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EFFECTIVENESS OF *BRASSICA JUNCEA* AS A BIOFUMIGANT IN CONTROLLING *FUSARIUM OXYSPORUM* fsp *TUBEROSI* IN POTATOES (*SOLANUM TUBEROSUM*)

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DECLARATION

I HATINANYIKA KNOWLEDGE do hereby declare that this dissertation was the result of my own original efforts and investigations, and such work has not been presented elsewhere for any degree or any university programme. All other supplementary sources of information have been acknowledged by means of references.

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ABSTRACT

Fusarium oxysporum fsp *tuberosi* is an important soilborne pathogen which causes vascular wilt on potato worldwide. However, toxicity and an increase of pollution caused by fungicides have led to the development of environmentally friendly control measures such as biofumigation. A laboratory study was conducted to determine the effects of *B. juncea* plant extract concentrations on growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro. The treatments were *B. juncea* plant extracts at 1, 2 and 3 g, Ridomil gold (positive control) and distilled water (negative control). Data on radial mycelium growth and inhibition percentage were collected. A glasshouse study was also done to evaluate the biofumigant effect of decomposed *B. juncea* residues on the incidence and severity of vascular wilt pathogen *Fusarium oxysporum* fsp *tuberos*i in potatoes. The treatments were decomposed *B. juncea* residues at 60, 40 and 20 g, Ridomil gold (positive control), no crop residues amendment and inoculation (negative control) and a healthy control. Data on disease incidence and severity were collected. Both experiments were conducted at Zimbabwe Fertilizer Company (ZFC Ltd). *Brassica juncea* plant extracts significantly reduced ($p < 0.05$) radial mycelium growth and had inhibitory effects on the development of *F. oxysporum* fsp *tuberosi.* Radial mycelium growth decreased as the amount of plant tissues added to each Petri dish increased. Mycelium inhibition percentage increased as the amount of plant tissue added to each Petri dish increased. *Brassica juncea* (at 60g) decomposed residues significantly reduced ($p < 0.05$) disease incidence and severity at 8 and 10 weeks after planting. However, all treatments failed to reduce disease incidence and severity at week 12. From the results it can be concluded that *B. juncea* has a biofumigant effect and farmers can use decomposed residues as they reduce disease incidence and severity at the early weeks of incorporation.

Keywords: Biofumigation; plant extracts; decomposed crop residues; *F. oxysporum* fsp *tuberosi*.

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Dedication

I dedicate this work to my parents Mr. and Mrs. Hatinanyika and my siblings Rejoice, Luckson and Joel Hatinanyika. I did it for you my dearest and compassionate family. Let this be the beginning for the rise of our family and for good things to come.

Table of Contents

List of Tables

List of Figures

List of Plates

List of Appendix

CHAPTER ONE

Introduction

1.1 Background

Potatoes (*Solanum tuberosum*) have gained popularity with Zimbabwean farmers and are cultivated due to its adaptability to different temperature ranges, its nutrition and many uses compared to other tuber crops, prices of the crop can be as high as $ 1.75/kg in favourable years and can earn foreign currency through export. However, the full economic value of this crop has not been achieved because of constraints caused by soilborne fungal diseases (Nxumalo, 2013). Poor agronomic practices by farmers such as cultivation of potatoes on the same piece of land every year and crop rotations with other solanaceous crops such as tomatoes and tobacco cause persistence, recurring and increased severity of soilborne diseases (Ayed *et al*., 2006). Soilborne fungal pathogens have now become an important concern for smallholder farmers in developing countries as they are difficult to control and cause a decline in quality, yield, productivity and profitability (Mudyiwa *et al*., 2016).

Vascular wilt caused by *Fusarium oxysporum* fsp *tuberosi* is one of the soilborne diseases which causes reduced plant growth, tuber quality and reduction in marketable yield of potato (Larkin and Griffin, 2007). Moreso, yield losses caused by *F. oxysporum* fsp *tuberosi* can be up to 70% in the field and in storage (Agrios, 2005). The disease has symptoms like chlorosis, rapid wilting of the whole plant and collapse of the stem (Makhlouf and Abdeen, 2015). These symptoms usually commence at the flowering stage and farmers are unable to detect the disease earlier as their aim is to increase flowers per plant to maximise yield which makes management of *F. oxysporum* fsp *tuberosi* difficult. Symptoms of yellow concentric rings on the leaves indicate the

production of toxic metabolites such as fusaric acid which increases virulence and pathogenecity of *F. oxysporum* fsp *tuberosi* (Agrios, 2005).

Most smallholder potato farmers in Zimbabwe grow varieties such as Amethyst, BP1 and Diamond which are susceptible to many pathogens including *F. oxysporum* fsp *tuberosi* (Ngadze, 2012). Therefore, lack of high yielding resistant varieties in Zimbabwe has led to the dependency on chemical control for the management of vascular wilt disease (Haroutunian, 2013). Frequent and increased applications of chemicals such as Ridomil and Mancozeb are not feasible and cost effective for poor resource farmers (Kuri *et al*., 2011).

Fusarium oxysporum fsp *tuberosi* produces three types of asexual spores which are macroconidia, microconidia and chlamydospores (Agrios, 2005). Chlamydospores have the ability to survive in the soil for a very long time of even up to 30 years (Smolińska and Kowalczyk, 2014). Therefore, disease management in the field is complicated as there is no effective fungicide treatment for its control (Agrios, 2005). Furthermore, pest control has mostly been dependent on fungicide use. However, some fungicides are extremely persistent, poisonous to plants, animals and humans (Kuri *et al*., 2011). Chemicals like chlorofluorocarbons are ozone depleting and the demand for blemish free produce is increasing (Karavina and Mandumbu, 2012). Lives of workers are now endangered during and after using fungicides which inversely necessitates the use of an alternative control measure which is sustainable and has less mammalian toxicity (Mudyiwa *et al.*, 2016 and Larkin and Griffin, 2007).

Biofumigation is an alternative disease management strategy which uses decomposed plant tissue from *Brassicas* for disease control (Matthiessen and Kirkegaard, 2006). Biofumigation is defined as the suppression of soilborne diseases resulting from isothiocyanates released in the

soil after incorporating glucosinolate containing plant tissue (Matthiessen and Kirkegaard, 2006). *Brassica juncea* has been used as a biofumigant in controlling *Fusarium oxysporum* fsp *tuberosi* in-vitro and has the highest biofumigant effect in the *Brassicaceae* family (Taylor, 2013 and Smolinska *et al.*, 2003). *Brassica juncea* has been strongly correlated with a reduction in yield losses caused by soilborne diseases and in improving quality of potatoes (Larkin and Griffin, 2007).

In light of the above information, adoption of biofumigation for the control of *F. oxysporum* fsp *tuberosi* by farmers would help reduce the dependency on synthetic fungicides and provide an alternative control which is environmentally friendly. Smallholder farmers and organic farmers who export potatoes should ensure that their produce has lower chemical toxicity levels to meet the international market requirements. Therefore, there is a research gap on the effectiveness of *B. juncea* as a biofumigant for controlling plant pathogens. This study seeks to assess the effectiveness of *B. juncea* as a biofumigant in controlling *F. oxysporum* fsp *tuberosi* in potatoes as an alternative control.

1.2 Problem Statement

Biofumigation is being recognised in the agricultural sector hence their concentrations are not yet known. The study was carried out to compare and evaluate the effects of *Brassica juncea* plant extract concentrations on the suppression of *Fusarium oxysporum* fsp *tuberosi* in potatoes.

1.3 Justification

Biofumigation is now considered worldwide as a component of integrated disease management (Taylor *et al.*, 2014). Biofumigation is an alternative that can be used to manage soilborne pathogens without damaging the environment (Karavina and Mandumbu, 2012). The use of biofumigation causes less toxicity and persistence in the soil (Matthiessen and Kirkegaard,

2006). Biofumigant crops are non pollusive and they can reduce greenhouse gas emissions and groundwater pollution (Pisa *et al*., 2015). Biofumigant crops are used in phytoremediation and extraction of heavy toxic metals such as lead and copper available in the soil (Moosavi and Seghatoleslami, 2013). Furthermore, biofumigant crops produce isothiocyanates which are chemically similar to methyl isothiocyanate an active ingredient found in metham sodium (Matthiessen and Kirkegaard, 2006). Isothiocyanates have the ability to suppress plant pathogens, weeds and nematodes (Matthiessen and Kirkegaard, 2006). Biofumigant crops can be used to break disease cycles through crop rotations and reduce chlamydospores present in the soil. Therefore, the adoption of biofumigation by farmers will reduce costs associated with chemical fumigants (Kuri *et al*., 2011).

Biofumigant crops can be used as green manure cover crops which can add organic matter and to the soil and improve soil fertility (Reddy, 2012). Biofumigant crops enhance mineralization of atmospheric nitrogen which becomes available for plant uptake (Reddy, 2012). Therefore, there is a yield increase and the dependency on inorganic fertilizers is reduced. In addition to that, biofumigation adds more organic carbon to the soil (Balesh *et al*., 2005). The presence of organic carbon in the soil increases the activities of biological control agents such as *Trichoderma* species (Karavina and Mandumbu, 2012). Therefore, biofumigant crops are available to farmers throughout the year as they can be included into the soil as green manures, dead green manures and seed meals (Taylor, 2013).

Biofumigation as an alternative control measure for soilborne pathogens enables smallholder farmers to grow higher yielding potato varieties which are susceptible to *F. oxysporum* fsp *tuberosi*. Biofumigation reduces yield losses and abandonment of fields caused by *F. oxysporum* fsp *tuberosi* and other secondary pathogens such as *Pectobacterium caratovora* (Manditsvara,

2014). *Brassica juncea* is a cultivated indigenous vegetable which is readily available to farmers in Zimbabwe. However, *B. juncea* has low marketability and is usually left to flower and rot in the field, hence farmers can manipulate their accessibility to excess *Brassica*s and use their antifungal properties and benefit potatoes in the next season which have high profitability and marketability (Karavina and Mandumbu, 2012). Biofumigation creates employment and research opportunities for horticultural breeders to breed for biofumigant crops that have the highest isothiocyanate content which can be used by smallholder farmers for disease control (Taylor, 2013)

1.4 Objectives

1.4.1 Main Objective

To assess the effectiveness of *Brassica juncea* as a biofumigant in controlling *Fusarium oxysporum* fsp *tuberosi* in potatoes.

1.4.2 Specific Objectives

I. To determine effect of *Brassica juncea* plant extract concentration on growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro.

II. To evaluate the biofumigant effect of decomposed *Brassica juncea* residues concentration on the incidence and severity of vascular wilt pathogen *Fusarium oxysporum* fsp *tuberos*i in potatoes.

1.5 Hypothesis

I. Different *Brassica juncea* plant extract concentrations can suppress the growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro.

II. Different *Brassica juncea* decomposed residue concentrations can effectively reduce the incidence and severity of vascular wilt pathogen *Fusarium oxysporum* fsp *tuberosi* in potatoes

CHAPTER 2

Literature review

2.1 Origin and Economic importance of Potatoes in Zimbabwe

Potatoes are the fourth main food crop in the world and are grown worldwide as they support a considerable proportion of the population (Fiers *et al*., 2012). Potatoes *(Solanum tuberosum)* originated from South America and are grown in Zimbabwe by categories of farmers which include commercial farmers, smallholder farmers and urban farmers. Potatoes are preferably grown in parts of the country with good fertile soils and sufficient rainfall regimes. Potatoes have become one of the most popular food crops grown as an alternate staple and are seen as a food security crop in Zimbabwe (Ngadze, 2012). Ngadze (2012), mentioned that potatoes are now a common household food since they are utilized and consumed in remote and urban homes. Therefore, potatoes have a great potential to reduce food shortages worldwide (Makhlouf and Abdeen, 2015).

Potatoes are adaptable to different temperatures and have a diversified use for human consumption and livestock feed (Manzira, 2010). Potatoes are widely used in the catering industry as a fast food and in the processing where they are canned or baked for value addition (Manditsvara, 2014; Manzira, 2010). Potatoes can be consumed baked, boiled, mashed or fried (Manzira, 2010). Potatoes are vastly tasty, nutritionous and are composed of carbohydrates, protein, iron, vitamins, fibre and minerals. Moreover, potatoes are highly recommended to people suffering from hypertension as they contain micronutrients which decrease blood pressure

such as phosphorus, potassium and iron. Potato production has increased over the years in Zimbabwe due to changes in lifestyle and consumption trends.

2.2 Zimbabwe Potato farmers and challenges experienced in production

In Zimbabwe potatoes grow well in Nyanga because of the cool climate desirable for potato growth and production, and preferably low disease pressure (Dyogo, 2012). Moreover, the majority of potato production in Zimbabwe come from smallholder farmers who are distributed in different parts of the country and sell their produce to local markets such as Mbare msika (Gashgari and Gherbawy, 2013). Ngadze (2012), mentioned that potatoes are cultivated in various parts of Zimbabwe but huge differences exist in yield due to biotic and abiotic factors because of different agrological regions which contribute in disease pressure. Potato production in Zimbabwe is significantly affected by physiological disorders caused by high temperatures, pest and diseases which decrease yield and quality (Ngadze, 2012). *Fusarium oxysporum* fsp *tuberosi* is one of the pathogens that cause economic losses in potato production in Zimbabwe. *Fusarium oxysporum* fsp *tuberosi* is intensifying in economic importance due to an increase in consumer habits which favour washed pleasant tubers which are disease free (Fiers *et al*., 2012).

2.3*Fusarium oxysporum*

Fusarium oxysporum fsp *tuberosi* is a soilborne fungus which causes vascular wilt disease in potatoes (Nxumalo, 2013). Vascular wilt pathogens which affect many crops including potato are *F. oxysporum* fsp *tuberosi, F. avenaceum, F. eumartii* and *F. javanicum*. However, *F. oxysporum* fsp *tuberosi* is the main causal agent of vascular wilts in potatoes and causes high yield losses as compared to other species (Wale *et al*., 2008). Moreso, *F. oxysporum* fsp *tuberosi* shows host specificity to potatoes and is referred to as a soil inhabitant as it survives in the soil for over thirty years (Nxumalo, 2013). However, most smallholder farmers in Zimbabwe are not aware of

the fact that *F. oxysporum* fsp *tuberosi* is the primary causal pathogen that is leading to high yield losses of potatoes (Ngadze, 2012). Contamination of potato tubers by *F. oxysporum* fsp *tuberosi* causes a large health problem to people due to the production of mycotoxins which are toxic and lead to death in severe cases (Ismaiel and Papenbrock, 2015).

2.3.1 Economic Importance and Yield Losses caused by *Fusarium oxysporum fsp tuberosi*

Potatoes have a vast potential to produce high yields per unit area 15 to 18t/ha for summer crop and 25t/ha yield per ha under optimum environments for winter crop, but they are vulnerable to vascular wilts which severely reduce yield (Manzira, 2010). Yield losses can be up to 70 % in the field and storage due to a rapid decline in quality and quantity (Agrios, 2005). Lack of effective control of vascular wilts has become a major constraint for poor resource farmers in sub-Sahara Africa and has contributed immensely to low yields (Mudyiwa *et al*., 2016; Wale *et al*., 2008). Moreover, it is reported that vascular wilts have caused financial problems to farmers in sub-Sahara Africa and other leading potato growing nations such as Iran (Saremi *et al*., 2011).

Fusarium oxysporum fsp *tuberosi* causes blemish on tubers by causing browning at the end of the stem and tuber which reduces their marketable value (Wale *et al*., 2008). *Fusarium oxysporum* fsp *tuberosi* causes wilting and yellowing of leaves before physiological maturity (Saremi *et al*., 2011). Yellowing of the leaves reduces the leaf area available for photosynthesis to take place in the plant. This leads to limited accumulation of assimilates that can be supplied to the tubers for growth and development (Saremi *et al*., 2011). Therefore, tubers become small in size and develop internal vascular browning (Agrios, 2005). However, internal vascular browning is not desirable as it reduces the cosmetic value of potatoes and makes tubers unattractive for use in the processing industry (Gashgari and Gherbawy, 2013). Therefore, a low grade of infected tubers reduces profits for communal farmers in Zimbabwe.

Sub- Saharan Africa has been experiencing climate change and farmers have received extreme temperatures and dry weather in unfavourable seasons (Smolińska and Kowalczyk, 2014). Moreover, spores of *F. oxysporum* fsp *tuberosi* have a prolonged survival period in air under dry weather conditions (Irshad and Naz, 2014). Therefore, farmers have experienced increased virulence of *F. oxysporum* fsp *tuberosi* and yield loss in the past decade. Increased virulence of *F. oxysporum* fsp *tuberosi* has led to repeated applications of fungicides and chemical fumigants (Haroutunian, 2013).

Potato productivity decreases among communal farmers each year due to synergistic disease infestation (Agrios, 2005). Synergism of *F. oxysporum* fsp *tuberosi* and *Pectobacterium caratovora* causes severe yield losses up to 80% in the field and storage among potato farmers in Zimbabwe (Ngadze, 2012). Farmers can produce up to 15t/ha a year when there is improved soil fertility, good rains and less disease manifestation (Manzira, 2010). However, due to synergism of pathogens farmers produce a yield of less than 1.3t/ha a year. Yield losses occur because soft rot pathogens release enzymes that degrade the pectin and lead to decay of potatoes under humid conditions in the field, transit and storage (Ngadze, 2012).

Production of mycotoxins by *F. oxysporum* fsp *tuberosi* affects the quality of potato tubers (Agrios, 2005). Mycotoxins cause a deviation in the biochemical processes of the plant and cause phytotoxicity in plants (Ismaiel and Papenbrock, 2015). Biochemical changes such as reduction in carbohydrate and protein occur (Agrios, 2005). Potato tubers are vulnerable to contamination by mycotoxins as they develop in the soil and are exposed to inoculum (Agrios, 2005). Mycotoxins have negative effects on human health and animal health and nutrition (Ismaiel and Papenbrock, 2015). Moreso; mycotoxins are highly carcinogenic to humans and can lead to tuber rejection on the international market upon detection and identification.

Farmers face decertification due to vascular wilts, as seed certification schemes do not allow more than 8% disease infestation (Gouws, 2006). The percentage of potato bags containing vascular wilts and common scab infected seed tubers were about 32% between 1996-2004 which led to decertification or rejection of seed in the SADC region (Gouws, 2006). Moreover, severe infestations by vascular wilts and common scab have lead to abandonment of fields in seed production areas in South Africa (Gouws, 2006). Sprouting of tubers, seed viability, germination and growth are affected immensely by *F. oxysporum* fsp *tuberosi*.

Furthermore, potato seedlings succumb to diseases such as damping off and root rot (Agrios, 2005). Therefore, there is a high risk of stunted growth which leads to seedling failure and farmers fail to reach the optimum plant population per hectare (Agrios, 2005). Moreso, damping off and root rot farmers cause farmers to waste resources indirectly as roots are unable to absorb inorganic nutrients such as nitrogen, phosphorus and calcium and water absorption is diminished (Agrios, 2005).

2.3.2 Disease cycle and biology *of Fusarium oxysporum fsp tuberosi*

Fusarium oxysporum fsp *tuberosi* inoculum arises from the soil, infected seed and infected plant debris (Wale *et al*., 2008). Overwintering structures in the form of chlamydospores are stimulated by the presence of a susceptible host potato plant. Penetration occurs in the root elongation zone through wounds or natural openings present on the plant (Wale *et al*., 2008). Wounds can be caused by insects, nematodes, production practices, farm implements and poor handling of seed potato (Agrios, 2005). Wounding of a susceptible host sends chemical signals to chlamydospores which breaks dormancy and stimulates germination (Nxumalo, 2013).

Spore germination is enhanced by the diffusion of nutrients in the roots and exposure to a dry environment with temperatures ranging from 25-35^{o}C (Wale *et al*., 2008). Mycelium develops in the roots and penetrates the cortex. The pathogen colonises the plant and grows upwards and blocks the vascular tissue in the form of microconidia and metabolites such as extracellular polysaccharides are released (Nxumalo, 2013; Wale *et al*., 2008). Extracellular polysaccharides increase the virulence of the pathogen by clogging the xylem tissue. The pathogen invades the xylem vessels and continues to spread throughout the plant and establishes itself to form spores (Agrios, 2005). Blockage of the vascular tissues in the lower stem results in the shortage of water and nutrients which cause a decline in the metabolic processes of the plant.

Fusarium oxysporum fsp *tuberosi* secretes secondary metabolites in the form of toxins. The toxins interfere with the metabolic system of the plant and can lead to an increase in the virulence of the pathogen (Agrios, 2005). Secretion of toxins has a negative effect on the plant such as decreased plant metabolisms which leads to death before physiological maturity in severe cases (Agrios, 2005). The tubers formed are of smaller as the plant dies before physiological maturity. Inoculum can be spread by planting contaminated seed or when resting spores in the soil are spread with farm machinery, by wind and irrigation (Wale *et al*., 2008). Crop stress, high temperatures, rapid growth, transpiration, excess nitrate application and potassium deficiency are conducive for pathogen development (Wale *et al*., 2008). The type of irrigation used by farmers has an effect on the rate at which the disease spreads from one location to another.

2.3.3 Disease Symptoms of *Fusarium oxysporum fsp tuberosi*

Plate 2. 1: Image showing a potato plant with yellowing symptoms after an infection with *Fusarium oxysporum* fsp *tuberosi*. Image was taken from Nxumalo, (2013).

Above ground symptoms usually start on the lower leaves showing chlorosis followed by wilting as seen in (Plate 2.1) (Wale *et al*., 2008). Wilting can occur on well irrigated plants and yellow concentric rings develop on the leaves due to toxin production (Agrios, 2005). Symptoms advance to the upper leaves and they become necrotic which causes the stem to collapse (Makhlouf and Abdeen, 2015). *Fusarium oxysporum* fsp *tuberosi* causes decay of roots and lower parts of the stem (Wale *et al*., 2008). Browning and drying of leaves occurs and leads to death of the whole plant (Makhlouf and Abdeen, 2015). Plants infected at seedling stage usually wilt and die soon after appearance of the first symptoms (Agrios, 2005). Moreover, older plants may wilt and die abruptly if infection is severe and the weather is favourable for the pathogen development (Agrios, 2005). According to Agrios (2005), there is evidence of vascular browning in the cross section of the tuber and stem of the infected plants.

2.3.4 Control of *Fusarium oxysporum fsp tuberosi*

Fusarium oxysporum fsp *tuberosi* is difficult to control and eradicate in potato fields as the entire field can be destroyed before harvesting (Agrios, 2005). Cultural control measures such as the use of resistant cultivars, crop rotation and planting uninfected seed tubers can reduce disease incidence (Nxumalo, 2013). Crop rotations with maize and Katambora Rhodes grass are not effective as they do not reduce disease severity (Ngadze, 2012). Little knowledge is known on biological control among communal farmers in Zimbabwe. Moreover, no biological control agent has been found to control *F. oxysporum* fsp *tuberosi*. Most farmers have been dependent on chemical control for management of vascular wilt disease (Haroutunian, 2013). However, resource poor farmers have limited access to chemical control due to high costs of fungicides (Kuri *et al*., 2011). Chemical control has been unsuccessful due to the production of chlamydospores which are resistant to chemical fumigation and fungicides (Smolińska and Kowalczyk, 2014). Moreso, chemical fumigants have been withdrawn from the market because they are dangerous to human health and the environment (Mudyiwa *et al*., 2016). There are no alternate methods available to farmers to completely eradicate *F. oxysporum* fsp *tuberosi* from the soil (Smolińska and Kowalczyk, 2014). Therefore, alternative crop protection measures have to be put in place to reduce pesticide use and guard the environment from depletion (Prasad *et al*., 2015).

2.4 Biofumigation as an alternative control

Biofumigation is the agronomic practice of using chemicals released from decomposed plant tissues to suppress pests and diseases (Brown and Morra, 2005). Biofumigation makes use of the glucosinolate hydrolysis products produced by *Brassica* plants (Taylor, 2013). In addition to that, it has been reported that plants in the *Caricaceae*, *Moringaceae*, *Salvadoraceae* and

Tropaeolaceae families have biofumigant properties (Brown and Morra, 2005; Reddy, 2012). Biofumigation has the ability to reduce disease incidence and severity of soilborne diseases such as vascular wilts and dry rots. From previous studies, black scurf was reduced by canola and rapeseed (Karavina and Mandumbu, 2012). In vitro assays with the *Brassica juncea* inhibited growth of *Rhizoctonia solani*, *Phytophtora erythroseptica*, *Pythium ultimum* and *Sclerotonia sclerotiorum* (Larkin and Griffin, 2007).

In Zimbabwe mustard (*Brassica juncea*) is commonly grown, consumed and readily available to farmers (Karavina and Mandumbu, 2012). Therefore, *B. juncea* can be grown and exploited as a biofumigant in control of soilborne pathogens by potato farmers (Manditsvara, 2014). Plant foliage and seeds have glucosinolate compounds that produce isothiocyanates upon hydrolysis which act as biofumigants (Prasad *et al*., 2015). Isothiocyanates have fungicidal, herbicidal, nematicidal, and insecticidal effects (Prasad *et al*., 2015). *Brassica juncea* can effectively reduce chlamydospores in the soil and can be useful as a biofumigant as it contains the highest level of glucosinolates. The use of *B. juncea* as a biofumigant has the ability to reduce the soil microbial community and encourage the growth and increase of biological control agents (Larkin and Griffin, 2007). Biofumigation is now considered worldwide as a component of integrated disease management (Taylor *et al*., 2014).

2.5 Glucosinolate-Myrosinase defense system in plants

Glucosinolates are sulphur containing chemicals that are produced in vacuoles of plant cells and have no biological activity (Agrios, 2005). Myrosinase is an enzyme located separately in myrosin cells within the plant (Prasad *et al*., 2015). Myrosinase carry out hydrolysis reactions with glucosinolate to release products which are toxic. Glucosinolate and myrosinase remain separated from each other while the plant tissues are intact (Taylor, 2013).

It is only upon physical disruption of the plant tissue that myrosinase reacts with glucosinolate in the presence of water (Prasad *et al*., 2015). This process releases biologically active products such as isothiocyanates, organic cyanide, nitriles and ionic thiocyanates as seen in (Figure 2.1) (Brown and Morra, 2005). Therefore, the glucosinolate-myrosinase system in plants is considered to be a defense system against soil borne pathogens.

Glucosinolate concentration and composition are affected by plant genetics, environmental and physiological factors such as age of plant, radiation, temperature and photoperiod (Taylor, 2013). Moreover, concentration and composition of glucosinolates can change significantly throughout the plant development and growth (Verkerk *et al*., 2009). Glucosinolate content within plants is affected by agronomic factors such as soil type, moisture and mineral availability and soil health status (Velasco *et al*., 2007). Short days, cool temperatures and frost temperatures during winter can reduce the glucosinolate content in plants (Taylor, 2013). On the other hand, pest attack by sap sucking insects and leaf eaters can increase the glucosinolate content in plants (Taylor, 2013).

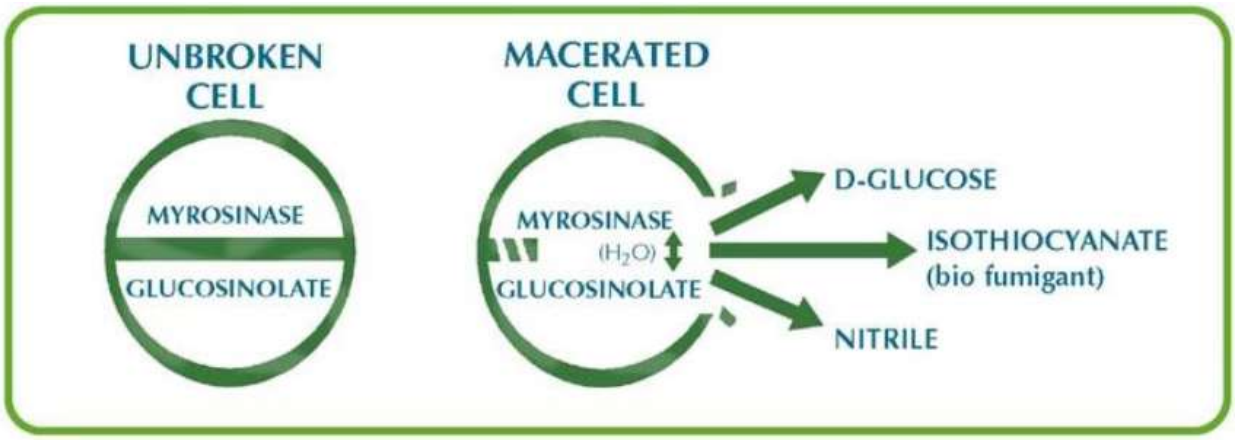


Figure 2. 1 Image showing the glucosinolate-myrosinase system in plants. Glucosinolate and myrosinase remain separated from each other while the plant tissues are intact. It is upon

physical disruption that a hydrolysis reaction occurs between glucosinolate and myrosinase to produce several products. Isothiocyanates can inhibit soil borne pathogens. Image taken from http://serve-ag.com.au/services/seed/sales-production/biofumigation-seed/.

2.6 Methods of incorporation

Biofumigant crops can be incorporated into the soil by several methods. Common methods include incorporation of green manures (Taylor, 2013). *Brassica* crops are grown on the land to be fumigated until flowering (Taylor, 2013). Prior to planting of the susceptible crops, *Brassicas* are chopped, mulched and ploughed into the soil (Matthiessen and Kirkegaard, 2006). The green manuring process will disrupt the *Brassica* tissues, allowing glucosinolate hydrolysis to take place (Taylor, 2013). Therefore, the use of green manure crops increases the fertility status and improves bulk density and water holding capacity of the soil.

Dried green manures contain high concentrations of isothiocyanates (Taylor, 2013). The plant material can then be ploughed into the ground and with addition of water (Lazzeri *et al*., 2009). Allelochemicals will be formed during decomposition of organic matter (Lazzeri *et al*., 2009). The use of extracted oil from canola seeds allows farmers to avoid growing *Brassica* crops (Taylor, 2013). The seeds are dried and crushed and the resultant seed meal can be ploughed into the soil (Taylor, 2013). Therefore, the seed meal is more beneficial than the green manure cover crop.

2.7 Physiological responses of fungi to isothiocyanates

Fungistatic and fungitoxic are the processes which describe fungal responses to toxic compounds (Taylor, 2013). Fungistatic occurs when the initial point of fungal growth is delayed in responses to the presence of toxic compounds (Taylor, 2013). Fungitoxic results in fungi being killed and unable to grow and develop in response to a toxic compound. Studies using *Fusarium oxysporum*

which affects nursery orchard seedlings by Smolinska *et al*. (2003), displayed both fungistatic and fungitoxic responses (Taylor, 2013).

2.8 Factors affecting effectiveness of isothiocyanates in the soil and environment

Isothiocyanates are short lived in the soil and they last for a few weeks (Brown and Morra, 2005). Volatile losses of isothiocyanates can occur at high temperature as this will not be beneficial as soilborne pests will not be suppressed. Moreso, studies showed that greater concentrations of volatile thiomethane and allyl isothiocyanates were observed at elevated temperatures (Brown and Morra, 2005). Soil sterilized with heat reduced the disappearance of methyl isothiocyanates (Brown and Morra, 2005). However, increased water content combined with cold temperatures resulted in increased lifetimes of methyl isothiocyanate (Brown and Morra, 2005).

Volatile losses are greater from coarse textured soils and less in soils with high organic matter (Brown and Morra, 2005). Soil pH influences the development and loss of glucosinolate hydrolysis product. Liming soil shortens residence times of isothiocyanates in soil (Brown and Morra, 2005). However, pH values of soils do not change isothiocyanate persistence times.

2.9 Adoption of Biofumigation by farmers

Biofumigation might harm non target beneficial soil biota such as biocontrol agents (Henderson *et al*., 2009). Furthermore, biofumigation might not eliminate all the harmful non target effects associated with synthetic chemicals (Manditsvara, 2014). Moreso, biofumigants are non-persistent thus they do not provide a long term control for pests (Manditsvara, 2014). Therefore, farmers might have to complement biofumigation with other control measures (Manditsvara, 2014)

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 Study Area

The research was carried out in the Plant Pathology Laboratory and glasshouse at Zimbabwe Fertilizer Company, Aspindale which is located 14,9km from Harare central business district (latitude 17° 52' South, longitude 30° 57' East). The research was carried out during the 2021/2 summer season and the soils used for the glasshouse experiment were red clay soils with more than 40% clay. The area received mean temperatures ranging from 20 - 26^{o}C and received a yearly rainfall ranging from 900mm to 1300mm.

3.2 Experimental Design

3.2.1 Laboratory Experiment

The laboratory experiment was laid out as a Randomised Complete Block Design (RCBD) with three blocks and five treatments. Blocking was done according to the shelves of the incubator.

Table 3. 1 Treatments used in the evaluation of antimicrobial properties of B. juncea in the laboratory.

Samples	Treatment
1	*B. juncea* at 1g
2	*B. juncea* at 2g
3	*B. juncea* at 3g
4	distilled water control
5	Ridomil positive control

3.2.2 Biomass Preparation

Clay soil was sterilized in the oven at 100^0C for 24 hours to destroy soil borne inoculum. A total of 10 pots were disinfected by using 5% sodium hypochlorite ($NaOCl_2$) and half filled with sterile soil. Three seedlings of *Brassica juncea* were grown in each pot until flowering stage.

3.2.3 Isolation of *Fusarium oxysporum* fsp *tuberosi* isolates

Isolation of *F. oxysporum* fsp *tuberosi* was done according to the method described by Mudyiwa *et al*. (2016), with minor modifications of the procedure involved. Infected potato tubers showing symptoms of vascular wilt diseases such as brown lesions and internal vascular discolouration

were collected from Mbare msika and were washed under running tap water for 3 hours. Infected tissues from potato tubers were cut into small pieces measuring 4 mm in diameter using a flame sterilised cork bore. Small infected tissues were transferred using a flame sterilised forcep into sterile Petri dishes containing 5% sodium hypochlorite. The infected tissues were surface sterilised in sodium hypochlorite for a time period of three minutes. After sterilisation, the infected tissues were rinsed 3 times in sterile distilled water and dried on sterile blotter paper for three minutes. The sterilised infected tissues were asceptically transferred to Petri dishes containing solidified Potato Dextrose Agar (PDA) and incubated at 27^{o}C in an incubator room for seven days to allow for sporulation (Ngadze, 2014).

3.2.4 Identification of *F. oxysporum* fsp *tuberosi* using Microscopy

The procedure of identifying *F. oxysporum* fsp *tuberosi* formed on PDA was done according to Nxumalo (2013), by using a microscope to identify colour of mycelium formed, spores and other fruiting structures. After seven days of incubation microscopic slides containing *F. oxysporum* fsp *tuberosi* were prepared and observed under a compound microscope model, (AusJena Laboval 4 and Leits Laboraux) at a magnification of X40 (Ngadze, 2014). The pathogen was identified using spore colour and fruiting structures such as chlamydospores, macroconidia and microconidia (Agrios, 2005). Moreso, spore identification was enhanced by books and images showing spores produced by the pathogen.

3.2.5 Subculturing

Following identification of spores, *Fusarium oxysporum* fsp *tuberosi* isolates were subcultured onto PDA plates and incubated at 30^{0}C for seven days to obtain pure cultures. After subculturing, a 0.5 cm diameter plug was taken and placed in the centre of a fresh PDA contained in a Petri dish measuring 9 cm in diameter.

3.2.6 Plant extracts preparation and mycelium growth

The process of plant extract preparation was done according to Smolinska (2003). Fresh *Brassica juncea* plant materials were frozen in a refrigerator at 4^{o}C and macerated using a blender. At least 1, 2 and 3 grams of *B. juncea* macerated plant tissue were placed on the lids of the individual Petri dishes immediately. All dishes including the control without any macerated plant tissue were incubated in an upside down position at 27^{o}C. The Petri dishes were covered with lids and sealed with parafilm. All these procedures were carried out under asceptic conditions.

3.2.7 Data collection

Radial mycelium growth was measured after three, seven and ten days of inoculation. Percentage inhibition was calculated using the formula with modifications (Biggs *et al*., 1997):

$$\frac{\text{Radial mycelium growth} \times 100}{\text{Control radial growth}}$$

3.3 Experimental Design

3.3.1 Glasshouse Experiment

The glasshouse experiment was laid out as a RCBD with six treatments. All treatments except the control contained chopped plant tissue at various rates. Blocking was done according to distance from the door. Treatments were replicated three times and boarder pots were included to subject treatments to the same conditions.

Table 3. 2 Showing treatments for glasshouse experiment

Treatment number	Treatment
1	*B. juncea* at 20g

2	*B. juncea* at 40g
3	*B. juncea* at 60g
4	Ridomil gold a positive control
5	Inoculated soil as negative control
6	Soil not inoculated as healthy control

3.3.2 Decomposition of crop residues

Brassica juncea seedlings were allowed to grow for 8 weeks until they reached the flowering stage. After 8 weeks crop residues were harvested and cut into 10 mm pieces which were incorporated into the soil. *B. juncea* residues were left to decompose for a month.

3.3.3 Agronomy:

Certified disease free tissue cultured seedlings of the cultivar Diamond were used to reduce plant pathogens such as viruses. Potato seedlings were planted in pots half filled with soil and seedfert (7:20:7) was applied as a basal fertiliser at a rate of 7g per pot. Ammonium Nitrate was applied as top dressing at a rate of 5g per pot 6 and 8 weeks of plant growth. Pots were filled at 63 days after planting to simultate the ridging (Manditsvara, 2014). Crops were irrigated using autoclaved water to reduce contamination.

3.3.4 Inoculation

Fusarium oxysporum fsp *tuberosi* lesions were cut from the infected tubers of the potato using a sterile scalpel. The lesions were viewed under a compound microscope (AusJena Laboval 4 and Leits Laboraux K), at a magnification of X40 for spore identification (Manditsvara, 2014). The

lesions containing the pathogen were peeled off, dried and ground to a powder in a buffer solution. The inoculum was sieved through 0.5 mm sieves and a haemocytometer was then used to check spores under a compound microscope and spores were diluted with sterile distilled water to a concentration of 1 x 10^4 spores per ml^{-1} (Dgobo, 2012). Inoculum was introduced into the soil 6 weeks after planting potato tissue cultured seedlings. Therefore, 100 ml of 1 x 10^4 spores per ml^{-1} concentration was applied per pot and mixed with moist soil (Dgobo, 2012).

3.4 Data Collection

3.4.1 Disease incidence of plants

Diseased plants were those showing symptoms of vascular wilts such as yellowing of lower leaves and wilting of the leaves and whole plant (Nxumalo, 2013). Disease assessment was carried out two weeks after inoculation and thereafter, every fortnight.

Disease incidence scoring was done using the formula (Ayed *et al*., 2006):

% of plants infected= number of plants infected/total number of plants*100

3.4.2 Disease severity of plants

Disease severity was recorded using the rating scale developed by Ayed et al. (2006), and was done two weeks after inoculation and thereafter, every fortnight per plant until they reached physiological maturity. The rating scale ranged from 1 to 5 according to (Ayed *et al*., 2006)

Table 3. 3 Showing disease rating scale

Number	Description
1	Leaf showing no symptoms

2	Leaf showing wilting
3	Leaf showing chlorosis
4	Leaf showing necrosis
5	Dead leaf

3.5 Data analysis

The data from the laboratory and glasshouse experiments were entered in Microsoft Excel and subjected to Analysis of Variance (ANOVA) using Genstat 14th Software. Mean separations were done using Fisher's protected Least Significance Difference level (LSD) at 5% significance level on all significant data. Data which did not meet the assumptions of ANOVA was transformed. Non parametric tests for disease severity were subjected to Kruskal and Wallis one way ANOVA.

CHAPTER FOUR

Results

4.1 Effects of *Brassica juncea* plant extracts on radial mycelium growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro.

Table 4.1 Means of radial mycelium growth of *Fusarium oxysporum fsp tuberosi* after exposure to *Brassica juncea* plant extracts at day 3, 6 and 9 post inoculation.

Treatments	Day 3 (mm)	Day 6 (mm)	Day 9 (mm)
***Brassica juncea* 1g**	10.17^{c}	17.83^{c}	19.83^{cd}
***Brassica juncea* 2g**	8.58^{b}	14.84^{bc}	16.50^{bc}
***Brassica juncea* 3g**	2.14^{a}	8.06^{a}	8.67^{a}
Positive control	1.06^{a}	8.22^{a}	10.94^{a}
Negative control	12.00^{d}	22.17^{d}	23.94^{d}
P-value	<0.001	<0.001	<0.001
SED	0.769	1.909	2.250
LSD (5%)	1.558	3.864	4.555
CV (%)	24	28.5	29.9

* means in the same column which share the same letter are not significantly different from one another at Fisher's LSD test at 5% significance level. Positive control represents fungicide (ridomil gold), negative control represents no addition of plant extracts (distilled water).

4.1.1 Effects of *Brassica juncea* plant extracts on radial mycelium growth of *F. oxysporum fsp tuberosi* at 3 days post inoculation.

Brassica juncea plant extracts had an effect on radial mycelium growth of *F. oxysporum* fsp *tuberosi* at 3 days post inoculation ($p < 0.05$) (Table 4.1 above). Radial mycelium growth in the *B. juncea* treatments (*B. juncea* at 1g; *B juncea* at 2g;, *B. juncea* at 3g) respectively were significantly lower ($p < 0.001$) than those recorded in the negative control. *Brassica juncea* 3 g recorded the lowest radial mycelium growth of 2.14 mm (Table 4.1 above) which was not significantly different from the positive control (Ridomil gold) treatment which recorded 1.06 mm. Radial mycelium growth decreased as the amount of plant tissues added to each Petri dish increased (Table 4.1above and Plate 4.1below).

4.1.2 Effects of *Brassica juncea* plant extracts on radial mycelium growth of *F. oxysporum fsp tuberosi* at 6 days post inoculation.

B. juncea plant extracts had an effect on radial mycelium growth of *F. oxysporum* fsp *tuberosi* at 6 days post inoculation ($p<0.05$) (Table 4.1 above). *Brassica juncea* plant extracts at 3g recorded the lowest radial mycelium growth of 8.06 mm while the negative control recorded a radial mycelium growth of 22.17 mm (Table 4.1 above). There was no significant difference between the positive control and *B. juncea* plant extracts at 3 g. There was a distinct reduction in radial mycelium growth as the amount of plant tissue added to each Petri dish increased. The same trend was also observed at 9 days post inoculation.

4.1.3 Effects of *Brassica juncea* plant extracts on radial mycelium growth of *F. oxysporum fsp tuberosi* at 9 days post inoculation.

Brassica juncea plant extracts at 2 and 3 g significantly reduced radial mycelium growth of *F. oxysporum* fsp *tuberosi* at 9 days post inoculation $p<0.05$. *Brassica juncea* 3 g consistently throughout the assessment period was not significantly different from the positive control (Table 4.1 above). Expected, *B. juncea* plant extracts at 1 g were not statistically different from the negative control and *F. oxysporum* fsp *tuberosi* had the ability to grow to fill the diameter of the Petri dish in 9 days.

Addition of *B. juncea* plant extracts into each Petri dish reduced radial mycelium growth of *F.oxysporum* fsp *tuberosi* (Plate 4.1).

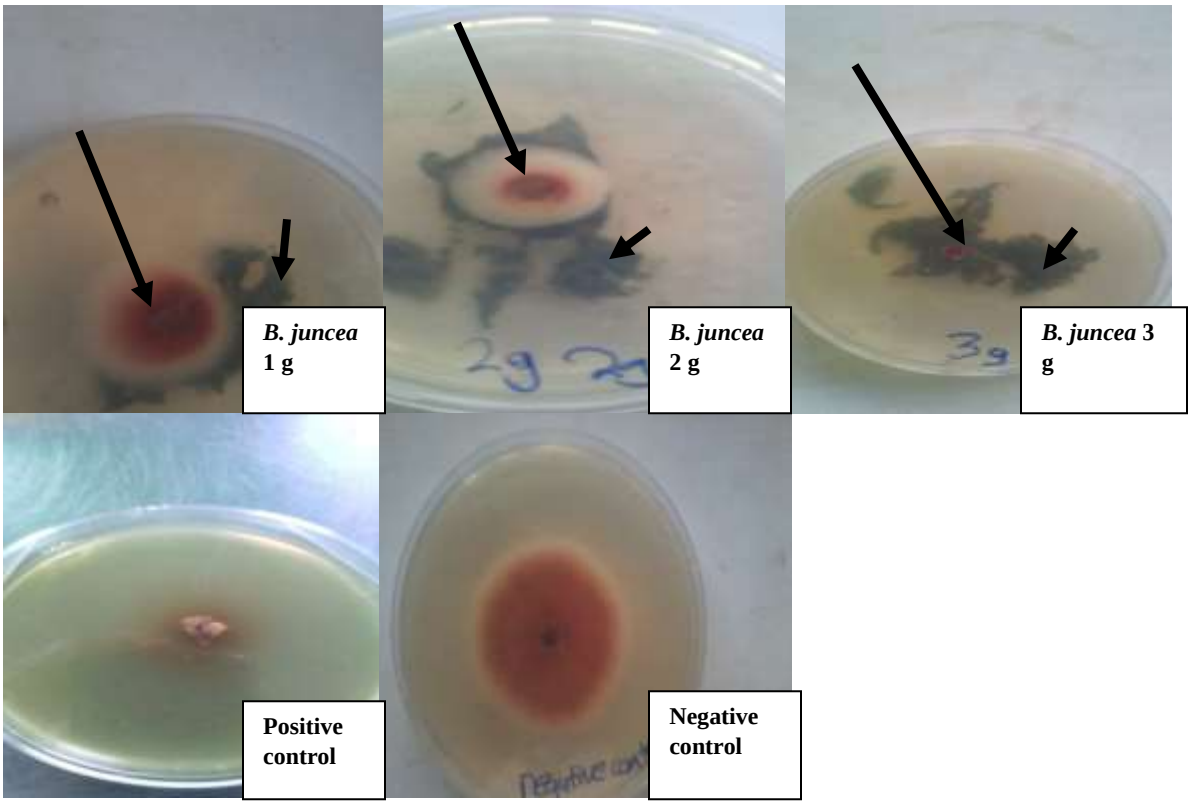
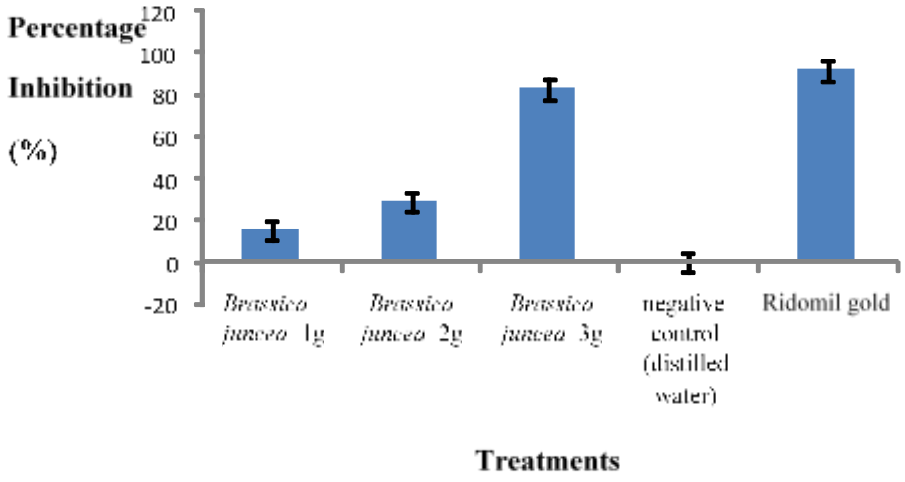


Plate 4. 1: Effects of *Brassica juncea* plant extracts on radial mycelium growth of *F. oxysporum* fsp *tuberosi* at 3 days post inoculation. The big arrows show mycelium growth of *F. oxysporum* fsp *tuberosi* and the small arrows show *B. juncea* plant extracts.

4.2 **Effects of *Brassica juncea* plant extracts on inhibition percentage of *Fusarium oxysporum* fsp *tuberosi* in-vitro**

4.2.1 Effects of *Brassica juncea* plant extracts on inhibition percentage at 3 days post inoculation.

Figure 4. 1 Effects of *Brassica juncea* plant extracts on inhibition percentage at 3 days post inoculation.



Brassica juncea plant extract concentration had an effect on inhibition percentage of *F. oxysporum* fsp *tuberosi* at 3 days post inoculation ($p<0.05$). *B. juncea* 3 g recorded the highest percentage inhibition of 82.3% (Figure 4.1). There was no significant difference between *B.*

juncea 3 g plant extracts and the ridomil gold (positive control). Distilled water (negative control) did not inhibit the pathogen and recorded the lowest percentage inhibition of 0% (Figure 4.1). Percentage inhibition increased as the amount of plant tissues added to each Petri dish increased.

4.2.2 Effects of *Brassica juncea* plant extracts on Inhibition Percentage 6 days Post Inoculation

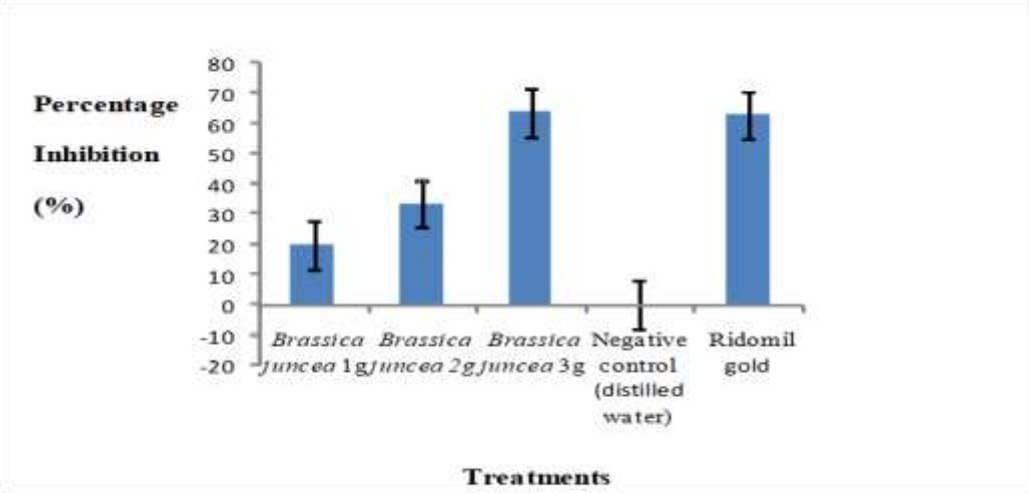


Figure 4. 2 Percentage Inhibition 6 days Post Inoculation.

Brassica juncea plant extract concentration had an effect on inhibition percentage of *F. oxysporum* fsp *tuberosi* at 6 days Post Inoculation ($p<0.05$). Distilled water did not inhibit the pathogen and recorded the lowest inhibition percentage of 0% (Figure 4.2). *Brassica juncea* 3 g plant extracts recorded the highest inhibition percentage of 63.6% while Ridomil gold recorded an inhibition percentage of 62.7% and there was no statistical difference between the two treatments. The general trend observed showed that inhibition of *F. oxysporum* fsp *tuberosi* increased as the amount of plant tissue added to each Petri dish increased

4.2.3 **Effects of *Brassica juncea* plant extracts on Percentage Inhibition 9 days Post Inoculation**

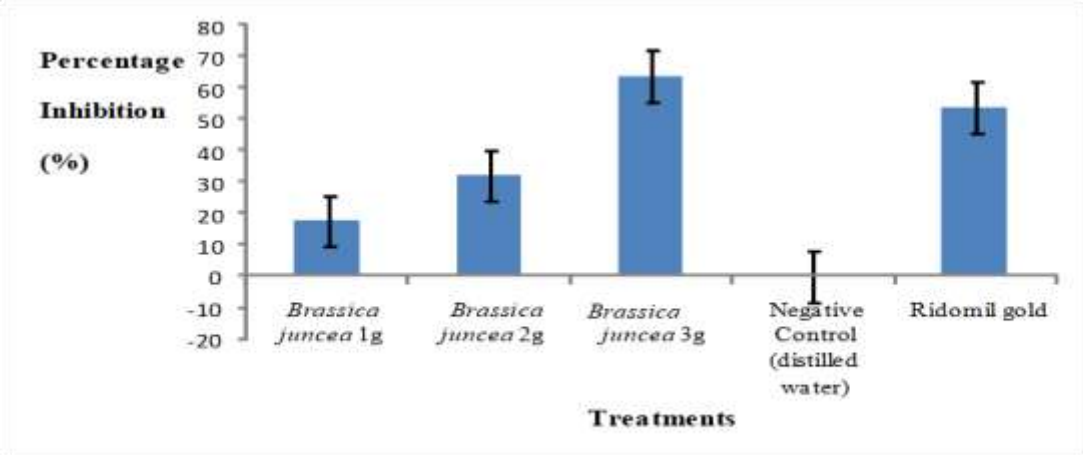


Figure 4. 3 Percentage Inhibition 9 days Post Inhibition.

Brassica juncea plant extract had an effect on inhibition percentage ($p < 0.05$) of *F. oxysporum* fsp *tuberosi* at 9 days Post Inoculation. *Brassica juncea* 3g recorded the highest inhibition percentage of 63.7% (Figure 4.3). Distilled water recorded the lowest inhibition of 0% (Figure 4.3). Percentage inhibition increased as the amount of plant tissues added to each Petri dish increased.

4.3 Biofumigant effects of *Brassica juncea* decomposed residues on the incidence and severity of vascular wilt pathogen *Fusarium oxysporum* fsp *tuberos*i in potatoes.

4.3.1 Effects of *Brassica juncea* decomposed residues incidence of vascular wilt pathogen *F. oxysporum fsp tuberosi* in potatoes.

Brassica juncea decomposed residues significantly reduced ($p < 0.05$) disease at 8 and 10 weeks after planting. *Brassica juncea* decomposed residues at 60 g recorded the lowest disease incidence of 5% at week 8 and was not statistically different from Ridomil gold (positive control). The negative control recorded the highest disease incidence of 75 % at 10 weeks after planting (Figure 4.4 below). However, all treatments failed to reduce disease incidence in week 12Disease incidence decreased as the amount of decomposed *B. juncea* decomposed residues increased (Figure 4.4).

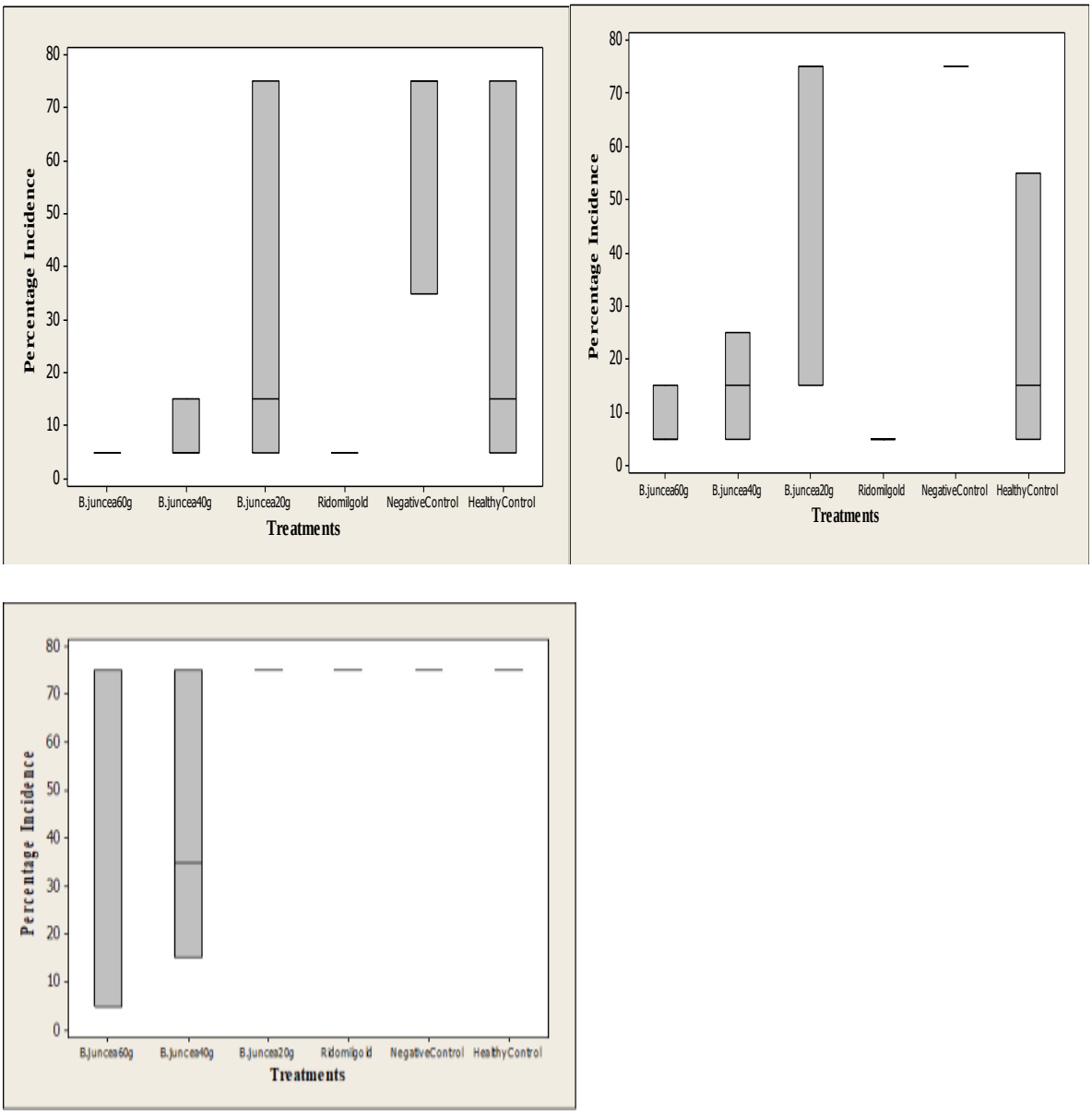


Figure 4. 4 Effects of *Brassica juncea* decomposed residues on disease incidence of vascular wilts measure at 8, 10 and 12 weeks after transplanting Diamond potato variety.

4.3.2 Effects of *Brassica juncea* decomposed residues on severity of vascular wilt pathogen *F. oxysporum fsp tuberosi* in potatoes.

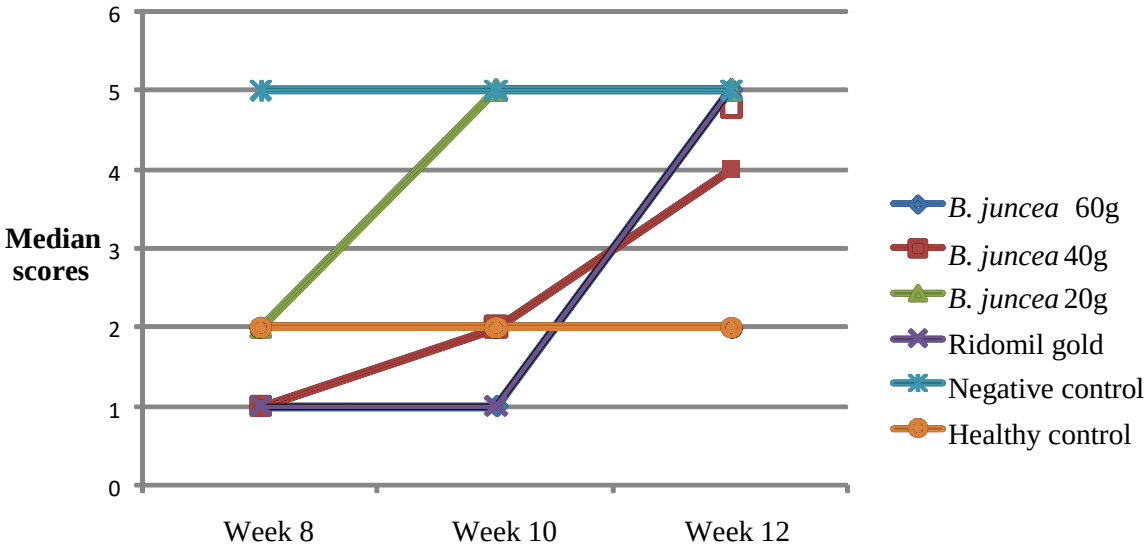


Figure 4. 5 Effects of *Brassica juncea* decomposed residues on disease severity at week 8, 10 and 12 of plant growth. Positive control represents fungicide (ridomil gold), Negative control represents inoculation with no amendments of *B. juncea* decomposed residues and Healthy control represents no inoculation and no amendments of *B. juncea* decomposed residues.

Kruskal and Wallis test with test static adjusted for ties revealed that *Brassica juncea* decomposed residues significantly reduced ($p < 0.05$) disease severity at 8 and 10 weeks after planting. *B. juncea* 60 g decomposed residues recorded the lowest severity score of 1 in week 8 while the negative control (distilled water), with inoculation and no amendments of *Brassica* residues recorded the highest severity score of 5 in week 10 (Figure 4.5 and Plate 4.2).

Addition of *B. juncea* decomposed residues reduced disease incidence and severity of *F. oxysporum* fsp *tuberosi* (Plate 4.2).

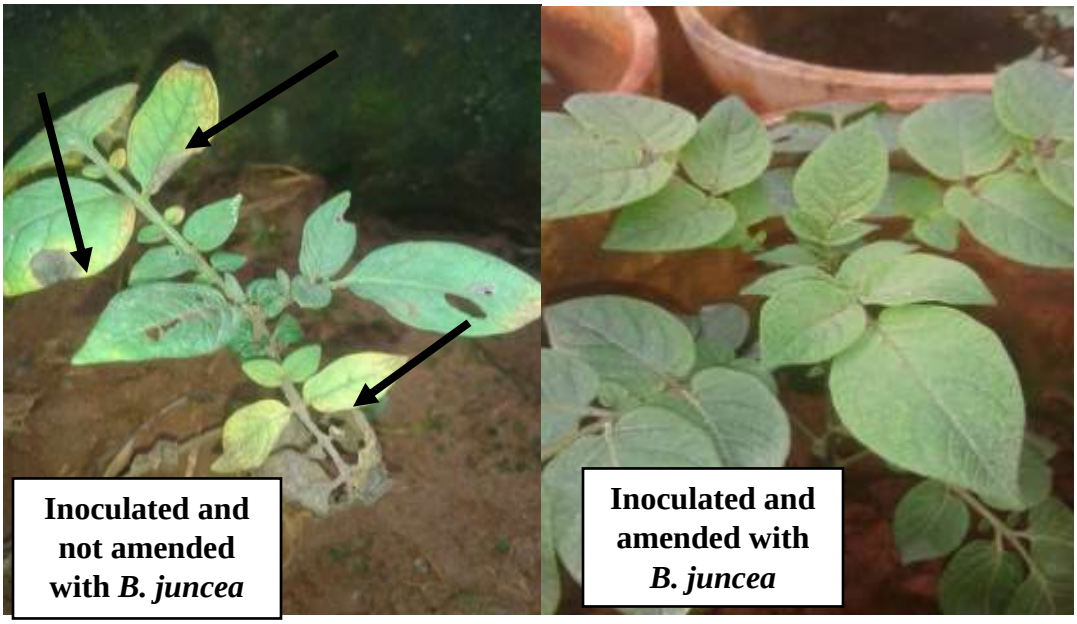


Plate 4. 2 Effects of *B. juncea* decomposed crop residues on disease incidence and severity of vascular wilt. Arrows show yellowing symptoms.

CHAPTER FIVE

DISCUSSION

5.1 Effects of *Brassica juncea* plant extracts on radial mycelium growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro.

The findings from this study showed that *B. juncea* plant extracts at 3g were effective in suppressing radial mycelium growth of *F. oxysporum* fsp *tuberosi* on day 3, 6 and 9 post inoculation. This suggested that *Brassica juncea* plant extracts had a biofumigant effect and produced high concentrations of isothiocyanates as hydrolysis products which were toxic to the pathogen. These results confirmed studies carried out by Handiseni *et al*. (2013), who observed that *B. juncea* plant extracts released high concentrations of isothiocyanates which significantly reduced the growth of *Rhizoctonia solani*. Reduction in pathogen populations resulting from a green manure crop are likely achievable since chlamydospores are sensitive to isothiocyanate. Pathogenic F. oxysporum isolates infesting nursery soils are mostly suppressed by plant species such *as Brassica carinata, B.nigra* and *B. juncea,* which contain glucosinolates that release high concentrations of propenyl isothiocyanate These results are also in line with similar studies carried out by Larkin and Griffin (2007), who observed that *B. juncea* produced isothiocyanates which resulted in the highest reduction in mycelium growth of fungal soil borne pathogens such as *Fusarium sambucinam* as compared to other crops such as ryegrass, barley, canola, rapeseed and turnip. Relevante and Cumagun (2013), concluded that *B. juncea* var *Monteverde* had a biofumigant effect which suppressed radial mycelium growth of *F. oxysporum*.

The findings of this study concur with literature that *B. juncea* is more effective as a biofumigant in-vitro at flowering. This could be attributed to the fact that *B. juncea* had a high

biomass accumulation at flowering as compared to the juvenile stage of the life cycle. High biomass accumulation resulted in increased concentrations and rapid release of isothiocyanates in-vitro. This attribute is supported with studies done by Fan *et al*. (2008), who observed that kohlrabi and *B. juncea* plant extracts were better suppressors of *F. oxysporum* at flowering as they had a higher concentration of isothiocyanates. Kirkegaard *et al.* (1996), mentioned that *B. juncea* had a higher suppressive effect than *B. napus* at flowering stages.

The extraction method done in the present study of freezing the tissues in a freezer and macerating plant extracts using a blender aided in the complete release of volatiles compounds which reduced radial mycelium growth of *F. oxysporum* fsp *tuberosi*. This could be attributed to the fact that maceration of plant extracts triggered a hydrolysis reaction between myrosinase and glucosinolates and produced hydrolysis products such as organic cyanades, aldehydes and sulphur compounds (Brown and Morra, 2005; Agrios, 2005). Therefore, maceration of plant tissues activated an enzyme which triggered the rapid release of secondary metabolites that were toxic. This is in agreement with studies done by Motisi *et al*. (2013), who showed that maceration of *B. juncea* leaves and roots aided in the complete release of toxic volatiles and enhanced suppression of the growth of *R. solani* in sugar beet. This explains the importance of macerating *Brassicas* in order to reduce radial mycelium growth and achieve the highest efficacy in biofumigation.

The results presented in this study showed a distinct reduction in mycelium growth similar to Ridomil gold which implied that *Brassica juncea* plant extracts produced isothiocyanates which had a mode of action that interfered with the biochemical pathways of *F. oxysporum* fsp *tuberosi* and reduced mycelium growth (Agrios, 2005). These results confirmed studies by Manici *et al*. (1997), who observed that isothiocyanates interfered with the biochemical and metabolic

pathways of *Pythium* fungal strains and reduced mycelium growth. Manici *et al*. (1997) and Taylor (2013), mentioned that a reduction in radial mycelium growth was caused by the inactivation of intracellular enzymes, inhibition of metabolic enzymes by radicals and inhibition of oxygen uptake by plant pathogens. This is also in agreement with studies done by Calmes *et al*. (2015), who observed a similar mode of action whereby exposure of *Alternaria brassicicola* to isothiocyanates led to a decreased oxygen consumption rate, intracellular accumulation of reactive oxygen species and mitochondrial membrane depolarization.

The results from this present study indicated that higher concentrations of plant extracts suppressed radial mycelium growth immensely as compared to lower concentrations. This suggested that high plant extract concentrations released high concentrations of toxic volatiles and enhanced the antifungal properties of PDA media as compared to lower concentrations of plant extracts and the negative control. Moreso, *F.oxsporum* was resistant to lower concentrations which resulted in lower suppression rate. The findings of this study are in agreement with Mari *et al*. (2008), who carried out research and concluded that the largest amount of isothiocyanates should be applied to achieve improved disease control against fungal pathogens.

Surprisingly, *B. juncea* plant extracts used at 1g were not suppressing radial mycelium growth of *F. oxysporum* fsp *tuberosi* at 9 days post inoculation and gave the same response as the negative control statistically. This is attributed to the fact that hormesis was taking place in lower concentrations whereby plant extracts had a stimulatory effect on the pathogen and only reduced radial mycelium growth of the pathogen at higher concentrations. These results are consistent with studies done by Manditsvara (2014), who found out that 10% *B. napus* plant extracts had the same effects on *Spongospora subterranea* as the negative control and concluded that lower

concentrations of isothiocyanates were not sufficient to cause biocidal effects on the pathogen. Moreover, failure of *B. juncea* 1g plant extracts to reduce radial mycelium growth of the pathogen 9 days post inoculation from the present study suggested

5.2 Effects of *Brassica juncea* plant extracts on inhibition percentage of *F. oxysporum* fsp *tuberosi.*

Brassica juncea plant extracts had inhibitory effects on *F. oxysporum* fsp *tuberosi* and were dosage dependent. The results from the present study concur with studies by Larkin and Griffin (2007), who evaluated the control of soil borne pathogens of potato using *Brassica* green manure crops. According to the results of Larkin and Griffin (2007), volatiles released from chopped leaf material of *Brassica juncea* at high concentrations resulted in almost complete inhibition (80 - 90%) of soil borne potato pathogens including *Fusarium sambucinam, Sclerotinia sclerotiorum* and *Pythium ultinum*. Furthermore, this is in agreement with work done by Sotelo *et al*. (2015), who evaluated the in-vitro activity of glucosinolates and their degradation products on *Brassica* bacterial and fungal pathogens. They found out that leaf methanolic extracts of *Brassica* crops suppressed growth of *Xanthomonas campestris* pv. *campestris*, *Pseudomonas syringae* pv. *maliculicola*, *Alternaria brassicae* and *Sclerotinia scletorium* and concluded that the inhibitory effects of glucosinolates were dosage dependent.

Moreover, a fungitoxic response was evident on day 3, 6 and 9 post inoculations at high concentrations of *B. juncea* extracts. This suggested that the pathogen was unable to grow and develop in response to the toxic volatiles (Taylor, 2013). The present study concurs with studies done by Smolinska *et al*. (2003), who carried out a similar experiment and observed that higher concentrations of isothiocyanates released by *B. juncea* inhibited *F. oxysporum* and resulted in a fungitoxic response. On the other hand, a fungistatic response was evident on day 3, 6 and 9 post inoculations at lower concentrations of *B. juncea* plant extracts. This suggested that the initial

point of fungal growth was delayed in responses to the presence of toxic volatiles (Taylor, 2013). These results are in agreement with studies done by Taylor (2013), who found out that lower concentrations of isothiocyanates inhibited *R. solani* by 49% and concluded that lower isothiocyanates gave a fungistatic response as they allowed growth of the pathogen.

5.3 Effects of *Brassica juncea* decomposed residues on severity of vascular wilt pathogen *F. oxysporum* fsp *tuberosi* in potatoes.

Brassica juncea decomposed residues reduced disease incidence and severity at 8 and 10 weeks after planting. Therefore, complete hydrolysis of glucosinolates to isothiocyanates had taken place at weeks 8 and 10. The results suggested that the isothiocyanates were toxic and interfered with the biochemical and metabolic pathways of *F. oxysporum* fsp *tuberosi* which reduced disease severity and incidence (Taylor, 2013). This is in line with work done by Hassan *et al*. (2016), who evaluated the biofumigant effects of crushed radish leaves to manage *F. oxysporum* and *Meloidogyne spp* in eggplants under greenhouse conditions. They observed that crushed radish leaves were effective in reducing disease incidence and severity. Moreover, a reduction in disease infection from the present study at weeks 8 and 10 was dosage dependent. Higher concentrations of isothiocyanates reduced disease incidence and severity compared to lower concentrations of isothiocyanates. The results from the present study confirmed research done by Gouws (2006), who observed that higher concentrations of isothiocyanates caused a reduction in common scab disease as compared to a lower concentration of isothiocyanates.

Surprisingly, *B. juncea* decomposed residues did not reduce disease incidence and severity in week 12. This suggested losses of isothiocyanates from the soil into the atmosphere through volatilisation. The findings of the present study support the work done by Rodríguez-Molina *et al*. (2016), who found out that the efficacy of biofumigation against soilborne pathogens was

reduced if *Brassica* green manure cover crops were incorporated without covering with plastics. Therefore, plastics reduce losses of isothiocyanates through volatilisation and promote high temperatures and anaerobic conditions in the soil which are detrimental to the growth of *F. oxysporum* fsp *tuberosi*. Moreover, studies done by Hansen and Keinath (2013), indicated that the concentration of isothiocyanates released by *B. juncea* cover crops increased when covered by a virtually impermeable film composed of polyethylene and reduced the infection of *R. solani* in pepper.

On the other hand, the experiment was done in red clay soils without organic matter which resulted in high losses of isothiocyanates. The findings of the present study concur with literature that volatile losses are greater from clay soils without organic matter (Brown and Morra, 2005). Clay soils have a high surface area and adsorb more isothiocyanates as compared to sandy soils which reduces the availability of isothiocyanates in the rhizosphere. Therefore, small amounts of isothiocyanates were available for disease control in clay soils and were prone to volatilisation. The results in the present study are in line with work done by Price *et al*. (2005), who quantified volatiles produced from *B. juncea* tissue incorporated in sandy loam soil and clay loam soil. They observed that sandy loam soils had more isothiocyanates as compared to clay loam soils. Moreso, leaching of isothiocyanates could have attributed to their unavailability to suppress disease incidence and severity at week 12. This is in line with studies done by Laegdsmand *et al*. (2007), who found out that isothiocyanates from rape plant material leached from the rhizosphere in both sandy and loamy soils after irrigation.

Moreover, the results showed that the efficacy of *Brassica* green manure decreased distinctly in week 12. This suggested that the incorporation method of green manure cover crops was not beneficial as compared to other methods such as the use of defatted seed meals. This is in

agreement with work done by Rodríguez-Molina *et al*. (2016), who found out that defatted seed meals of *Brassicaceae* in the form pellets reduced disease severity and incidence of *Phytophthora nicotiane* which causes crown root in pepper as compared to the green manure cover crops.

Generally, this study has proved that *B. juncea* reduces radial mycelium growth and has inhibitory effects on *F. oxysporum* fsp *tuberosi. Brassica juncea* has the ability to reduce disease incidence and severity in the early stages of incorporation. *B. juncea* has the potential to be used as an alternative to fungicides by farmers. However, there is need to reduce loss of isothiocyanates in order to reduce disease incidence and severity at late stages of incorporation.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study it can be confirmed that *Brassica juncea* has biofumigant effects. The results from this study proves that *Brassica juncea* plant extracts at 3g works very well to reduce radial mycelium growth of *Fusarium oxysporum* fsp *tuberosi* in-vitro. Furthermore, *B. juncea* plant extracts used at 3g are fungitoxic and *B. juncea* plant extracts used at 1 and 2 g are fungistatic. Brassica juncea extracts at 1g and distilled water had no effect on the pathogen .*Brassica juncea* decomposed residues at 60g reduce disease incidence and severity of vascular wilt pathogen *F. oxysporum* fsp *tuberos*i in potatoes at week 8 and 10. However, *B. juncea* decomposed residues do not reduce disease incidence and severity of vascular wilt pathogen *F. oxysporum* fsp *tuberos*i in potatoes at late stages of crop residue incorporation.

6.2 Recommendations

- Farmers are recommended to use Brassica juncea at extract at 3g rate in control *of F. oxysprosum fsp tuberosi* in potatoes. It is cheaper as compared to Ridomil Gold and it is cheaper.
- Farmers are recommended to add another Brassica juncea residues after 12 weeks to avoid reduction of its action in control of *F. oxsprosum fsp tuberosi*.
- Farmers are recommended to practice biofumigation during the summer season to ensure complete hydrolysis of isothiocyanates and must be used in combination with soil solarisation in order to avoid volatilisation of isothiocyanates.

- Smallholder farmers can harvest leaves and sell them and leave the plants to flower and use them as a biofumigant.
- Biofumigation trials should be carried out under field conditions and in different soil types.
- There is need for further research in agronomy to find ways to increase the Brassica juncea isothiocyanates concentrations as their control are dosage dependent.

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APPENDICES

Appendix 1 Analysis of variance for the effects of *Brassica juncea* plant extracts on Radial Mycelium Growth 3 days Post inoculation

Source of variation	DF	SS	MS	F	P
Block	2	6.011	3.006	1.13	
Treatments	4	866.508	216.627	81.32	<.001
Residual	38	101.225	2.664		
Total	44	973.744			

Appendix 2 Analysis of variance for the effects of *B. juncea* plant extracts on Radial mycelium growth 6 days post inoculation

Source of variation	DF	SS	MS	F	P
Blocks	2	63.97	31.99	1.95	
Treatments	4	1355.12	338.78	20.67	<.001
Residual	38	622.95	16.39		
Total	44	2042.04			

Appendix 3 Analysis of variance for the effects of *B. juncea* plant extracts on Radial mycelium growth 9 days Post inoculation

Source of variation	DF	SS	MS	F	P
Block	2	71.34	35.67	1.57	
Treatments	4	1416.53	354.13	15.55	<.001
Residual	38	865.60	22.78		
Total	44	2353.48			

Appendix 4 Analysis of variance for the effects of *B. juncea* plant extracts on Inhibition Percentage 3 days Post Inoculation

Source of variation	DF	SS	MS	F	P
Block	2	107.9	54.0	0.29	
Treatments	4	60393.6	15098.4	81.77	<.001
Residual	38	7016.7	184.7		
Total	44	67518.2			

Appendix 5 Analysis of variance for the effects of *B. juncea* plant extracts on Inhibition percentage 6 days post inoculation

Source of variation	DF	SS	MS	F	P
Block	2	899.2	449.6	1.57	
Treatments	4	27458.0	6864.5	24.03	<.001
Residual	38	10856.3	285.7		
Total	44	39213.6			

Appendix 6 Analysis of variance for the effects of *Brassica juncea* plant extracts on Inhibition Percentage 9 days Post Inoculation

Source of variation	DF	SS	MS	F	P
Block	2	258.8	129.4	0.43	
Treatments	4	24283.3	6070.8	20.20	<.001
Residual	38	11419.5	300.5		
Total	44	35961.6			

Appendix 7 Analysis of variance for the effects of decomposed *B. juncea* residues on Incidence Percentage 8 weeks

Source of variation	DF	SS	MS	F	P
block stratum	2	2544.4	1272.2	2.94	
treatment	5	7511.1	1502.2	3.48	0.044
Residual	10	4322.2	432.2		
Total	17	14377.8			

Appendix 8 Analysis of variance for the effects of *B. juncea* plant extracts on Incidence percentage 10 weeks

Source of variation	DF	SS	MS	F	P
Block	2	544.4	272.2	0.77	
Treatments	5	11977.8	2395.6	6.80	0.005
Residual	10	3522.2	352.2		
Total	17	16044.4			

Appendix 9 Analysis of variance for the effects of *B. juncea* plant extracts on Incidence Percentage 12 weeks

Source of variation	DF	SS	MS	F	P
block stratum	2	1477.8	738.9	2.02	
block.*Units* stratum					
treatment	5	3361.1	672.2	1.84	0.193
Residual	10	3655.6	365.6		
Total	17	8494.4			

Appendix 10 Kruskal-Wallis Test for the effects of decomposed *B. juncea* residues on severity at 8 weeks.

treatment	N	Median	Ave Rank	Z
1	3	1.000	5.5	-1.42
2	3	1.000	7.5	-0.71
3	3	1.000	8.8	-0.24
4	3	1.000	5.5	-1.42
5	3	5.000	15.5	2.13
6	3	5.000	14.2	1.66
Overall	18		9.5	

H = 9.92 DF = 5 P = 0.078
H = 12.51 DF = 5 P = 0.028 (adjusted for ties)

Appendix 11 Kruskal-Wallis Test for the effects of decomposed *B. juncea* residues on severity at 10 weeks

Treatment	N	Median	Ave Rank	Z
1	3	1.000	5.8	-1.30
2	3	2.000	8.5	-0.36
3	3	5.000	13.5	1.42
4	3	1.000	4.0	-1.95
5	3	5.000	15.5	2.13
6	3	2.000	9.7	0.06
Overall	18		9.5	

H = 10.18 DF = 5 P = 0.070
H = 11.37 DF = 5 P = 0.045 (adjusted for ties)

Appendix 12 Kruskal-Wallis Test for the effects of decomposed *B. juncea* residues on severity at 12 weeks

treatment	N	Median	Ave Rank	Z
1	3	5.000	8.2	-0.47
2	3	4.000	6.2	-1.18
3	3	5.000	11.5	0.71
4	3	5.000	8.2	-0.47
5	3	5.000	11.5	0.71
6	3	5.000	11.5	0.71
Overall	18		9.5	

H = 2.81 DF = 5 P = 0.730
H = 5.30 DF = 5 P = 0.380 (adjusted for ties)