EFFECT OF *Tagetes minuta* AND *Capsicum frutescens* EXTRACTS ON *Pectobacterium carotovorum*, GROWTH, YIELD AND QUALITY OF POTATOES (*Solanum tuberosum*)

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented before in any institution for any other award.

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DEDICATION

This work is dedicated to my dear children Lucky, Faith and Billy; you are the reason I look forward to tomorrow.

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ABSTRACT

Potato production is hindered by pathogens like *Pectobacterium carotovorum* that cause blackleg in the field and soft rot after harvest. The purpose of this study was to determine the possibility of integrating *Tagetes minuta* and *Capsicum frutescens* extracts in the management of soft rot and blackleg in potatoes. The research was conducted at the horticulture research and teaching laboratory and field of Egerton University, Njoro. *In vitro* and *in vivo* experiments were set up in a Completely Randomized Design and Randomized Complete Block Design with three replications using aqueous extracts of *T. minuta* and *C. frutescens* each at 40%, 30% and 20% concentrations. Water and streptomycin sulphate/Copper oxychloride were negative and positive controls respectively. Data collected was analyzed using Genstat edition 4 and significantly different means were separated using Tukey's HSD test. The treatments were applied on nutrient agar (using a modified disc diffusion method) and on potato chips to test for bacterial growth inhibition and their effect on potato tissue maceration. *T. minuta* (40%, 30% and 20%) showed zones of inhibition of 7.17 mm, 6.67 mm and 6.10 mm; streptomycin sulphate 8.83 mm which were significantly different from *C. frutescens* and distilled water that recorded 0.00 mm zones of inhibition. *T. minuta* and streptomycin sulphate showed a significant difference in the number of days (9 days) to total tissue maceration and percent weight loss from *C. frutescens* and water that took only 5 days for the potato chips to be completely macerated.

For *in vivo* experiments, the treatments were applied on seed potato before sprouting, sprouted potato before planting and ware potato before storage. The plant extracts showed significant differences on disease incidence and severity on the plants and tubers in the field but showed no significant difference on growth parameters. The potato plants treated with 40% and 30% *T. minuta*; and copper oxychloride recorded low disease incidents (2 plants/plot) and low severity (40-54%) while those treated with water and *C. frutescens* showed high disease incidents (4 plants/plot) and high severity (57-93%). Copper oxychloride and *T. minuta* recorded low percent postharvest infections (6.5-12.11%) while *C. frutescens* had high infections (40-95%). The extracts had no significant effects in the dry matter content and total soluble solids of the potatoes. It was found that *T. minuta* is effective against *Pectobacterium* both *in vitro* and *in vivo* and therefore it can be incorporated in the integrated disease management systems as an affordable bio-control component to manage blackleg and soft rot in potatoes.

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ACRONYMS

ADC	Agricultural development corporation
ANOVA	Analysis of variance
CFU	Colony forming units
CO	Copper oxychloride
CRD	Completely randomized design
DAI	Days after inoculation
DAP	Days after planting
DAS	Days after storage
DM	Dry matter
HSD	Honestly significant difference
MOA	Ministry of agriculture
NA	Nutrient agar
RCBD	Randomized completely block design
SM	Streptomycin sulphate
TSS	Total soluble solids

CHAPTER ONE

INTRODUCTION

1.1. Background information

Potato (*Solanum tuberosum)* produces tubers that are consumed the world over as crisps, mashed potato, chips (French fries) and even mixed with other foods. Traditionally, potato is grown from tubers but can also be grown from stems (sprouts) and true seed (Donnelly *et al.,* 2003). Potato is the fourth main food crop in the world after rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*), (Cunnington, 2008; Czajkowski *et al.,* 2011). In Kenya, it is the second most important food crop after maize in terms of bulk harvested. It is an important staple and cash crop for smallholder growers in the Kenyan highlands and in the wider Eastern Africa (Gildemacher, 2012) where it has a high potential to raise smallholder income and improve food security. Grown by more than 800,000 farmers, potato plays an important role in employing over 2.5 million people indirectly as market agents, transporters and processors (Onditi *et al*., 2012).

Despite its importance, on-farm potato yields in Kenya fall below 10 t ha^{-1} compared to the potential yields of 40-60 t ha^{-1} attainable by progressive growers (Kaguongo *et al.,* 2008) because of production constraints that include low soil fertility, inadequate supply of certified seeds, use of low yielding varieties and diseases (Muthoni and Nyamongo, 2009). High quality seed potatoes account for less than 7% of the whole potato seed market and this encourages the common practice among potato growers of planting own-saved tubers from previous harvests or from neighbours (Gildemacher, 2012). Few seed potatoes are currently sourced from specialized multipliers as growers largely rely on farm/home-saved seed potato which makes economic sense in the absence of affordable high quality seed potato and limited market security (Kumar *et al.,* 2013). It is reported that in 2009/2010, economic losses due to blackleg, soft rot and seed piece decay ranged from 23% to 40% in Zimbabwe (Ngadze and Icishahayo, 2014). More than 50% of the potato farms across Kenya also reported cases of soft rot and blackleg diseases which accounted for as much as 25% of the annual potato losses in 2012/2013 (Onkendi and Moleleki, 2014).

Some of the methods that could be used to manage soft rot include reduction of wounding during harvesting, promoting wound healing after harvest, proper ventilation in storage, storing potatoes below 15 °C, use of tissue culture propagation material (seed potato), crop rotation,

planting in well drained soils, use of copper based bactericides, use of chlorinated water to clean potato after harvest and sanitizing tools and equipment used during field management practices. These methods have shown low success because the grower has no control over the environmental conditions and the chemicals may be fairly expensive besides their negative impacts on the environment. The growers, marketers and middlemen also lack knowledge on pre-harvest and post-harvest handling of potatoes to help minimize predisposing the potatoes to soft rot infections.

Secondary plant products including flavonoids, steroidal alkaloids and saponins have shown antibacterial activity against plant pathogenic bacteria **(**Oguwike *et al.,* 2013). Plant extracts and oils from neem (*Azadirachta indica*), pepper (*Capsicum spp*.) and *Acassia spp*. have been used against plant diseases (Bowers and Locke, 2004). Plant extracts used to control phytopathogens in plants have also been obtained from *Eucalyptus spp*., garlic (*Allium sativum*), mint (*Mentha spicata*), ginger (*Zingiber officinale),* turmeric (*Cucurm longa)* and basil (*Ocinum tenuiflorum)* (Stangarlin *et al*., 2011). Essential oils and crude extracts from other plants like guava (*Psidium guajava),* rosemary (*Rosimarinus oficinalis),* thyme (*Thymus vulgaris*) and coriander (*Coriandrum sativum)* have also been reported to inhibit growth of bacteria (Nezhad *et al*., 2012; Sarah *et al*., 2012).

Studies show that *Tagetes minuta* has been used in pest control. Leaf extracts were used to reduce root-knot nematode infestation and increase the number of fruits and total yield of tomato (Taye *et al.,* 2012). Essential oils from *Tagetes minuta* (dihydrotagetone, ocimene, terpinolene, piperitone, β-caryophyllene) have also been shown to exhibit antimicrobial activity against bacteria and fungus (Rondon *et al.,* 2006). Aqueous extracts of chilli (*Capsicum spp*.), onion (*Allium cepa*), garlic (*Allium sativum*) and leek (*Allium porrum*) have been proved to be good antibacterials against *Pectobacterium carotovorum* (Ortego *et al.,* 2003).

The current study examined the prospects of using the extracts from *Tagetes minuta* and *Capsicum frutescens* in the management of soft rot in potatoes and the resulting effects on growth and yield of potatoes. The results showed that *Tagetes minuta* extracts inhibit the growth of *Pectobacterium* bacteria both *in vitro* and *in vivo*. Application of *Tagetes minuta* extracts to seed potato before planting significantly reduced incidences of soft rot both in the crop plants in the field and the resulting tubers. *Tagetes minuta* also significantly reduced soft rot infections in stored potato. There was overall higher potato yield from the plots treated with *T. minuta*.

However, *Capsicum frutescens* did not inhibit the bacteria both *in vitro* and *in vivo* and there was significantly lower yield from the plots treated with *C. frutescens*. The plant extracts did not show significant effects on the plant height, number of stems and number of stems of potatoes.

1.2. Statement of the problem

Potato, the fourth most important food crop in the world and the second most important food crop in Kenya experiences high losses caused by the black leg and soft rot bacterial pathogen, *Pectobacterium carotovorum.* On-farm potato yields in Kenya remain below 10 t ha^{-1} compared to the potential yields of 40-60 t ha^{-1} attainable by progressive growers due to production constraints that include soft rot infections. Contamination of tubers by the bacteria starts from the field and continues during storage. As a result of the challenges caused by the bacteria, potato yields have remained low leading to loss of income for the growers and insufficient supply of quality potatoes for the consumers.

1.3. Objectives

1.3.1. Broad objective

To contribute to increased food security through management of potato black leg and soft rot caused by *Pectobacterium carotovorum*.

1.3.2. Specific objectives

i. To determine the effect of *T. minuta* and *C. frutescens* extracts on *Pectobacterium carotovorum, in vitro*.
ii. To determine the effect of *T. minuta* and *C. frutescens* extracts on potato blackleg and soft rot, *in vivo.*
iii. To determine the effect of *T. minuta* and *C. frutescens* extracts on sprouting, plant growth, yield and quality of potatoes

1.4. Null hypotheses

i. *T. minuta* and *C. frutescens* extracts have no effect on *Pectobacterium* bacteria, *in vitro.*
ii. *T. minuta* and *C. frutescens* extracts have no effect on potato blackleg and soft rot, *in vivo*.
iii. *T. minuta* and *C. frutescens* extracts have no effect on sprouting, plant growth, yield and quality of potatoes.

1.5. Justification of the study

Potato being the fourth main food crop in the world after rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*) and second most important staple food crop in Kenya after maize, requires attention in terms of yield, proper storage, nutritional quality, pest and disease management. The importance of potato in Kenya continues to rise due to increased urbanization, change of eating habits and uptake of processed potato products.

Good quality potato is important in ensuring high returns for the growers and food security for consumers. This is because diseased and pest infested potatoes fetch low prices or even do not sell at all and the losses are felt by the growers at the same time reduces food security. At the same time, pests and diseases lead to low supply of potato to the market meaning potato consumers lack potatoes or pay high prices for the little available. Incorporating *Tagetes minuta* extracts in the integrated disease management to control blackleg and soft rot will lead to low cost of production, less wastages and ample supply of potato to the market. Control of soft rot in potatoes will also ensure high quality potatoes and prevent fluctuations of potato supply in the market thereby contributing to the national food security. The use of *Tagetes minuta* extracts is also advantageous because of its rapid degradation with no persistence and bio-accumulation in the environment which are major problems associated with the use of synthetic chemicals to control pests and diseases. Besides, *Tagetes minuta* is locally available as it grows as a weed in most fields and can therefore be easily and locally processed for use in the farms.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of potato

Potato is an annual plant in the family *Solanaceae* that originated from the Andes near the border of Peru and South America (Hijmans and Spooner, 2001). Potato shares the genus *Solanum* with many other plant species among them *Solanum nigrum* (black night shade), *Solanum esculentum/Lycopersicon esculentum* (tomato), *Solanum incunum* (sodom apple), *Capsicum spp* (pepper) and *Solanum melanongena* (eggplant). It is a succulent, dicotyledonous plant with alternate leaves above ground and three kinds of stems; sprouts, stolons and tubers (Struik and Wirsema, 1999).

Potato requires cool night temperatures that are important in accumulation of carbohydrates and dry matter which are essential in enhancing starch storage in tubers. The number of tubers set per plant is high at lower temperatures whereas higher temperatures favour development of foliage but retard tuberization (Haverkort 1990; Hausler *et al.,* 2002). This means that extremely cool and high temperatures reduce net assimilation in tubers while high temperatures may prevent tuber initiation. Worthington and Hutchinson (2005), also confirm that potato is best adapted to cool climates with mean daily temperatures ranging from 15 °C-18 °C. These are the conditions in the mid-highlands of altitudes ranging from 1,400 m to 3,000 m above sea level in Kenya where potato is largely grown (Onditi *et al*., 2012). Potato is highly susceptible to diseases such as late blight (*Phytophthora infestans*) bacterial wilt (*Ralstonia solanacearum*) and *Pectobactrium carotovorum* which cause blackleg and soft rot.

2.2. Uses of the potato

According to Khurana *et al*. (2003), over a billion people in different parts of the world consume potato. The potato tuber contains important dietary constituents such as carbohydrates, proteins, vitamins and minerals needed for healthy growth (Burt, 1989; Fernie and Willmitzer, 2001; Camire *et al.,* 2009). Potato tubers contain 77.7% water, 2% proteins, 19.4% carbohydrates, 0.6% crude fibre, 0.1% fats and 1% ash (Buckenhuskes, 2005). Silva and Simon (2006) also state that potato contains the three forms of sugar (sucrose, fructose and glucose) and other nitrogenous compounds. All of the above confirm that potato contain important nutrients

and therefore good as a staple food. In Kenya, recipes for preparing potato are diverse; ranging from boiling, roasting, frying to mixing with other foods like meats, grains and cereals.

Non-food industries use potato to produce animal feeds, starch and alcohol among other products (Struik and Wiersema, 1999). Potato starch is used by the pharmaceutical, textile, wood and paper industries as an adhesive, binder, texture agent and filler, and by oil drilling firms to wash boreholes (Ugonna *et al.,* 2013). Besides, potato farming in Kenya employs about 2.5 million people at all levels of the value chain (MOA, 2008) from the farm to the point of consumption. Potato is also used as a cash crop especially in Kenya where many growers depend on it as their source of income and therefore has raised livelihoods of many people as emphasized by Gildemacher *et al.* (2009) who say that enhanced productivity of potatoes can improve the livelihood of smallholder farmers as well as meet the growing consumer demand.

2.3. The *Pectobacterium carotovorum* bacteria

Yield and quality of potato is affected by many factors including bacterial diseases such as bacterial wilt (*Ralstonia solanacearum*), ring rot (*Clavibacter michiganensis*), soft rot and blackleg that are caused by *Pectobacterium carotovorum* (Bibi *et al.*, 2013). As alluded by Barabote *et al*. (2003) and Costa *et al.* (2006), the main bacteria causing blackleg in the growing plant and soft rot in tubers are the bacteria belonging to the genus *Pectobacterium* (formerly *Erwinia*). These are *Pectobacterium carotovorum* subsp. *carotovora* and *Pectobacterium carotovorum* subsp. *atroseptica*. These bacteria are pectinolytic Gram-negative, facultative, anaerobic, non-sporing, motile, straight rods with peritrichous flagellae and they belong to the c-Proteobacteria subdivision in the Enterobacteriaceae family (Ismael *et al.,* 2012).

Pectobacterium carotovorum bacteria may affect vines and tubers in the field or after harvest. The disease is expressed as blackleg and aerial stem rot during the growing period whereas soft rot affects tubers in the field, during transit and storage (Riyad *et al*., 2013). Blackleg is characterized by blackening of the stem base and an inky black decay of potato plants. The plants' foliage yellow and the plants finally die (Bibi *et al.,* 2013; Marquez-Villavicencio *et al*., 2011). On the other hand, soft rot in tubers is characterized by a watery slimy rot with a whitish, cloudy, slimy liquid that ooze from breaks in the tuber tissues with a rotted odour.

Wounded potato tubers tend to be more susceptible than non-wounded ones especially in stored potato**.** Natural openings in the tuber such as lenticels, stolon ends and abscission tissues are also points of entry for *Pectobacterium* soft rot bacteria initiating infection that may advance to total destruction of the tuber and spread to adjoining tubers as ooze from the rotting tubers (Czajkowski *et al.,* 2011).

Soft-rot *Pectobacterium* bacteria have built-in redundancy for pathogenicity expressing four pectin-degrading extracellular enzymes: polygalacturonase, protease, pectin methylesterase and pectate lyase which is primarily responsible for extensive potato decay (Barth *et al.,* 2009; Prajapat *et al*., 2013). Pectate lyase cleaves pectate trans-eliminatively and randomly due to its high virulence factor (Tsuyumu *et al*., 2014). The enzymes allow infiltration and maceration of plant tissues on which they feed on and destroy the "cementing" material between cells resulting in the watery slimy rot with a whitish, cloudy, slimy liquid oozing from breaks in the plant tissue with a rotted odour (Austin *et al.,* 1988; Elphinstone and Perombelon, 1987)

Soft rot bacteria use quorum sensing; a population density-dependent regulatory mechanism (employed by most bacteria) to effect infection (Poonguzhali *et al*., 2007). *Pectobacterium carotovorum* bacteria use at least two quorum sensing systems; N-acylhomoserine lactone (AHL) based as well as autoinducer-2 (AI-2) dependent signaling systems (Põllumaa *et al*., 2012). This explains its aggressiveness once infection has occurred which may clear a whole stock of potatoes within a short time.

Conditions associated with handling and harvesting of produce are also means by which the soft rot bacteria pathogen enter tissues. These include sunscald, staking, tying plants, tissue bruising, inadequate suberization of potato seed pieces, insect damage and latent infections in seed tuber (Hannukkala and Segertedt, 2004). Extensive contamination may occur during harvest and grading when the bacteria from rotting tubers spread to fresh wounds and natural openings of other tubers. Environmental conditions may also induce soft rot development. These include high humidity, high rainfall or over irrigation, poor drying conditions and warm temperatures of 22 ^{o}C - 33 ^{o}C. Under field conditions, bacteria from rotting seed tubers are transmitted through soil water to the lenticels of progeny tubers (Pérombelon and Kelman, 1987).

A study carried out by Ali *et al*. (2010) regarding soil, diseased plant debris or seed potatoes as sources of inoculum for *Pectobacterium* bacteria found that seed potatoes were the

most important source of primary inoculum because the bacterial population considerably increased in numbers throughout the monitoring period.

2.4. Management of potato blackleg and soft rot

Several approaches have been employed to control blackleg and tuber soft rot in potato but the degree of success has been variable. According to Czajkowski *et al.* (2011), methods like avoidance of contamination, seed certification, improved store management, hot water treatment, chemical methods and breeding for resistance have been explored. As *Pectobacterium carotovorum* contamination of potatoes is widespread and incidences are related to seed tuber contamination, seed health has been assessed by inspecting parental crops for blackleg plants and selecting crops with no or a low disease incidence for seed (Pérombelon, 1992) to avoid planting seed that is already infected as this will enhance the disease incidences. Potato seed can also be treated against the bacteria before planting and ware potato before storage to reduce the incidences and severity of the disease in the crop before and after harvesting.

2.4.1. Chemical control strategies

Copper based compounds are used to control bacterial infections but once infection occurs, they cannot control the disease. It has been observed that application of copper-based chemicals was effective in reducing soft rots in Calla lilies (Gracia-Garza *et al.*, 2002). As stated by Elphinstone *et al*. (1987), copper sprays may be used to prevent infection of wounded plant stems and leaves but once the plant is colonized there is no chemical control for this pathogen. Antibiotics like streptomycin sulphate could also diminish disease incidence but there are dangers of development of resistance with continued use as indicated by Upma *et al*. (2014) who say that more strains of pathogens have become antibiotic resistant and some have become resistant to several antibiotics.

However, the use of synthetic chemicals to control crop diseases has been criticized the world over because of their adverse effects on human health and the environment (Babana *et al.*, 2011). Besides, indiscriminate use of synthetic chemical pesticides to control various pests and pathogenic microorganisms of crop plants have been implicated in causing health hazards both in terrestrial and aquatic systems through their residual toxicity (Rahman *et al.*, 2012). As such, the current trend is to use products with as little synthetic chemicals as possible, or use of no synthetic chemicals at all. Therefore an eco-friendly method is needed in the control of the crop

diseases (Poudyal, 2012) and more so in the control of *Pectobacterium* blackleg and soft rot in potatoes.

2.4.2. Avoidance methods

Proper sanitation aimed at avoiding contamination of plants and tubers has been used to control diseases in potatoes. This involves rogueing of infected progeny tubers and foliage to reduce the source of the pathogen inoculum and avoid spread during mechanical handling at harvest and postharvest (Czajkowski *et al.*, 2011). Use of well-drained fields also reduces the risk of tubers being surrounded by a water film that can result in anaerobiosis and consequent tuber decay in the field. Late harvesting allows bacterial multiplication on leaves and in debris left on the ground following haulm flailing. Studies show that dehaulming could expose the crop to a lesser period to diseases and pests which results in healthy seed crop **(**Mahmud *et al.*, 2009). This is because tubers that are left in the soil for an adequate time after vine death to allow sufficient skin set are less subject to wounding and consequently less likely to be candidates for bacterial infections while tubers harvested from green vines are more susceptible to postharvest soft rot**.**

Washing and disinfection of machines used when spraying, haulm flailing, harvesting and grading in store also help to reduce risks of introducing soft rot bacteria in a pathogen-free crop (Perombelon, 1992). Correct machinery adjustment during harvesting and grading reduces the risk of wounding. Drying the tubers rapidly by forced ventilation with warm air also favours wound healing by formation of a wound periderm which forms a barrier to disease microbes and water loss. This not only prevents tuber decay but also avoids increasing the tuber inoculum load thereby no subsequent disease risks. Using true seeds instead of seed tubers can also reduce the incidence of tuber infections with blackleg and soft rot because most seed-borne diseases are not transmitted through the true seed (Muthoni *et al.*, 2013). This however may not be the best option as the resulting crop lacks uniformity, low yields and the seedlings lack the capacity to tolerate environmental stresses. It may not be a viable venture in the farms as the farmers may not have the knowhow in producing tubers from true seed of potatoes.

2.4.3. Plant nutrition

Proper plant nutrition may induce natural disease tolerance and reduce susceptibility to soft rot pathogens. Calcium fertilization is thought to increase calcium level in plant cell that

contributes to plant resistance to bacterial soft rot as demonstrated in Kunming where calla lily growers apply lime (CaO) as calcium fertilizer (Ni *et al.,* 2010). Calcium plays an important role in the tolerance of plants against bacterial pathogens by improving the structure and integrity of plant cell wall components resulting in higher resistance to diseases involving tissue maceration. This is surported by Czajkowski *et al.* (2011) who noted that calcium fertilization reduced soft rot caused by *Pectobacterium spp.* in Chinese cabbage, beans and potato tubers which may have been related to calcium concentration in the tissues. This is also in agreement with Gracia-Garza *et al*. (2004) who indicate that appropriate calcium uptake by plants can reduce soft rot due to improved plant tolerance.

According to Bartz *et al.* (1992), reduction of bacterial soft rot in tubers has been associated with enhanced levels of calcium in the tissues which can be achieved by fertilization with gypsum. This was seen in tubers with higher content of calcium which had lower soft rot potential than those with low levels of calcium. This is echoed by Arvin *et al*. (2005) who carried out an experiment to evaluate the effects of calcium concentration in medium on microtuberization of potato and found that supplemental calcium is important for tuber development. According to Mantsebo *et al.* (2014), increasing calcium concentration in the potato tubers helps to reduce the incidence of tuber soft rot during storage thereby increasing their shelf life. In this regard, soils low in Calcium can be amended by adding $CaSO_4$ (gypsum) to increase resistance not only to blackleg, but also to soft rot in progeny tubers.

Nitrogen is an important component of many structural, genetic and metabolic compounds in plants (Mahmood *et al.,* 2006). Therefore the level of nitrogen may also be another factor that affects susceptibility to soft rot pathogens. The effect of nitrogen levels on blackleg and soft rot in potato has shown that blackleg incidence caused by *Pectobacterium carotovorum* is lower in field plots treated with high than with low levels of Nitrogen fertilizers (Czajkowski *et al.,* 2011). However, this is contrary to studies by Ali *et al*. (2014) who suggest that disease severity increases with increasing levels of nitrogen but found out that potash plays a role in decreasing blackleg and soft rot severity and increasing yield. This may be by cross-linking, strengthening of the host cells and depressing the pathogen genes responsible for the production of bacterial enzymes that degrade host plant tissue.

2.4.4. Breeding for resistance

Breeding against *Pectobacterium carotovorum* has been used in *Zantedescia* and gave less susceptible to resistant hybrids (Snijder, 2004). Attempts to breed potato cultivars with increased levels of resistance have been partially successful. Wild *solanum speci*es (*Solanum brevidens*) crossed with the *S. tuberosum* showed partial resistance to bacterial soft rot (Austin *et al.,* 1988). However, such resistance does not last long probably because of the narrow range of genetic diversity in parental breeding material of potato.

Genetic engineering has also been tested in potatoes for control of soft rots where genes that confer soft rot resistance in potato have been identified (gene *ubiquitin7* or *ubi7*) in tubers (Garbarino *et al*., 1995). Attaching ubiquitin7 gene to an anti-rot gene was effective when the tubers are damaged and it gave 85% to 96% effective results according to Wood (1998). However, introduction of genes into crops from non-crossable species to improve their quality is not readily accepted by the wider society. Due to ignorance, people tend to think that the modified genes in the genetically modified crops (commonly referred to as GMO) might be transferred into their bodies and therefore may cause disease conditions. Only in March 2010 was the first genetically modified potato cultivar with increased starch content for industrial use allowed in Europe (Poudyal, 2012*).* As much as it may be ignorant perception, it requires a bit of time to convince the society otherwise.

As noted by Czajkowski *et al.* (2011), breeding has not succeeded in producing potato cultivars immune to *Pectobacterium carotovorum* and breeding is a long term process taking close to 10 years of screening and carrying out trials to ensure that the selected material does not carry over undesirable traits. Therefore breeding for resistance and genetic engineering may not give an immediate viable solution to the problem of potato blackleg and soft rot. As such, use of plant extracts in management of *Pectobacterium* infections in potatoes comes in handy.

2.4.5. Biological control methods

Antimicrobial properties of substances of natural origin derived from animals, plants and micro-organisms have been recognized. Antagonistic plant-associated bacteria, actinomycetes (*Streptomyces* spp.) isolated from rhizosphere soil have been reported to produce siderophores and inhibit the growth of phytopathogens (Gopalakrishnan *et al*., 2011)**.** Plant extracts are also important for the control of soil-borne plant pathogens and have the potential to control

Pectobacterium infections in potatoes (Babana *et al*., 2011*).* The use of bio-agents is therefore an alternative, as well as an eco-friendly and durable tool for protecting crops against pathogens.

2.4.5.1. Bio-control methods using pathogen antagonists

Antagonistic bacteria and yeasts have been used to control various plant pathogens. They are known to affect life cycles of different plant pathogens or pests by diverse mechanisms including the production of extracellular metabolites and intracellular proteinaceous toxins. Li *et al*. (2011) reported that the genus *Bacillus* and *Pseudomonas* have antagonistic activity against various plant pathogenic bacteria including soft rot bacterium *Erwinia carotovora* subsp. *carotovora* (*Pectobacterium carotovorum* subsp. *carotovororum*), *in vitro.* This is echoed by Rashid *et al*. (2013) who reported significant inhibition of *Erwinia carotovora* subsp. *carotovora* by *Bacillus subtilis, Pseudomonas fluorescens, Rhizobium leguminosarum* and *Trichoderma harzianum.* A study by Algeblawi and Adam (2013) on the effect of *Pseudomonas fluorescens, Bacillus subtilis* and *Bacillus thuringiensis* as a biocontrol agent on isolates of *Erwinia carotovora* subsp. *carotovora* also showed positive significant effects on the bacteria.

Antagonistic bacteria act by antibiosis, competition for space, nutrients, siderophore-mediated suppression, parasitism and induce systemic resistance in the host (Sharma *et al*., 2009). Yeasts are also known to colonize the wound surfaces for long periods even under dry conditions, producing extracellular polysaccharides. This enhances their survivability, restrict the growth of pathogen propagules, use nutrients rapidly and proliferate at a faster rate than the pathogen and they are the least affected by the pesticides (Ray and Swain, 2013). Lactic acid bacteria (*Lactobacillus plantarum*, *La. acidophilus*, *La. buchneri*, *Leuconostoc spp*. and *Weissella cibaria)* isolated from fresh fruits, vegetables and milk products showed *in vitro* antagonistic activity towards *Pectobacterium carotovora* in assays which was attributed to the production of hydrogen peroxide and acidification of the medium (Czajkowski *et al.*, 2011).

Communication between cells occurs via small, diffusible signal molecules and in Gram-negative bacteria it is mediated mainly by Acyl-Homoserine Lactones (AHLs) which regulate virulence gene expression and serve as the vital quorum-sensing signals that regulate the virulence of pathogenic bacteria like in *Pectobacterium species* (Li *et al.*, 2011). This is possibly to ensure that infection will start only if the bacterial density is large enough to overwhelm the plant's response (Hense *et al.,* 2007). Bacteria of the genus *Bacillus* and *Pseudomonas* possess an AHL lactonase gene which blocks the quorum sensing mechanism by enzymatic degradation

of signal molecules (Li *et al.,* 2011). When that gene was cloned in commercial potato cultivars, the transgenic plants showed a high level of resistance against *Pectobacterium carotovora*. Either symptom expression was entirely blocked or symptom development was significantly reduced (Czajkowski *et al.,* 2011). A study conducted on chilli endophytic bacteria showed *in vitro* antagonistic response to *S. rolfsii, C. capsici, F. oxysporum* and *Pythium sp.* (Amaresan *et al.,* 2014).

2.4.5.2. Bio-control using plant products

Many secondary plant products including flavonoids, steroidal alkaloids and saponins show antibacterial activity against plant pathogenic bacteria **(**Oguwike *et al.,* 2013). These natural plant products influence antimicrobial activity by inhibiting peptidoglycan synthesis, damage microbial membrane structures, modify bacterial membrane surface hydrophobicity and modulate quorum sensing (Shayan and Saeidi, 2013).

Many researchers have successively used plant products against plant and animal disease micro-organisms. Saeed *et al*. (2013) found antibacterial activity of *Juglans regia* bark and leaf extract against *Staphylococcus aureus* and *Agrobacterium tumefaciens.* Studies carried out on potato in Bangladesh showed that dried jute leaf (*Corchorus capsularis*) and cheerota plant (*Swertia chirata*) extracts had antibacterial activity against soft rot bacteria *in vitro* and effectively reduced the bacterial soft rot disease of different potato varieties under storage conditions (Rahman *et al.,* 2012). In a study by Ravikumar and Rajkumar (2013), it was found that *Crotalaria trichotoma, Citrus aurantifolia, Azadirachta indica, Polyalthia longifolia, Datura metel, Muntingia calabura* and *Oxalis latifolia* extracts significantly reduced the growth of mycelia in *Alternaria solani*, the causal agent of early blight of tomato. A review by Ali (2015) revealed the antibacterial activity of many plants among them *Allium spp., Arachis hypogaea, Artemisia campestris, Asparagus officinalis, Avena sativa, Brassica rapa, Canna indica, Capsicum annuum, Capsicum frutescens, Casuarina equisetifolia* and *Chenopodium alba.*

Spices and aromatic herbs contain compounds that have been shown to have bactericidal and antifungal properties that exert a large spectrum of effects including *thymol* from thyme and Oregon, *cinnamic aldehyde* from cinnamon and *eugenol* from clove. Extracts from the three chilli varieties (Habanero, Serrano and bell pepper) used by Ortego *et al.* (2003) showed growth inhibition against *Pectobacterium carotovorum* subsp.*carotovora*. The inhibition was attributed

to the compounds in chilli that include meta-coumaric acid, ortho-coumaric acid and trans-cinnamic acid. Opara *et al*. (2013) used plant extracts consisting of *Azadirachta indica* seed, *Garcinia kola, Zingiber officinale, Piper guineense* seed *and Myristica fragrans* seed against bacterial soft rot *(Erwinia carotovora) and* fruit spot *(Xanthomonas vesicatoria)* and the extracts were effective in inhibiting the growth of bacteria *in vitro.*

Mexican marigold (*Tagetes minuta*) has been reported to contain 5-(3-buten-1-ynyl) 2, 2-bithienyl and alpha terthienyl (Krishnamurthy *et al.,* 2012**)** which may have antimicrobial activity. Leaves and flowers of *Tagetes minuta* also contain essential oils that contain β-ocimene, dihydrotagetone, terpinolene, piperitone, β-caryophyllene, tagetenone, cis-tagetenone and trans-tagetenone which have been shown to exhibit antibacterial and antifungal activity (Rondon *et al.,* 2006; Chamorro *et al.*, 2008).

Tagetes species has been used widely to treat human ailments. Priyanka *et al*. (2013) reported the use of *Tagetes* in kidney troubles, ulcers, wounds and liver complications. In folk medicines, extracts from *T. erecta, T. tenuifolia T. minuta* and *T. Patula* have been used to treat various stomach and intestinal disorders and some of them have biological activity as well (Lubna and Naeem, 2012). The oil from *Tagetes patula* has been reported to show antibacterial activity against various human pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Snehali *et al.,* 2014).

In their study, Ramya *et al*. (2012) found compounds like alkaloids, flavonoids, steroids, tannins and phenolic compounds which are purported to have bioactive properties like bactericidal action. In the same study, *Tagetes erecta* proved to be bactericidal against *Staphylococcus sp*., *Escherichia coli* and *Pseudomonas aeruginosa.* Liu *et al*. (2015) used α-triple thiophene extracts from Marigold (*Tagetes spp*.) to test for antimicrobial activity in common food spoilage microbes (*Colibacillus spp., Penicillium spp., Salmonella spp., Staphylococcus aureus and Bacillus subtilis*) and found it to be active against *Escherichia coli, Salmonella spp.* and strains of *Penicillium spp.* Das and Mishra (2012) found *Tagetes erecta* to have antibacterial activity against *S. mutans, S. sorbrinus, S. sanguis and E. coli* that cause sore throat, scarlet fever, rheumatic fever and toxic shock syndrome in humans. A review by Gupta and Vasudeva (2012) revealed that the essential oil of the flowers of *Tagetes minuta* had

antimicrobial activity against *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Aspergillus niger* and *Trichoderma viride.*

In pest control studies have shown that *Tagetes minuta* leaf extracts reduced root-knot nematode infestation and increased the number of fruits and total tomato yield (Taye *et al.*, 2012). The leaves of *T. minuta* have also been used in Pakistan to repel safari ants, blowflies, mosquitoes and to kill mosquito larvae. The volatile *Tagetes* oil has also been shown to be highly suppressive against plants and animals (Sehrish *et al.*, 2013). According to Sylvia *et al.* (2014), *Tagetes minuta* has been shown to have insecticidal and repellent properties against red spider mite (*Tetranychus urticae*) and black bean aphids *(Aphis fabae*). In view of these, the current study sought the possibility of including *T. minuta* in the management of *Pectobacreium carotovorum* disease causing pathogen in potatoes.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Research site description

The experiment was conducted at the horticulture research and teaching laboratory and field station of Egerton University, Njoro, Kenya. The potatoes were grown in the open field in two seasons in 2015. The field lies at a latitude of 0°23′ S and longitude 35°35′ E in the lower highland III (LH3) agro-ecological zone at an altitude of ~2238 m above sea level. The soils in the field are well-drained sandy loam-vintric, mollic androsols. The field receives between 902 mm to 1012 mm of rainfall per annum and temperature ranges of 15.6 °C to 23 °C (Jaetzold *et al*., 2005). *In vitro* and some *in vivo* experiments were conducted in the laboratory where the temperatures ranged between 17 °C and 22 °C and the relative humidity ranged from 75% to 85%.

3.2. Plant material for extracts

Whole above ground plant material of *Tagetes minuta* growing as weeds was collected from the farm fields at Egerton University, Nakuru, Kenya. Red ripe *Capsicum frutescens* fruits (Tabasco variety) were purchased from Nakuru farmers' market. The materials were positively identified and confirmed at the Department of Biological Sciences, Egerton University.

3.3. Preparation of the plant extracts

Leaves and stems of *Tagetes minuta*; and *Capsicum frutescens* fruits were used to produce the two crude extracts that were evaluated. The material was air dried under shade for three weeks then ground using an electric grinder (SB-808 by SAYONA PPS). Two hundred grams of the ground material from the two (*Tagetes minuta* and *Capsicum frutescens*) were separately homogenized in distilled water in the ratio of 1:10 (W: V) and steeped for 24 hours at 30 °C on a rotary shaker (THZ-C-1 Hangzhou, China). The materials were filtered through a muslin cloth and centrifuged (KUBOTA 6800, Japan) at 5000 rpm for 15 minutes. The supernatant was collected and concentrated in a water bath at 70 °C to make the final volume one-fifth of the original volume which served as 100% concentration of each crude extract. The two extracts were then stored at 4 °C until evaluation (Parekh *et al.,* 2005). The two extracts were each diluted to 40%, 30% and 20% (V:V) concentration for the *in vitro* experiments and subsequent use in the *in vivo* experiments.

3.4. Preparation of the agar plates and bacterial nutrient broth

Nutrient agar and nutrient broth were each prepared then sterilized for 15 minutes at 121°C. After cooling to 45-50 °C, nutrient agar was poured aseptically into sterilized petri plates of diameter 9 cm (10 ml). The media was allowed to solidify in petri plates for about an hour in a laminar flow hood and then placed in an inverted position (to avoid evaporation of water from the medium within the plates). After 24 hours, uncontaminated plates were used to culture the bacteria. The nutrient broth in glass bottles was later used for shaking incubation of the bacteria to obtain the bacteria in liquid suspension form.

3.5. Isolation of the pathogen

Pectobacterium carotovorum colonies were obtained from naturally infected potato tubers. After surface sterilization with 70% ethanol solution and washing three times in sterile water, the potato sections were ground in sterile water to obtain 5 ml of potato paste containing the bacteria. A sterile loop was used to pick and streak the bacteria onto nutrient agar plates which were incubated at 22 °C for three days according to Schaad (1988) to produce single, round, convex, creamy-translucent, raised and shiny colonies on nutrient agar as (Plate 1). These were sub-cultured 3 times to obtain pure cultures which were maintained well covered for subsequent uses (Perombelon and Wolf, 1998).

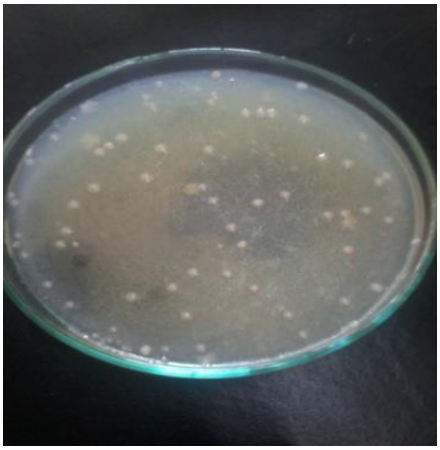


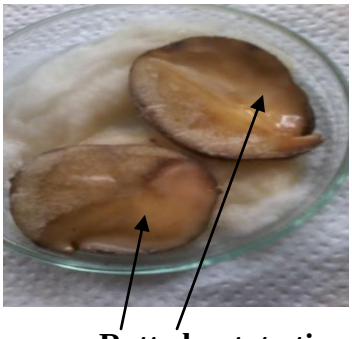
Plate 1: Bacterial colonies on nutrient agar

The top of single and well-isolated colonies were picked with a sterile loop and inoculated into the 250 ml of nutrient broth. The broth culture was then incubated for 12 hours to obtain young cultures. The turbidity of actively growing broth cultures was then adjusted to a 0.5

McFarland standard (McFarland, 1907), comparable to bacterial suspension of 10^8 CFU ml^{-1}. The bacterial suspension was later used to inoculate the sterilized agar plates.

3.6. Pathogenicity test on potato tubers

Potato tubers were washed thoroughly in running water; air dried then dipped in 70% ethanol for 3 minutes, washed in sterile water then air dried. Eight 5 mm thick slices of potato were cut and placed on wet sterile serviette in petri dishes. Using a micro pipette, 50 µl of 10^8 CFU ml^{-1} bacterial suspensions was placed on the surface in the centre of four slices for inoculation with four control slices treated with sterile water. The slices were incubated at 23 ºC and examination for the presence of rots was done after 3 days of incubation. By day 3, the slices inoculated with the bacterial suspension had soft rotted tissue (Plate 2a) while those treated with sterile water had no rotted tissue (Plate 2b). Re-isolation of bacteria from rotted tissue (Plate 2a) was done and inoculated on NA medium (Mikicinski *et al*., 2010) to ascertain that it was the bacteria in question.



a. Rotted potato tissues

b. Unrotted control potato slice

Plate 2: (a) Rotted potato tissue 3 days after inoculation (b) Control potato slice 3 days after inoculation

3.7. *In vitro* study: Experimental design and application of treatments

The experiments were set up in a completely randomized design (CRD) with each extract at three levels. Streptomycin sulphate and sterile water were used as the positive and negative controls respectively.

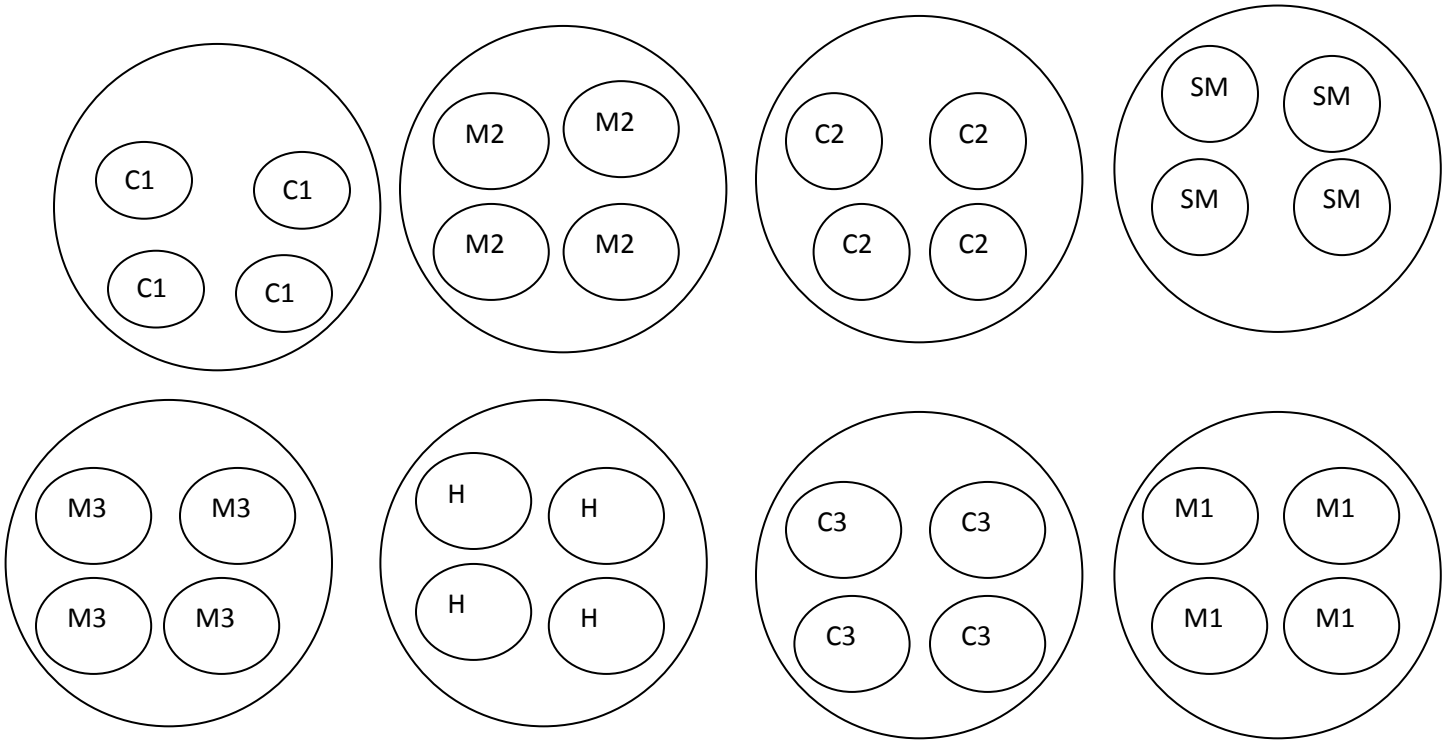


Figure 1: Experimental layout for the *in vitro* bacterial growth inhibition experiment

KEY: M1: 40% *T. minuta*, M2: 30% *T. minuta*, M3: 20% *T. minuta*, H: Sterile water, SM: 100 ppm Streptomycin sulphate, C1: 40% *C. frutescens*, C2: 30% *C. frutescens*, C3: 20% *C. frutescens*.

3.7.1. *In vitro* bacterial growth inhibition

A sterile cotton wool swab was used to inoculate the pathogen from the nutrient broth culture onto the surface of sterile nutrient agar plates. The cotton wool with the pathogen was swabbed over the entire surface of the nutrient agar medium in the petri plates and then allowed to dry at 22 °C in a laminar flow hood.

Eight treatments were used in this experiment; three concentrations (40%, 30%, and 20%) of *Tagetes minuta* extract and three concentrations (40%, 30% and 20%) of *Capsicum frutescens* extract, 100 ppm streptomycin sulphate (positive control) and sterile water (negative control). The extract concentrations were obtained by mixing the 100% concentration crude extracts with distilled water at the ratios of; 1:2.5, 3:7 and 1:5 (extract: water). *T. minuta* and *C. frutescens* extracts at 40%, 30% and 20% concentrations were applied by placing four drops of each at the centre of the inoculated agar plates using a sterile micropipette. Four drops of

Streptomycin sulphate and water were also applied as positive and negative control respectively. Each treatment was applied in a separate agar plate with the four drops serving as replications (Figure 1). The plates were kept at 22 °C in the laminar flow hood for 1 hour to allow for diffusion of the test material into the medium. After 12 hours, the agar plates were examined for bacterial growth inhibition and the inhibition zone diameter was measured in millimeters (mm) using a pair of calipers and recorded as a measure of the antibacterial activity.

3.7.2. Effect of *T. minuta* and *C. frutescens* extracts on the number of days to total tissue maceration of potato chips

This experiment was set up to test the number of days it took for complete tissue maceration of potato chips treated with the different concentrations of the plant extracts. Potato chips weighing 5 g were immersed in 10 ml of distilled water in test tubes with two drops of the bacteria from the nutrient broth suspension equivalent to 100 µl of 10^8 CFU ml^{-1}. The treatments were then applied by placing three drops, two drops and one drop of 100% *Tagetes minuta* and 100% *Capsicum frutescens* extracts respectively into the test tubes containing the potato chips in the 10 ml bacterial suspension. Three drops of 100 ppm streptomycin sulphate and distilled water were also used as positive and negative controls respectively. These were replicated three times and incubated at 22 ºC then checked for tissue maceration and recorded in number of days taken for tissues to be completely macerated. Tissues were considered to be completely macerated when there were no visible potato chips in the test tube but only a whitish, cloudy, slimy material was observed floating on the test solution.

3.7.3. Effect of *T. minuta* concentration on percent weight loss of potato chips due to tissue maceration

Potato chips weighing 5 g were immersed in 10 ml of each of the test material (40%, 30% and 20% *T. minuta*, distilled water and 100 ppm streptomycin sulphate) in test tubes with 100 µl of 10^8 CFU ml^{-1} of the *Pectobacterium* bacteria. The experiment was set up in a completely randomized design and each of the treatments was replicated three times. The potato chips were incubated at 22 °C then checked for weight loss due to tissue maceration by washing off the macerated tissues and recorded as percent weight loss from the initial 5 g at two days' interval. This was done until all tissues were completely macerated when no more solid potato chips were left for weighing.

3.8. *In vivo* study

3.8.1. Inoculation of healthy tubers

Certified uniform sized seed potato tubers of *Cangi* variety was obtained twice from the Agricultural Development Corporation (ADC) at Molo for the two seasons. The potatoes were surfaced-sterilized for 10 minutes with 5% Sodium hypochlorite, rinsed thoroughly in sterile water and allowed to air dry. A bacterial suspension of 10^8 CFU ml^{-1} was sprayed on the tubers and then incubated for 12 hours at room temperature. The *Cangi* variety of potato was chosen because it is the most commonly grown by the local growers probably due to its short growth period of about 2½ months to 3 months.

3.8.2. Application of treatments in the *in vivo* experiments

The eight treatments in these experiments consisted of the two plant extracts each at 40%, 30% and 20% concentration with water as the negative control and copper oxychloride as the positive control. For the field experiment, inoculated tubers were sprayed with the respective treatments, then left to dry for 24 hours in single layers in vented plastic crates to enhance sticking of the compounds before they were planted at a depth of 10 cm and labeled. For the stored and chitting (sprouting) experiment, the potatoes were sprayed with the respective treatments in a completely randomized design replicated three times before storage.

3.8.3. Experimental design for the *in vivo* experiments

The field experiment was laid out in a Randomized Complete Block Design (RCBD). The whole experimental field measured 20.5 m by 13 m such that each block measured 20.5 m by 3 m. Each plot measured 3 m by 2 m to make eight plots per block. A path of 1 m was maintained between the blocks and 50 cm between the plots within the blocks. The potatoes were spaced at 75 cm by 30 cm in furrows of about 10 cm deep to give four rows each with six plants giving a plant population of 24 plants per plot, 192 plants per block and 576 plants in the whole experimental field. The storage and sprouting (chitting) experiments were set up in a completely randomized design replicated three times.

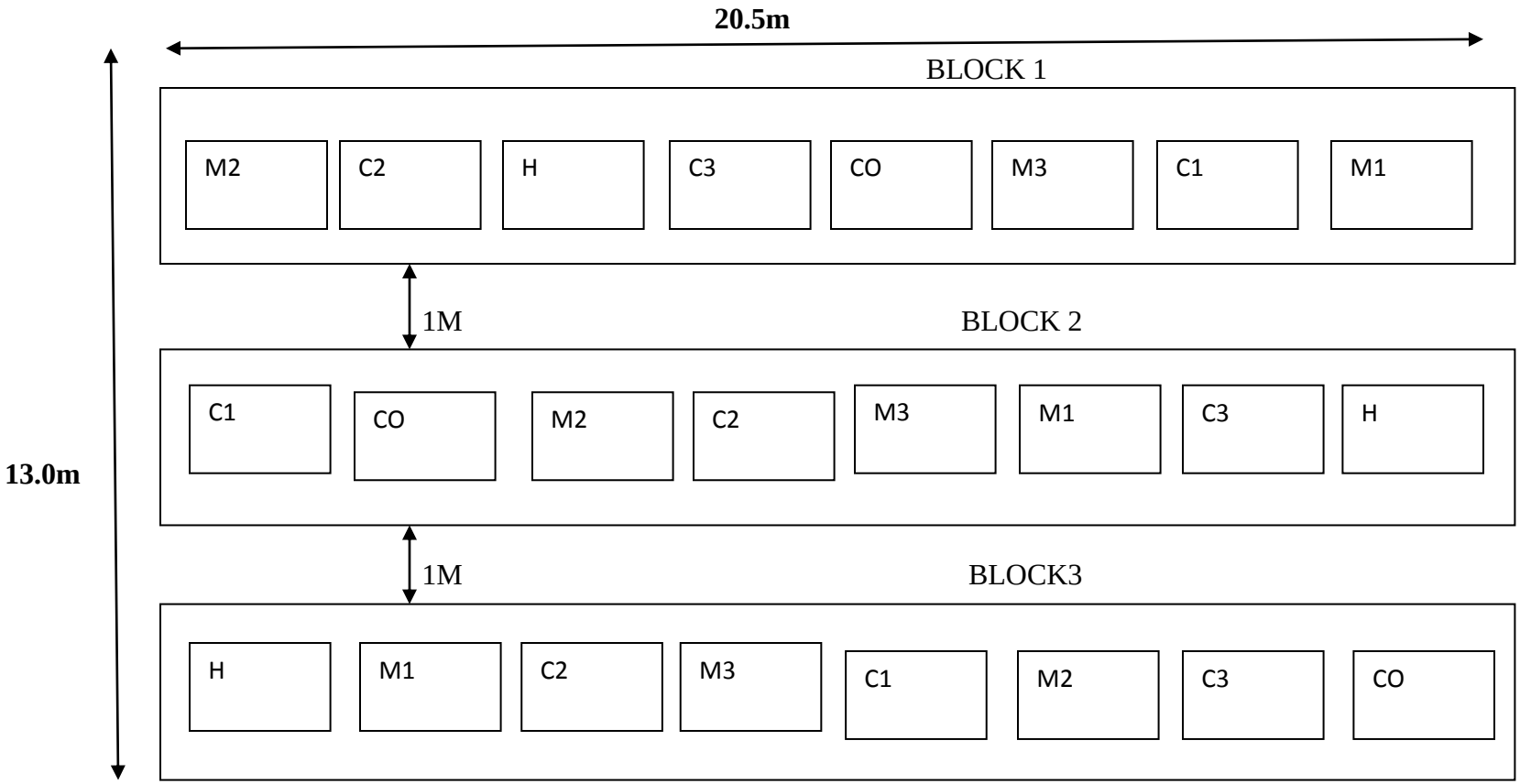


Figure 2: Experimental layout in the field

TREATMENTS: H: Distilled Water (-ve control), **C1**:40 % *C. frutescens*, **C2**:30% *C. frutescens*, **C3**:20% *C. frutescens*, **M1**: 40% *T. minuta*, **M2**:30% *T. minuta*, **M3**:20%*T. minuta*, **CO**: Copper oxychloride (+ve control).

3.8.4. Field management practices

The potato tubers were planted with 200 kg/ha NPK fertilizer at a depth of about 10 cm with the dominant sprouts facing upwards for faster and uniform emergence. After emergence, earthing up was done during the first weeding. Irrigation was done twice a day; in the morning and evening to maintain the moisture content in the soil during the dry season to avoid water stress that may hinder tuberization. The potatoes were sprayed against late blight (*Phytophthora infestans)* when the disease symptoms appeared (60 days after planting) using Ridomil at 3 kg/ha and repeated after one week. Dynamec was sprayed against the aphids and leaf hoppers at 100 g/L. Moles were controlled by trapping and digging a trench around the experimental field to keep them off the experimental field.

3.9. Data collection

3.9.1. *In vitro* data collection

In the *in vitro* study, antibacterial activity of *Tagetes minuta* and *Capsicum frutescens* extracts were evaluated against *Pectobacterium* bacteria. The antibacterial activity of these extracts was determined by a modified disc diffusion method (Alzoreky and Nakahara, 2003) and on potato chips. The results of the *in vitro* tests were expressed in mm, number of days to total tissue maceration and percent weight loss due to tissue maceration.

3.9.1.1. Effect of *T. minuta* and *C. frutescens* extracts on *in vitro* bacterial growth inhibition

The diameter on the zone of inhibition produced by each of the treatments was measured in millimeters using a pair of calipers and recorded as a measure of the antibacterial activity of each plant extract after 12 hours of incubation at 22 ºC. The diameter of bacterial growth inhibition was reported in comparison with the inhibition to bacterial growth caused by streptomycin sulphate and sterile water.

3.9.1.2. Effect of *T. minuta* and *C. frutescens* extracts on the number of days to total tissue maceration

The number of days it took for the potato tissues to be totally macerated was recorded for each of the treatments by observing through the glass (test tube) until no potato chips were visible. That is when only a whitish, cloudy, slimy material floating on the surface of the test chemical was observed.

3.9.1.3. Effect of *T. minuta* concentration on percent weight loss of potato chips due to tissue maceration

The effect of the concentration of *T. minuta* on percent weight loss on the potato chips was tested against that of streptomycin and water at two days' interval for 11 days. Five measurements were taken from day 2 to day 11. Weight loss of the potato chips due to tissue maceration was measured by washing off the rotted tissues then subtracting from the original weight of 5 g and recorded as percent weight loss. The tissues were considered completely macerated when there were no more solid potato chips to be weighed.

3.9.2. *In vivo* data collection

Data was collected on the effect of the plant extracts on growth and development of potatoes and the effect of the extracts on disease components in both season 1 and 2. Plant data collected was on percentage sprout emergence, number of stems, plant height, number of leaves, number of stems and flowering. Data collected on disease components was on disease incidence and severity on potato plants and tubers. Data was also collected on yield and quality of potato tubers.

3.9.2.1. Effect of *T. minuta* and *C. frutescens* extracts on sprouting of potatoes

The percent sprouting was taken for every treatment by chitting the potatoes and sprouts counted at 18 days, 25 days, 32 days and 39 days after chitting. The number of sprouts was determined by counting the sprouts per tuber in the six sampled tubers per treatment. Sprouting capacity was expressed as the number of developed sprouts as a percentage of total sprouts per tuber and later percent sprouting determined by dividing the number of sprouts by the number of tubers considered.

3.9.2.2. Effect of *T. minuta* and *C. frutescens* extracts on the sprout emergence percentage

The number of sprouts that emerged per plot was counted twice; first at 28 days after planting and at 35 days after planting (DAP) and the sprout emergence percentage calculated by dividing the number of emerging sprouts per plot by the number of tubers planted multiplied by 100.

3.9.2.3. Effect of *T. minuta* and *C. frutescens* extracts on plant height of potatoes

Potato height was measured using a tape measure on four sampled plants picked in every plot. The height was measured from the soil level to the tip of the tallest leaf weekly at 28 days, 35 days, 42 days and 48 days after planting.

3.9.2.4. Effect of *T. minuta* and *C. frutescens* extracts on the number of leaves of potato plants

The number of leaves from the sampled plants was also recorded weekly from 28 days, 35 days, 42 days and 48 days after planting.

3.9.2.5. Effect of *T. minuta* and *C. frutescens* extracts on the number of stems of potatoes

The number of stems per plant was determined on the four sampled plants in each treatment at 28 days and 35 days after planting by counting the number of main stems within an individual plant. Only stems arising from the mother tuber were considered as main stems.

3.9.2.6. Effect of *T. minuta* and *C. frutescens* extracts on the number of flowers of potatoes

The number of flowers was taken by counting the number of flowers at two days' interval from 48 days after planting for eight days. This was stopped when there was 50% flowering and recorded as percent flowering. A treatment plot was considered to have attained 50% flowering when at least half (12 plants) of the plants within the plot had flowered.

3.9.2.7. Effect of *T. minuta* and *C. frutescens* extracts on blackleg disease incidence

Plants were considered diseased if they exhibited mushy, watery breakdown of the tissue and the foul pungent odour characteristic of *Pectobacterium* soft rot. Stem wilting, yellowing and toppling with black discoloration were also an indicator of a diseased plant. Data on the number of infected plants was recorded at 5 days' interval by taking simple counts of infected plants in every plot.

3.9.2.8. Effect of *T. minuta* and *C. frutescens* extracts on blackleg disease severity on stems

The diseased plants per plot were assessed for disease severity by blackleg. Severity on the stems was calculated by dividing the number of infected stems by the total number of stems of the infected plants, multiplied by 100.

Disease severity = $\frac{n}{N} \times 100$, where; n is the number of stems with blackleg symptoms and N is the total number of stems of infected plants per plot examined.

3.9.2.9. Effect of *T. minuta* and *C. frutescens* extracts on soft rot incidence on tubers

Tubers were considered diseased if they contained watery, slimy rot with a whitish, cloudy, slimy liquid oozing from breaks in the tissues with a rotted odor. Tuber disease incidence was calculated by dividing the number of infected tubers by the total number of inspected tubers multiplied by 100;

Tuber disease incidence = $\frac{n}{N} \times 100$, where; n is the number of infected tubers and N is the number of tubers inspected.

3.9.2.10. Effect of *T. minuta* and *C. frutescens* extracts on soft rot severity on tubers

Disease severity on tubers was estimated as a percentage of the rotten tissue weight according to the change in weight of tuber before and after washing off the diseased portion. The weight before washing was obtained by weighing the diseased potatoes. The weight after washing was obtained by washing off the diseased portion then the remaining potato was weighed again (Plate 3a and 3b) according to Kelman and Dickey, (1980):

Disease severity = $\frac{Weight\ of\ tuber\ after\ washing\ off\ diseased\ portion}{Total\ weight\ of\ tuber\ before\ washing\ off\ diseased\ portion} \times 100$.

a.  b. 

Plate 3: Infected tubers (a) before washing and (b) after washing off infected portion.

3.9.2.11. Effect of *T. minuta* and *C. frutescens* extracts on the number of potato tubers

Potatoes were harvested from the four sampled plants per plot and placed separately on the ground for determination of yield per plot and calculated for the whole plot. Tubers produced

from the four plants were counted and recorded to determine treatment effects on the number of tubers per plot.

3.9.2.12. Effect of *T. minuta* and *C. frutescens* extracts on total and marketable yield

After counting the number of tubers per plot, all the tubers from the 4 middle plants per treatment were combined together and placed in one khaki paper bag and weighed using a spring balance to determine the total yield and marketable yield per plot. This was later converted to yield in t ha^{-1}.

3.9.2.13 Effect of *T. minuta* and *C. frutescens* extracts on the potato tuber sizes

Ten tubers were picked at random per plot after harvesting and their tuber sizes determined using a ruler and a pair of calipers. The average tuber size was calculated and the measurements recorded in millimeters.

3.9.2.14. Effect of *T. minuta* and *C. frutescens* extracts on dry matter content

Dry matter content was determined by taking 100 g of tubers from each plot from the sampled tubers, oven dried at 70 ºC to constant weights then weighed using an electronic weighing balance. The readings were recorded in grams per tuber.

3.9.2.15. Effect of *T. minuta* and *C. frutescens* extracts on the total soluble solids (TSS)

The TSS was determined using a hand-held refractometer (0-30% Brix) from the ten tubers from every plot. The tubers were grated and a drop of the juice squeezed and placed on the refractometer and the light refracted through a prism measured the total dissolved soluble solids (which is mainly sugar but also does include minerals) in the plant sap. The refractometer measurement was from zero to 30% Brix (Model 1974). The refractometer was cleaned and standardized with distilled water between readings to read 0% soluble solids content.

3.9.2.16. Effect of *T. minuta* and *C. frutescens* extracts on the percent postharvest soft rot infections

After harvesting 6 potatoes were picked randomly from every plot to be used to test the effect of the extracts on postharvest soft rot. The potatoes were inoculated with the bacteria, treated with the plant extracts, copper oxychloride and water, and then stored in polythene sleeves. The treatments were applied without sterilizing the potatoes just like a grower would do

in the farm. Observation for infected potatoes was made at two days' interval from 7days after storage and infected potatoes recorded as percent postharvest soft rots.

3.10. Data analysis

Laboratory and field data were subjected to Analysis of Variance using Genstat edition 4 and where the F-test was significant ($p \leq 0.05$), the means were separated using the Tukey's HSD test.

Laboratory, sprouting and postharvest data were fitted to the following linear model;

$Y_{ij} = \mu + \Gamma_i + \varepsilon ij$ where*;*

Y_{ij} – *observations made*

μ – *Overall mean*

Γ_i – *Effect due to the* i^{th} *treatment (extracts)*

εij - *Random Error*

Field data were fitted to the following model:

$Y_{ij} = \mu + \Gamma_i + \beta_j + \varepsilon ij$ where;

Y_{ij} - is the potato response due to the i^{th} treatment in the j^{th} replication

μ- is the overall mean

Γ_i – is the treatment effect (extracts)

β_j – is the block effect (replication)

εij – is the random error component.

CHAPTER FOUR
RESULTS

4.1. *In vitro* study

4.1.1. Effect of *T. minuta* and *C. frutescens* extracts on *in vitro* bacterial growth inhibition

Tagetes minuta extracts and streptomycin sulphate showed a significant difference in antibacterial activity against *Pectobacterium* bacteria compared to *Capsicum frutescens* extracts and water at $p \leq 0.05$ (Table 1). *T. minuta* (Plate 4b) showed zones of inhibition of 7.167 mm, 6.667 mm and 6.1 mm for the three concentrations (40%, 30% and 20%) respectively that was close to that of streptomycin sulphate (Plate 4a) that had an inhibition zone of 8.83 mm. On the other hand, *Capsicum frutescens* extracts (Plate 4c) and water (Plate 4d) showed 0.00 mm inhibition on the growth of the *Pectobacterium* bacterial colonies.

Table 1: Effect of *T. minuata* and *C. frutescens* on minimum inhibition zone on bacterial growth (mm)

Treatment	Minimum inhibition zone(mm)
Streptomycin sulphate	8.833a
40% *T. minuta*	7.167b
30% *T. minuta*	6.667b
20% *T. minuta*	6.100c
40%*C. frutescens*	1.000d
30%*C. frutescens*	1.000d
20%*C. frutescens*	1.000d
Distilled water	1.000d
p-value	<0.001

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

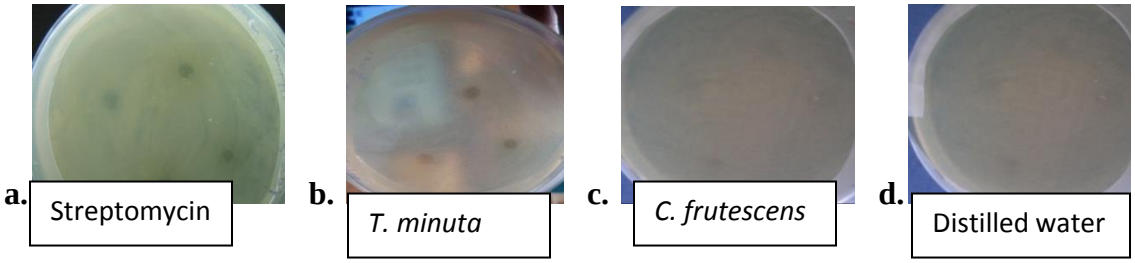


Plate 4: Antibacterial activity of the treatments by modified disc diffusion assay against *Pectobacterium carotovorum*.

4.1.2. Effect of *T. minuta* and *C. frutescens* extracts on the number of days to total tissue maceration of potato chips

The number of days to total tissue maceration differed significantly in the chips treated with *T.minuta* extracts from those treated with *C. frutescens* extracts at $p \leq 0.05$ (Table 2 and Plate 5). Potato chips treated with *T. minuta* extracts took the longest time (9 days) after streptomycin sulphate (10 days) for the tissues to be completely macerated. On the other hand, the potato chips treated with *C. frutescens* fruit extracts were completely macerated by day 5 as was the case with chips treated with water. By the day 8, there was only a whitish, cloudy, slimy material floating on the surface of the test chemical in the test tubes with *C. frutescens* extracts and water (Plate 5c and 5d) but the test tubes with *T. minuta* extracts and Streptomycin sulphate still had some potato chips visibly seen in the test tubes (Plate 5a and 5b).

Table 2: Effect of *T. minuta and C. frutescens* on number of days to total potato tissue maceration

Treatment	Days to total tissue maceration
Streptomycin sulphate	10.00a
40% T. minuta	9.00a
30% T. minuta	9.00a
20% T. minuta	7.00b
40% *C. frutescens*	5.33c
30% *C. frutescens*	5.00d
20% *C. frutescens*	4.67d
Distilled water	5.00d
p-value	<0.001

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

a. 

b. 

c. 

d. 

Plate 5: Extent of tissue maceration by day 9 after application of treatments

4.1.3. Effect of *T. minuta* on percent weight loss due to tissue maceration

There was a significant difference in percent weight loss due to tissue maceration at P≤0.05 (Table 3). Potato chips that were treated with water had the highest percent weight loss (100%) while those treated with 40% *T.minuta* had the lowest percent weight loss (50.60%) by day 11. Increasing the concentration of *T.minuta* led to reduced percent weight loss due to tissue maceration; the potato chips treated with 40% *T. minuta* had the lowest percent weight loss (50.60%) followed by those treated with 30% *T. minuta* (78.80%) and those treated with 20% *T. minuta* (83.87%).

Table 3: Effect of *T. minuta* concentration on percent weight loss after 2 to 11 DAI (Days after inoculation)

Treatment	2DAI	5DAI	7DAI	9DAI	11DAI
40% *T. minuta*	10.27ab	11.80a	16.60a	27.07a	50.60a
30% *T. minuta*	6.67ab	13.60ab	32.87ab	50.13bc	78.80b
20% *T. minuta*	7.93ab	22.80ab	40.47ab	62.80bc	83.87bc
100 ppm Streptomycin sulphate	3.00a	36.87bc	58.93bc	80.40d	93.97bc
Distilled water	20.80b	53.73c	74.27d	92.40d	100.00d
p- value	0.050	0.008	<0.001	<0.001	<0.001

Means followed by the same letter are not significantly different at *p*≤0.05 according to Tukey's HSD test

4.2. *In vivo* Study

4.2.1. Effect of *T. minuta* and *C. frutescens* extracts on sprouting of potatoes

The plant extracts had no significant effect on the potatoes on sprouting in the chitting experiment. Potatoes treated with *Tagetes minuta* and *Capsicum frutescens* extracts had almost the same number of sprouts after chitting for 30 days.

4.2.2. Effect of *T. minuta* and *C. frutescens* extracts on sprout emergence percentage

The plant extracts had no significant effects on the sprouting (emergence percentage) of potatoes in both season 1 and season 2. Due to drought experienced in season 2, there was an overall lower sprouting percentage than there was in season 1 by day 28 and day 35 after

planting. Season 1 potatoes started growing under high amounts of rainfall while season 2 potatoes started growing under sprinkler irrigation (using a hose pipe) and continued for almost one and half months under irrigation.

4.2.3. Effect of *T. minuta* and *C. frutescens* extracts on potato plant height

There was no significant difference in plant height among the treatments during both growth seasons although generally, the plants in season 2 were taller than those in season 1. This may be attributed to the high rainfall in season 1 for the first one month which was followed by drought whereas season 2 was started on irrigation then followed by high rainfall.

4.2.4. Effect of *T. minuta* and *C. frutescens* extracts on the number of leaves of potatoes

The plant extracts had no significant effects on the number of leaves per plant. The number of leaves for all the treatments was statistically the same in both season 1 and 2.

4.2.5. Effect of *T. minuta* and *C. frutescens* extracts on the number of stems of potatoes

The plant extracts had no significant effect in the number of stems per plant in both season 1 and season 2. The number of stems was statistically the same in all the treatments.

4.2.6. Effect of *T. minuta* and *C. frutescens* extracts on the number of flowers of potatoes

The plant extracts had no significant effect on the number of flowers and the number of days to 50% flowering.

4.2.7. Effect of *T. minuta* and *C. frutescens* extracts on disease components on potatoes

4.2.7.1. Effect of *T. minuta* and *C. frutescens* extacts on blackleg incidence of potatoes

Plots treated with *Tagetes minuta* extracts and copper oxychloride showed a significant difference in disease incidence compared to those treated with *Capsicum frutescens* and water in both season 1 and 2 at $p \leq 0.05$ (Table 4). The plots treated with 40% and 30% *Tagetes minuta*; and copper oxychloride had low disease incidents (2 plants per plot) while those treated with water and *Capsicum frutescens* had high disease incidents (4 plants per plot). The plants that had blackleg infections exhibited mushy watery breakdown of the tissue with a foul pungent odour characteristic of *Pectobacterium* soft rot. The stems also exhibited wilting, yellowing with black discoloration and finally the plant toppled (Plate 6b).

a.  b. 

Plate 6: Potato plants (a) in the field and (b) a potato plant showing symptoms of blackleg infection

Table 4: Effect of *T. minuta* and *C. frutescens* extracts on blackleg incidence (number of plants infected) at 35, 40 and 44 days after planting (DAP)

Treatment	Season 1			Season 2		
	35DAP	40DAP	44DAP	35DAP	40DAP	44DAP
Copper oxychloride	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
40% *T. minuta*	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
30% *T. minuta*	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
20% *T. minuta*	1.33ab	1.67ab	1.67ab	1.33ab	1.67a	2.00b
40%*C. frutescens*	3.00d	3.00bc	3.67c	2.00c	3.00c	3.00c
30%*C. frutescens*	2.33bc	2.67c	3.00c	2.00c	2.67b	2.67c
20%*C. frutescens*	1.67ab	2.33c	2.67bc	2.33d	3.00c	3.33c
Distilled water	2.67c	2.67c	2.67bc	1.67bc	2.33b	2.00b
p-value	<0.001	<0.001	<0.001	<0.001	0.004	0.008

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

4.2.7.2. Effect of *T. minuta* and *C. frutescens* extracts on blackleg severity of potatoes

The extracts showed a significant difference in disease severity on the stems at $p \leq 0.05$ with less severity in plots treated with *T. minuta* (54.33%) compared to those treated with *C.*

frutescens and water (92.67%) both in season 1 and 2 (Table 5). Potatoes treated with 40% and 30% *T. minuta* had very few infections. Those treated with 20% *T. minuta* showed no significance difference from those treated with 20% and 30% *C. frutescens*. However, disease severity was generally higher in season 2 than in season 1 which could be attributed to the high rainfall experienced towards the end of season 2 which may have enhanced spread of the bacteria in the soil.

Table 5: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems (% infection)

Treatment	Season 1			Season 2		
	35DAP	40DAP	44DAP	35DAP	40DAP	44DAP
Copper oxychloride	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
40% *T. minuta*	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
30% *T. minuta*	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
20% *T. minuta*	9.33a	26.00b	42.67ab	17.67b	40.00b	54.33b
40%*C. frutescens*	56.67b	82.67c	69.33bc	34.33b	61.00bc	92.67d
30%*C. frutescens*	48.00b	56.33b	69.33bc	84.33d	77.67c	84.33bc
20%*C. frutescens*	47.00b	59.33b	72.67bc	51.00c	79.00c	78.67bc
Distilled water	71.00b	100c	100c	42.67c	37.00b	37.00ab
p-value	<0.001	<0.001	0.048	0.005	<0.001	<0.001

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

4.2.7.3. Effect of *T. minuta* and *C. frutescens* extracts on soft rot disease incidence and severity on tubers

Disease incidence on tubers harvested from the plots treated with copper oxychloride and *Tagetes minuta* was signicantly different from those treated with *Capsicum frutescens* and water at $p \leq 0.05$ (Table 6). There were more diseased tubers (4.67 tubers) among the tubers treated with *Capsicum frutescens* and water as compared to those treated with 40% and 30% *Tagetes minuta* (1.33 tubers). However, there was no significant difference on disease incidence among

the tubers treated with 20% *Tagetes minuta*, water and 40%, 30% and 20% *Capsicum frutescens* in both season 1 and 2. Tubers treated with *Tagetes minuta* (40% and 30%) and copper oxychloride also had a significant difference in the disease severity compared with those treated with *Capsicum frutescens* and water.

Table 6: Effect of *T. minuta* and *C. frutescens* extracts on soft rot disease incidence and disease severity on tubers

Treatment	Season 1		Season 2	
	Incidence (number of plants)	Severity (%)	Incidence (number of plants)	Severity (%)
Copper oxychloride	1.00a	1.00a	1.33a	3.23a
40% *T. minuta*	1.00a	1.00a	1.00a	1.00a
30% *T. minuta*	1.00a	1.00a	1.00a	1.00a
20% *T. minuta*	1.00a	1.00a	1.33a	3.99a
40%*C. frutescens*	3.33b	37.00b	3.33b	19.85c
30%*C. frutescens*	2.33b	22.00ab	4.67c	17.27c
20%*C. frutescens*	2.67b	45.09b	3.67c	10.09b
Distilled water	3.33b	57.36c	3.00b	12.10b
p-value	0.007	0.005	0.022	0.029

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

4.2.8. Effect of *T. minuta* and *C. frutescens* extracts on tuber yield of potatoes

Potato tuber yield (t ha^{-1}) significantly differed among the treatments at $p \leq 0.05$ (Table 7). The total yield was significantly higher in the potatoes treated with *Tagetes minuta* and copper oxychloride (21.50 t ha^{-1}) than those that were treated with *Capsicum frutescens* and water (8.97 t ha^{-1}). The marketable yield was also significantly higher from the plots treated with *T. minuta* extracts and copper oxychloride (18.50 t ha^{-1}) compared to those treated with *C. frutescens* and

water (7.50 t ha^{-1}). The overall total yield and marketable yield was higher in season 2 than season 1 which may be attributed to the fact that season 1 potatoes started off in high rainfall and later experienced drought, while season 2 potatoes started off under irrigation and experienced high rainfall later in the season. However, the number of tubers did not differ significantly among the treatments in both season 1 and 2.

Table 7: Effect of *T. minuta* and *C. frutescens* extracts on yield of potatoes

Treatment	Season 1			Season 2		
	No. of Tubers/6m^2	Total Yield (Ton/ha)	Marketable Yield(ton/ha)	No. of Tubers/6m^2	Total Yield (ton/ha)	Marketable Yield(ton/ha)
Copper oxychloride	20.30a	10.56a	9.66abc	31.33a	20.22ab	18.28a
40% *T. minuta*	34.00a	13.67a	12.56a	29.33a	21.50a	18.50a
30% *T. minuta*	32.00a	11.61a	10.72ab	28.67a	18.50bc	16.83a
20% *T. minuta*	26.00a	10.89a	9.78abc	27.00a	16.59cd	14.78ab
40%*C. frutescens*	30.00a	8.97a	6.39d	28.33a	13.72de	10.89c
30%*C. frutescens*	27.30a	8.28a	6.83d	30.00a	13.44de	9.72d
20%*C. frutescens*	26.00a	9.00a	7.50bc	24.67a	14.94de	11.56c
Distilled water	31.30a	11.61a	9.44abc	27.33a	15.55de	11.95c
p-value	0.359	0.087	<0.001	0.735	<0.001	<0.001

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

4.2.8.1. Effect of *T. minuta* and *C. frutescens* on tuber size, total soluble solids and dry matter content of potatoes

There was no significant difference in the dry matter content and total soluble solids among the treatments (Table 8). However, there was a significant difference in potato size measured in diameter whereby the potatoes treated with *T. minuta* and copper oxychloride were

larger in size (59.67 mm) compared to those treated with *Capsicum frutescens* and water (41.33 mm) in both season 1 and 2.

Table 8: Effect of *T. minuta* and *C. frutescens* extracts on tuber size, TSS and dry matter content

Treatment	Season 1			Season 2		
	Tuber size(mm)	Dm content(g)	TSS(Brix)	Tuber size(mm)	Dm content(g)	TSS(Brix)
Copper oxychloride	50.00a	23.70a	5.83a	59.33a	20.25a	5.00a
40% *T. minuta*	50.00a	23.22a	6.00a	59.67a	20.13a	5.17a
30% *T. minuta*	47.00ab	22.37a	5.83a	59.67a	20.39a	5.17a
20% *T. minuta*	46.67ab	24.13a	6.17a	51.00ab	18.62a	5.07a
40%*C. frutescens*	43.33cd	22.65a	6.17a	49.67ab	21.69a	5.27a
30%*C. frutescens*	42.67d	22.48a	6.17a	47.33b	19.07a	5.03a
20%*C. frutescens*	41.33d	24.45a	6.17a	44.00b	19.78a	5.43a
Distilled water	45.33bc	22.80a	6.00a	53.00ab	19.12a	5.0a
p-value	<0.001	0.230	0.44	<0.001	0.312	0.591

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

4.2.8.2. Effect of *T. minuta* and *C. frutescens* extracts on postharvest percent soft rot infections of potatoes

In the postharvest evaluation, the number of infected tubers differed significantly among the treatments at $p \leq 0.05$ (Table 9). *Capsicum frutescens* had the highest number of infected tubers even compared to water. On the other hand, *Tagetes minuta* and copper oxychloride had the lowest number of infected tubers. The number of infected tubers increased progressively from 7 days to 11 days and by day 11, the tubers treated with 40% *Capsicum frutescens* were all rotten oozing slimy liquid with a bad odour (Plate 7e and 7f). Those treated with *Tagetes minuta* and copper oxychloride were all intact (Plate 7a, 7b, 7c and 7h). Those treated with water were 23.22% rotten (7d) but not as much as those treated with *Capsicum frutescens* that were more

than 84.33% rotten. Increasing the concentration of *Capsicum frutescens* led to increase in percent rots progressively from 28.78%, to 45.45% and 95.44% (Table 9).

Table 9: Effect of *T. minuta* and *C. frutescens* extracts on postharvest percent soft rot infections at 7, 9 and 11 days after storage (DAS)

Treatment	Season 1			Season 2		
	7DAS	9DAS	11DAS	7DAS	9DAS	11DAS
Copper oxychloride	1.00a	1.00a	1.00a	1.00a	1.00a	6.56ab
40% *T. minuta*	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
30% *T. minuta*	1.00a	6.56a	6.56a	1.00a	6.56a	6.56ab
20% *T. minuta*	1.00a	6.56a	6.56a	6.56a	6.56a	12.11ab
40%*C. frutescens*	23.22c	56.56c	84.33c	39.89c	67.67c	95.44d
30%*C. frutescens*	6.58b	34.33b	45.45c	28.78b	39.89c	39.89bc
20%*C. frutescens*	6.58b	23.22b	28.78b	17.67b	23.22b	28.78bc
Distilled water	6.58b	23.22b	23.22b	17.67b	17.67b	23.22abc
p-value	0.015	0.004	<.001	0.006	<.001	<.001

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test

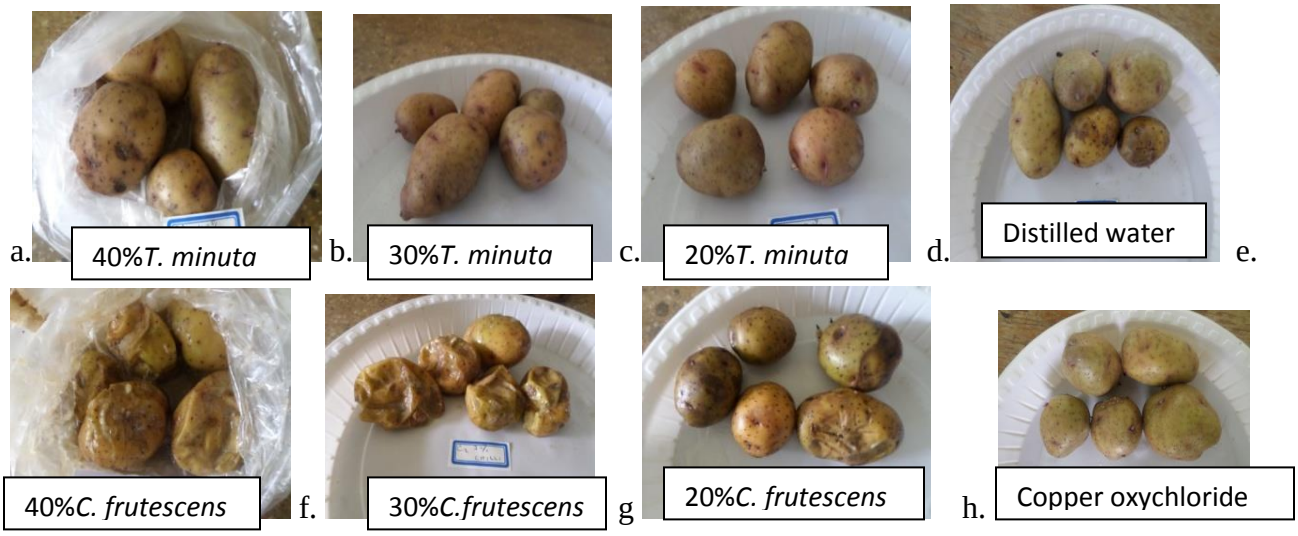


Plate 7: Post harvest infections of potatoes after treatment with the plant extracts

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1. Effect of *T. minuta* and *C. frutescens* on *in vitro* bacterial growth inhibition, tissue maceration and percent weight loss of potato chips due to tissue maceration

Tagetes minuta extracts at 40%, 30% and 20% concentration used in this study showed antibacterial activity against the *Pectobacterium carotovorum* bacteria *in vitro*. This is in agreement with previous research where crude plant extracts have been reported to have significant antibacterial activity, *in vitro*. Opara and Agugo (2014) observed *in vitro* antimicrobial activity of Z. *officinale* and *C. citratus* extracts against *Erwinia*, *Ralstonia* and *Flavobacteria*. Pfoze *et al*. (2011) reported significant antibacterial activity of *Mahonia manipurensis* crude extracts against gram-positive bacteria; *Bacillus cereus, E. cloacae and E. faecalis* and one gram-negative bacterium *S. flexneri* which was attributed to the alkaloids berberine, palmatine and jatrorrhizine present in the plant species used. This is echoed by Oguwike *et al.* (2013) who reported that secondary plant products like flavonoids, steroidal alkaloids and saponins show antibacterial activity against plant pathogenic bacteria. The secondary products in *T. minuta* extracts may have influenced antimicrobial activity on *Pectobacterium carotovorum* by inhibiting the peptidoglycan synthesis, damaged microbial membrane structures and/or even modified the bacterial membrane surface hydrophobicity of the *Pectobacterium carotovorum* as indicated by Shayan and Saeidi (2013).

Pouvova *et al*. (2008) reported *in vitro* effectiveness of *Tagetes bipinata* against *Clavibacter michiganensis* which causes ring rot in potato and bacterial wilt in Lucerne. The essential oils in *Tagetes bipinata* which had inhibitory effects on the *Clavibater michiganensis* may have been present in *T. minuta* causing the same inhibitory effects on *Pectobacterium carotovorum* in the current experiments. The phenolic compounds in *T. minuta* are capable of dissolving within the bacterial membrane thus penetrating inside the cell where they interact with cellular metabolic mechanisms thereby killing the bacteria (Ramya *et al*., 2012) as confirmed in this study.

Capsicum frutescens showed no activity against *Pectobacterium carotovorum* contrary to the findings by Ortega *et al.* (2003) who reported that growth of *Erwinia carotovora (Pectobacterium carotovorum)* is inhibited by extracts from the three varieties of *Capsicum annuum* and compounds found in the extracts like *meta*-coumaric and *trans*-cinnamic acids.

However, in the same experiment, it is reported that other compounds; Capsaicin and dihydrocapsaicin compounds do not affect the growth of the same bacteria which agrees with the findings in the current study.

Potato chips treated with *T. minuta* lasted 9 days before total maceration while those treated with streptomycin lasted 10 days, implying that *T. minuta* had almost the same antibacterial effects as streptomycin sulphate. On the other hand, potato chips that were treated with *Capsicum frutescens* and distilled water were completely macerated by day 5. In an experiment by Akbar *et al.* (2015), five isolates of *Pectobacterium* (*Erwinia*) evaluated for aggressiveness on tomato fruits, found isolates from chilli to be the most aggressive followed by tomato and potato isolates producing 22.3 mm, 7.9 mm and 7.8 mm diameter of soft rot lesions on the fruits respectively. This shows that the *Pectobacterium carotovorum* infects *Capsicum sp* and isolates from *Capsicum* could actually be the most aggressive strain. As such the *Capsicum frutescens* extracts in the current experiment may have acted more of a nutrient boosting growth of the bacteria instead of inhibiting it. Studies also show that *Capsicum chinense* has been used as an enrichment host for *Pectobacterium* during its isolation before culture (Zia *et al.,* 2011; Ali *et al.,* 2014) which supports this theory given that *C. frutescens* is in the same genus.

Evaluation of the three concentrations (40%, 30% and 20%) of *T. minuta* for percent weight loss due to tissue maceration by the *Pectobacterium* bacteria showed that *T. minuta* had the lowest percent weight loss compared to water and even streptomycin sulphate (Figure 7). The results corroborate with the results obtained by Ramya *et al.* (2012) who observed that the inhibitory effect of flower extracts from *Tagetes erecta* and *T*agetes *patula* on *Salmonella aureus* and *Escherichia coli* was higher than that of streptomycin. Kotan *et al. (*2007) also report a significant antibacterial activity by the essential oils of *Thymus canoviridis, Satureja hortiensis, Melissa officinalis* ssp. *inodora, Helichrysum pilicatum, Thymus haussknechtii and Thymus sipyleus* on inhibition of *X. axonopodis* which proved to be stronger than the standard antibiotic (streptocycline) used. Findings by Hussain *et al.* (2014) too indicate that in comparison to the antibiotics used in their study, the plant extracts were far more active against the test bacterial strains.

Tagetes oil is used as a flavor component in food products including cola and alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments and relishes and it has antibacterial activity (Al-Musayeib *et al.,* 2014). This is echoed by studies by

Shirazi *et al.* (2013) that showed that *Tagetes minuta* has been used as a food colorant in foods such as pasta, vegetable oil, margarine, mayonnaises, salad dressing, baked goods, confectionery, dairy products, ice cream, yogurt, citrus juice, mustard and poultry feed because of the rich orange-yellow carotenoid. Based on the present results and others, *T. minuta* can therefore be used to extend the shelf life of potato chips under ambient temperature conditions and still be used for human consumption.

5.2. Effect of *T. minuta* and *C. frutescens* on disease incidence and severity on potato plants and tubers

Tagetes minuta significantly managed blackleg and soft rot as seen from the fewer plants that showed blackleg symptoms and the reduced infections on the potato tubers. These results corroborate with other studies that indicate that plant extracts work in various ways to inhibit microbial and bacterial growth and can therefore be used to manage bacterial infections. A study by Upadhyay *et al*. (2010) showed that essential oils from citrus (*Citrus limon),* olive (*Olea europaea),* neem (*Azadirachta indica*) and ajwain (*Trachiyspirum ammi*) had strong antimicrobial potential against *Staphylococcus aureus, Streptococcus pneumoniae, Lactobacillus acidophilus, Micrococcus luteus, B. cereus, Klebsiella pneumoniae* and *Escherichia coli.* Upadhyay *et al*. (2010) also found that the leaves and bark extract of *Juglans regia* showed high activity against the gram positive bacteria and gram negative bacteria. War *et al.* (2015) observed that plant extracts from *Datura stramonium,* onion (*Allium cepa*) and garlic (*Allium sativum*)had antibacterial activity on *E. carotovora* of king chilli.

Results obtained by Ramya *et al.* (2012) showed the inhibitory effect of flower extracts from *T.erecta* and *T. patula* on *S. aureus*, *S. epidermidis* and *E. coli.* Kotan *et al.* (2007) also reported a significant antibacterial activity by the essential oils of *T. canoviridis, S. hortiensis, M. officinalis, H. pilicatum, T. haussknechtii and T. sipyleus* against of *X. axonopodis.*

In their study, Shirazi *et al*. (2013) found that *Tagetes minuta* and *Ocinum basilicum* extracts contain phenol-containing monoterpenes which had significant antibacterial activity against both Gram-positive and Gram-negative bacteria. The two plant extracts had a significant antibacterial activity against *Salmonella aureus, Salmonella enteritidis* and *E. coli* and antiseptic against *Proteus vulgaris, B. subtilis* and *Salmonella parathypha.* Jain *et al*. (2012) note that antibacterial activity observed with *T. erecta* in their study may be associated with compounds such as quercetagetin, phenolics, syringic acid, methyl-3, 5- dihydroxy-4- methoxy benzoate,

quercetin, thienyl and ethyl gallate which are known to have curative activity against several pathogens. Lubna and Naeem (2012) also show that *Tagetes sp.* contains secondary metabolites including flavonoids and terpenes that have pharmacological and antibacterial activity. The same compounds may have had antibacterial activity on *Pectobacterium carotovorum* in the current study.

A study by Hajhamed *et al*. (2007) showed that *Tagetes minuta* induces systemic resistance against *Pectobacterium* or even suppresses enzyme production which decreases the maceration of the tissues. This is in agreement with the current results that showed suppressed activity of *Pectobacterium carovorum* on the potatoes in the plots treated with *Tagetes minuta* extracts. In addition, Czajkowski *et al.* (2011) indicate improved resistance of potato tubers against *Pectobacterium carovorum* in *in vitro* experiments where the resistance to soft rot was two-fold higher in transgenic lines than in non-transformed control tubers. In the current study, the *T.minuta* may have induced resistance in the potatoes to the *Pectobacterium carotovorum* which had minimal infections compared to potatoes treated with *Capsicum frutescens*.

The antibacterial activity in the essential oils of several medicinal plants could be related to the attack on the phospholipids in the cell membranes of the microbes which causes increased permeability and leakage of cytoplasm thereby killing the bacteria (Al Abbasy *et al.,* 2015). This may have been the case with *T. minuta* used in the current study.

In the current study *Capsicum frutescens* extracts did not inhibit the *Pectobacterium* bacteria. This may be because *Capsicum* is a host to the bacteria as indicated by Akbar *et al.* (2015) who isolated *Erwinia carotovora* (*Pectobacterium carotovorum*) from pepper, tomato and potato. The inactivity of *Capsicum* against *Pectobacterium carotovorum* in the current research may also be attributed to the use of a different species of *Capsicum*. *Capsicum annuum* was used by Ortega *et al.,* (2003) while in the current study, *Capsicum frutescens* was used which may have had differences in the amount of the inhibitory compounds like *meta*-coumaric and *trans*-cinnamic acids in each of them. In the current study, the results may have also been different because the extracts were obtained using the aqueous extraction instead of the Soxhlet extraction method (using Isopropyl alcohol) used by Ortega *et al*. (2003).

However, the current results are in agreement with Amruthraj *et al.* (2013) who reported that acetonitrile and acetone extracts from *Capsicum chinense* were found to be ineffective against *E.coli* and *Erwinia sp.* However, Koffi-Nevry *et al*. (2012) found *Capsicum annuum* and

Capsicum frutescens to be effective against *Vibrio cholerae*, *Staphylococcus aureus* and *Salmonella typhimurium*. The extract from *Capsicum annuum* showed a higher antibacterial activity than the one from *Capsicum frutescens.* This shows that the inhibitory components in the two species of Capsicum significantly varied. This may have been the case in the current study.

5.3. Effect of *Tagetes minuta* and *Capsicum frutescens* extracts on yield and quality of potatoes

The total yield and consequently marketable yield was higher in the plots treated with *T. minuta* than those treated with *C. frutescens.* Potatoes treated with *C. frutescens* had higher infections which impacted negatively on yield as the infected potatoes had to be discarded. This agrees with many reports by other researchers. Prajapat *et al*. (2013) indicate that potato yield losses up to 98.8% have been experienced under artificial epiphytotics. Opara and Agugo (2014) noted that in Nigeria, over 60% of white yam varieties rot and the *Pectobacterium carotovorum* is among the micro-organisms implicated.

In a review by Czajkowski *et al*. (2011), it was stated that approximately 22% of potatoes are lost per year due to viral, bacterial, fungal and pest attack in potato tuber and potato plant incurring an annual loss of over 65 million tones and bacterial soft rot alone accounts for 30-50% of this huge loss. The results in this study concur with their findings as indicated by the lower yields due to soft rot infections in the plots treated with water and *C. frutescens* and water.

5.4. Effect of *T. minuta* and *C. frutescens* on percent postharvest soft rot infections

In the stored potatoes, infections were significantly low in potatoes treated with *T. minuta* (Plate 7a, b and c). This corroborates with the results obtained by Obey *et al*. (2015) who reported that the essential oils from aerial parts of *T. minuta* and *T. lucida* contain flavonoids that showed antibacterial activity against Gram-positive and Gram-negative bacteria. Hussain *et al.* (2014) found out that extracts of *Tagetes* were active against mult-drug resistant bacteria which included *Escherichia coli, V. cholerae, Providencia, Shigella, P. vulgaris, Salmonella typhi, Klebsiella pneumoniae* and *Citrobacter freundii.* Studies by Lubna and Naeem (2012) showed that *Tagetes* extracts had antibacterial activity against *S.typhi, E.coli, B. Lichniferous, K. pnomoniae, P.aeruginosa, S. viridians* and *B. subtilis* which may have been due to the presences of different flavonoids and terpenes especially in leaf extract.

Opara and Nwokocha (2015) reported up to 89.44% control of *Erwinia* (*Pectobacterium*) using ethanol and water extracts from *T. erecta*, *Jathropha curcas* and *Costus afa*r in control of the post-harvest rot in yam. This is in agreement with the current findings where *T. minuta* had well over 93% control of post-harvest rot caused by *Pectobacterium* bacteria.

Afrodet **(**2006) showed that the oil extract from *C. annuum* had antimicrobial activity against gram positive and gram negative bacteria and attributed it to the presence of capsanthin which is considered one of the major carotenoids of red pepper fruits. This is however contrary to the current findings where the potatoes treated with *C. frutescens* had almost 100% rots in the postharvest percent soft rot infections. Given that *C.frutescens* and *Solanum tuberosum* belong to the same family, solanaceae they are likely to be affected by the same diseases including *Pectobacterium.*

As stated previously, Akbar *et al.* (2015) evaluated five isolates of *Pectobacterium* (*Erwinia*) for aggressiveness on tomato fruits and chilli isolate was found to be the most aggressive followed by tomato and potato isolates producing 22.3 mm, 7.9 mm and 7.8 mm diameter of soft rot lesions on the fruits respectively. *Capsicum* isolate was the most aggressive which agrees with the current results where almost all the tubers treated with capsicum were completely macerated. This shows that the *Pectobacterium carotovorum* infects *Capsicum sp.* and as such, the *Capsicum frutescens* extracts in the current experiment may have acted as a source of nutrients instead of killing the bacteria as shown by Zia *et al.* (2011); Ali *et al.* (2014) who used *Capsicum chinense* as an enrichment host for *Pectobacterium* during isolation before culture.

Omolo *et al*. (2014) noted that the extraction methods inconsistency between analyzed plant materials strongly affect the observed levels of bacterial growth inhibition by chilli. This may also have been the case in the current research in that aqueous extraction was used instead of alcohol extraction.

Studies by Koffi-Nevry *et al*. (2012) found *Capsicum annuum* and *Capsicum frutescens* to be effective against *Vibrio cholerae, Staphylococcus aureus* and *Salmonella typhimurium* but extract from *Capsicum annuum* showed a higher antibacterial activity than the one from *Capsicum frutescens.* This shows that the inhibitory components in the two pepper extracts used may have been different and this may influence their antimicrobial effect. As deduced by Patricia *et al*. (2013) the difference in antibacterial activity of the *Capsicum* extracts used could

be explained by variations in the sample preparation, extraction and quantification methods; the diversity of the varieties and genotypes of peppers (as sweet or hot peppers); the maturity stage and the use of fresh or dehydrated fruits.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

6.1.1. Effect of *T. minuta* and *C. frutscens* extracts on *Pectobacterium carotovorum*, *in vitro*

Tagetes minuta extracts have antibacterial activity against *Pectobacterium carotovorum* bacteria *in vitro.* This is evidenced by inhibition of growth of bacterial colonies, extended days to total tissue maceration and the reduced percent weight loss due to tissue maceration. On the other hand *Capsicum frutescens* extracts did not show antibacterial activity *in vitro*.

6.1.2. Effect of *T. minuta* and *C. frutescens* extracts on potato blackleg and soft rot, *in vivo*

Tagetes minuta managed blackleg in the field and soft rot of tubers at harvest and after harvest. This can be seen from the reduced incidence and severity of blackleg on potato plants and soft rot of tubers in the field and percent postharvest soft rots. *Capsicum frutescens* on the other hand did not manage blackleg and soft in the potatoes either in the field or after harvest.

6.1.3. Effect of *T. minuta* and *C. frutscens* extracts on sprouting, growth, yield and quality of potatoes

Tagetes minuta increased the potato yield by reducing soft rot infections while *Capsicum frutescens* reduced the yields because of the increased soft rot infections. The plant extract however had no significant effects on the size, dry matter content and total soluble sugars of the potatoes.

6. 2. Recommendations

Tagetes minuta can be incorporated in the integrated disease management (IDM) of potatoes to manage bacterial soft rot infections both in the field and in storage. However, further research needs to be done to determine the exact chemicals in *Tagetes minuta* that inhibits the growth of bacteria.

Even though *Capsicum frutescens* did not inhibit the bacteria in the current study, more research is recommended to determine the method of extraction, species and the exact chemical component that may have inhibited bacteria in previous studies**.**

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APPENDICES

Appendix 1: Minimum inhibition zone on bacterial growth (mm) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value.
Treatments	7	242.5096	34.64423	989.84	<.001
Residual	16	0.56000	0.03500		
Total	23	243.06958			

Appendix 2: Effect of *T. minuta* and *C. frutescens* on number of days to total tissue maceration ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value.
Treatments	7	99.2917	14.1845	28.04	<.001
Residual	14	7.0833	0.5060		
Total	21	106.6250			

Appendix 3: Effect of *T. minuta* concentration on percent weight loss due to tissue maceration 2days after inoculation ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value.
Treatments	4	542.21	135.55	3.43	0.050
Residual	10	394.72	39.47		
Total	14	936.93			

Appendix 4: Effect of *T. minuta* concentration percent weight loss due to tissue maceration 5days after inoculation ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatment	4	3283.5	820.9	6.50	0.008
Residual	10	1262.5	126.2		
Total	14	4546.0			

Appendix 5: Effect of *T. minuta* concentration percent weight loss due to tissue maceration 7days after inoculation ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatment	4	7182.10	1795.53	26.88	<.001
Residual	10	668.05	66.81		
Total	14	7850.16			

Appendix 6: Effect of *T. minuta* concentration percent weight loss due to tissue maceration 9days after inoculation ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatment	4	7905.46	1976.37	23.12	<.001
Residual	10	854.81	85.48		
Total	14	8760.28			

Appendix 7: Effect of *T. minuta* concentration concentration percent weight loss due to tissue maceration 11days after inoculation ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	4	4396.06	1099.02	20.46	<.001
Residual	10	537.03	53.70		
Total	14	4933.10			

Appendix 8: Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 35 DAP (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	0.2500	0.1250	0.72	
Treatments	7	13.8333	1.9762	11.45	<.001
Residual	14	2.4167	0.1726		
Total	23	16.5000			

Appendix 9: Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 40 DAP (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	0.5833	0.2917	1.96	
Treatments	7	15.1667	2.1667	14.56	<.001
Residual	14	2.0833	0.1488		
Total	23	17.8333			

Appendix 10: Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 44 DAP (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	0.5833	0.2917	1.96	
Treatments	7	23.1667	3.3095	22.24	<.001
Residual	14	2.0833	0.1488		
Total	23	25.8333			

Appendix 11: Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 35 DAP (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	0.5833	0.2917	2.88	
TRT	7	5.9583	0.8512	8.41	<.001
Residual	14	1.4167	0.1012		
Total	23	7.9583			

Appendix 12: Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 40 DAP (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	3.5833	1.7917	3.91	
Treatments	7	16.9583	2.4226	5.29	0.004
Residual	14	6.4167	0.4583		
Total	23	26.9583			

Appendix 13: **Effect of *T. minuta* and *C. frutescens* on blackleg incidence on stems 44 DAP (season 2) ANOVA**

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	3.0000	1.5000	2.52	
Treatments	7	18.6667	2.6667	4.48	0.008
Residual	14	8.3333	0.5952		
Total	23	30.0000			

Appendix 14: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 35DAP (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	1144.8	572.4	1.44	
Treatments	7	17856.3	2550.9	6.42	0.002
Residual	14	5564.6	397.5		
Total	23	24565.6			

Appendix 15: **Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 40 DAP (season 1) ANOVA**

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	935.1	467.5	3.32	
Treatments	7	32797.0	4685.3	33.31	<.001
Residual	14	1968.9	140.6		
Total	23	35701.0			

Appendix 16: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 44 DAP (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	975.0	487.5	1.59	
Treatments	7	32695.8	4670.8	15.24	<.001
Residual	14	4291.7	306.5		
Total	23	37962.5			

Appendix 17: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 35DAP (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	2968.8	1484.4	2.79	
Treatments	7	18724.0	2674.9	5.03	0.005
Residual	14	7447.9	532.0		
Total	23	29140.6			

Appendix 18: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 40 DAP (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	1353.1	676.5	1.52	
Treatments	7	23671.3	3381.6	7.61	<.001
Residual	14	6219.6	444.3		
Total	23	31244.0			

Appendix 19: Effect of *T. minuta* and *C. frutescens* on blackleg severity on stems 44 DAP (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	1080.1	540.0	1.06	
Treatments	7	34102.0	4871.7	9.53	<.001
Residual	14	7155.2	511.1		
Total	23	42337.3			

Appendix 20: Effect of *T. minuta* and *C. frutescens* on soft rot incidence on tubers (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	0.0833	0.0417	0.13	
Treatments	7	24.2917	3.4702	10.60	<.001
Residual	14	4.5833	0.3274		
Total	23	28.9583			

Appendix 21: **Effect of *T. minuta* and *C. frutescens* on soft rot incidence on tubers (season 2) ANOVA**

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	15.083	7.542	4.35	
Treatments	7	42.500	6.071	3.51	0.022
Residual	14	24.250	1.732		
Total	23	81.833			

Appendix 22: Effect of *T. minuta* and *C. frutescens* on soft rot severity on tubers (season 1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replications	2	38.4	19.2	0.06	
Treatments	7	11274.4	1610.6	5.10	0.005
Residual	14	4422.5	315.9		
Total	23	15735.3			

Appendix 23: Effect of *T. minuta* and *C. frutescens* on soft rot severity on tubers (season 2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	527.45	263.72	5.21	
Treatments	7	1145.27	163.61	3.23	0.029
Residual	14	708.89	50.64		
Total	23	2381.61			

Appendix 24: Effect of *T. minuta* and *C. frutescens* on total yield in season 1 (tons/ha) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	8.973	4.486	0.86	
Treatments	7	84.544	12.078	2.31	0.087
Residual	14	73.256	5.233		
Total	23	166.773			

Appendix 25: Effect of *T. minuta* and *C. frutescens* on marketable yield in season 1 (tons/ha) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	6.757	3.379	2.27	
Treatments	7	91.576	13.082	8.78	<.001
Residual	14	20.867	1.490		
Total	23	119.200			

Appendix 26: Effect of *T. minuta* and *C. frutescens* on total yield in season 2 (tons/ha) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Replications	2	3.579	1.790	1.75	
Treatments	7	187.493	26.785	26.12	<.001
Residual	14	14.356	1.025		
Total	23	205.428			

Appendix 27: Effect of *T. minuta* and *C. frutescens* on marketable yield in season 2 (tons/ha) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	p-value
Replications	2	4.906	2.453	1.04	
Treatments	7	256.024	36.575	15.50	<.001
Residual	14	33.029	2.359		
Total	23	293.958			

Appendix 28: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 7DAI (season1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	1192.26	170.32	3.68	0.015
Residual	16	740.81	46.30		
Total	23	1933.07			

Appendix 29: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 9DAI (season1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	7916.8	1131.0	4.89	0.004
Residual	16	3703.1	231.4		
Total	23	11619.9			

Appendix 30: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 11DAI (season1) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	17359.9	2480.0	19.48	<.001
Residual	16	2036.9	127.3		
Total	23	19396.8			

Appendix 31: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 7DAI (season2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	4433.3	633.3	4.56	0.006
Residual	16	2221.9	138.9		
Total	23	6655.1			

Appendix 32: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 9DAI (season2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	10728.57	1532.65	16.55	<.001
Residual	16	1481.52	92.59		
Total	23	12210.09			

Appendix 33: Effect of *T. minuta* and *C. frutescens* on percent postharvest infection 11DAI (season2) ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	*p*-value
Treatments	7	19802.11	2828.87	34.91	<.001
Residual	16	1296.37	81.02		
Total	23	21098.48			

Appendix 34: Abstract page of published paper No. 1.

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In vitro antibacterial activity of *Tagetes minuta* and *Capsicum frutescens* extracts against *pectobacterium carotovorum*

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Abstract

Pectobacterium carotovorum bacteria cause soft rot and vascular wilt of vegetables in the field and post-harvest decay. The aim of this study was to determine *in vitro* antibacterial activity of *Tagetes minuta* and *Capsicum frutescens* extracts against *Pectobacterium carotovorum.* Aqueous extracts of *T. minuta* and *C. frutescens* were tested against *Pectobacterium* by modified disc diffusion method on agar plates and potato chips assay. Streptomycin sulphate and distilled water were the positive and the negative controls respectively. The experiment was set up in a completely randomized design. Data was collected on zone of growth inhibition, number of days to total tissue maceration and %weight loss of potato chips due to tissue maceration. The three concentrations of *T. minuta* (40%, 30% and 20%) recorded zones of inhibition of 7.167mm, 6.667mm and 6.1mm; and streptomycin sulphate recorded 8.83mm which were significantly different from *C. frutescens* and distilled water that recorded 0.00mm. In the potato chips assay, *T. minuta* and streptomycin sulphate showed a significant difference in the number of days (9days) to total tissue maceration and % weight loss from *C. frutescens* and water that took only 5days for the potato chips to be totally macerated. It can be concluded that extracts from *T. minuta* have antibacterial activity against *Pectobacterium carotovorum*.

Key: words*: In vitro*, growth inhibition, *Pectobacterium carotovorum*, *Tagetes minuta*, *Capsicum frutescen*s