand the potential risk they may have in the cultivation of plants.

CHAPTER FIVE

5 Conclusion and recommendations

Clonostachys rosea f. *catenula* had no effect on the chemical composition of the end-product of composting of solid cabbage waste compared to a control treatment over the period of composting. However, the *C. rosea* f. *catenula* treatment enhanced the germination of tomato seeds and improved the physical characteristics of the composts. The *C. rosea* f. *catenula* strain did not confer protection against *T. urticae* infestations of tomatoes. Interestingly, the conidia of *C. rosea* f. *catenula* persisted in the composted materials for several months and was able to endophytically colonise tomato seedlings. We proposed that organic wastes should be used as substrates for long term storage of endophytic entomopathogenic fungi. These findings also contributed to our understanding of the mechanisms involved in the fungus-plant residue-insect relationship. The effects of fungal inoculation on changes in enzyme activities during composting should be considered in the future.

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