THE EFFECTS OF *OCIMUM GRATISSIMUM, IPOMOEA BATATAS* AND *BRASSICA OLERACEA* VAR. *BOTRYTIS* EXTRACTS ON THE GROWTH AND DISEASE-CAUSING CAPABILITY OF *RALSTONIA SOLANACEARUM* IN *SOLANUM TUBEROSUM*

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ABSTRACT

Irish potato (*Solanum tuberosum* L.) is a major cash and food crop worldwide. The production of this crop has in a great way been negatively affected by the extensive spread of bacterial wilt that is caused by *Ralstonia solanacearum* with over 50% crop yield losses reported in the last two decades. In addition, no bactericide has been developed to combat the disease and exercise of integrated control measures have not resulted to a sustainable solution. Scientists are putting a lot of efforts in research to get a solution that is effective, environmental friendly and that will enable the capacity of food production be improved to cater for the increasing human population. Plants have been shown to contain principles which are active against pathogens and among them are Brassica, Ipomoea and Ocimum species. It is on this basis that this research was aimed at determining the efficacy of locally available plants extracts in controlling the bacterial wilt disease in potatoes. The study was carried out in Maseno University Botany & Horticulture Department laboratory and the institution's Botanic Garden in 2007. The pathogen was isolated from infected potato tubers and cultured on nutrient agar medium. Ethyl acetate and methanol extracts of leaves of *Ocimum gratissimum, Ipomoea batatas* and *Brassica oleracea* var. *botrytis* and essential oils of *Ocimum gratissimum* were evaluated for their efficacy at concentrations of 0.4, 0.2, 0.1, 0.05 and 0.025 mg/ml dissolved in dimethylsulphoxide in inhibiting *in vitro* growth of wilt bacteria. The experiment was extended to greenhouse where aqueous extracts of the three plants at concentrations of 2.5, 5 and 10% were evaluated for their efficacy in controlling the development of bacterial wilt symptoms on inoculated potato plants with control plants being inoculated with no treatment subjected to them. The extracts were also evaluated for their effect on different potato plants parameters. The experimental set up was

randomized completely block design with 12 treatments and three replications. Data collected was subjected to Analysis of Variance (ANOVA) using SAS statistical package and the effects declared significant at 5% level. Linear correlation was used to compare the relationship between variables. The study has proved that the three plants contains principles that are active against wilt bacteria with essential oils from *Ocimum gratissimum* at 0.4mg/ml exhibiting highest activity (10.1mm inhibition diameter) and *Ipomoea batatas* methanol extract at 0.025mg/ml exhibiting zero inhibition. The laboratory study has also shown a highly significant ($P < 0.0001$) interaction among plants, solvents and the various concentrations in the inhibitory activity. Aqueous extracts of the three plants have also exhibited varying levels of controlling wilt symptoms and promoting potato growth. The extracts of Ocimum at 10% and Brassica at 5% were the most effective in controlling the development of wilt symptoms with average wilting index of 1.33 meaning less than 50% wilting occurrence. Potato plants treated with Brassica extracts at 10% exhibited an abnormal character of its leaf tips drying and thus affecting their growth. In assessing the effect of potato wilting, the interaction between the plant and various concentrations was shown not to be significant ($P > 0.05$) in the first four days after inoculation. However it was significant ($P<0.001$) in the following three days and highly significant ($P< 0.0001$) after and until the end of the study period. The crude extracts had a significant effect on the growth parameters of potato. Based on the findings of this study the three plants contains compounds that are active against the growth of wilt bacteria and we recommend further evaluation of the crude extracts to determine the actual active ingredients which can be used to develop an effective biocontrol agent for this disease.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Irish potato or cultivated potato is an important cash and food crop in Kenya and most parts of the world and is scientifically referred to as *Solanum tuberosum* L. The crop is a perennial plant of the Solanaceae or night shade family commonly grown for its starchy tuber. Potato plants grow high to the ground and bear yellow to silver flowers with yellow stamens. They thrive well in loose, easily tilled and high in organic matter soils, if drainage is adequate. They are cross-pollinated mostly by bumble bees that carry pollen from other potato plants but a substantial amount of self fertilizing occurs as well (Waithaka, 1976). Any potato variety can also be propagated vegetatively by planting tubers or pieces of tubers, cut to include at least one or two eyes (a bud or node from which emerge future shoots). Potatoes to be eaten need to be stored at low temperatures to prevent buds from growing and to keep starch levels constant. Keeping them at higher temperatures make them sprout. New varieties are grown from seeds/true seeds or botanical seeds obtained from potato fruits which are usually small, green in colour resembling cherry tomatoes (Kinyae *et al.*, 1994).

1.1.1 Origin and distribution of potato

The crop originated in the highlands of South America where it has been used for over 8000 years and it is from this area that Spanish explorers brought the plant to Europe in the late 16th century. By 19th century, the crop had spread throughout the continent providing abundant food for workers of the industrial revolution. The crop became of great importance especially in regions which were too cold for maize as people would

turn to growing potatoes. The crop is often referred to as Irish potato in the English speaking world because of the association with the Great Irish Famine which began in 1845 and lasted for six years. This was caused by infection of the crop by *Phytophthora infestans* which destroyed the plants. The Irish peasant population had become highly dependent on the potato because of the relatively large amount of food that could be produced on fairly small holdings After the infestation of the disease, people were left with nothing to eat and no way to make money to support themselves. Many wandered the countryside, begging for food or work while others ate grass and weeds to survive and those who could afford left the country in search of a better life (Kinyae *et al.*, 1994).

Worldwide, the Irish potato is grown in more than 70% of all countries which include both the industrialized and developing countries. The crop has been bred into many standard or well known varieties each of which have particular agricultural attributes. They include; Desiree, Pink eye, Russet Burbank, Kipfler, Nicola, Pontiac and Spunta among others. Today potato is the fourth most important food crop in the world after wheat, maize and rice with annual production approaching 300 million tons (Otipa *et al.*, 2003). In addition more than one third of the global potato output now comes from developing countries. In Kenya potato ranks as the second most important food crop after maize (F.A.O., 2006; Ministry of Agriculture, 2007). It grows in parts of the country which are cool and of high altitude where rainfall is well distributed.

1.1.2 History of potato in Kenya

It is reported that appropriately one-third of all farmers in Kenya grow potatoes while 30-40% of the population consumes potatoes regularly (Mwangi, 2003). Potato is mainly grown by small-scale farmers in Central, Rift valley and Eastern provinces. The crop was introduced in Kenya and other areas of East Africa primarily by British farmers and colonial officials during the 1880s. The British colonial government encouraged potato cultivation during the First World War as a means to feed troops stationed in East Africa. Seed imports for research purposes and formal variety trials date from this period (Waithaka, 1976; CIP, 2006).

In the 1930s Kenyan potato production suffered both from economic problems associated with the global depression and from pests and diseases (Waithaka, 1976; CIP, 2006). But during the 2nd world war, production was maintained to meet the needs of British army. In 1967, a potato development project was established by the Kenyan Government with an aim to establish programmes in variety screening, plant breeding, seed multiplication and agronomy (Durr and Lorenzel, 1980). The distribution of Kenya's potato crop is typical for a tropical country; concentrated at mid elevations and highland areas where population density is high, farms are small and agricultural productivity is challenged to meet the demands of a growing population (Lutaladio *et al.*, 1995). The most favourable elevation is usually between 1500m – 2500m above the sea level.

Most of the growing regions are found in Central, Eastern and Rift valley provinces. In Central Province almost all districts produce potato with Nyandarua district, which lies

along the Aberdare mountain ranges being the largest producer. Central province produces more than half the total amount of potatoes consumed in Kenya.

In Eastern province, Meru district is the main potato-growing region since it lies along the slopes of Mount Kenya and in the Rift Valley, potatoes are grown in the Mau escarpment region of Dundori, Mau Narok and Molo and in the Western highlands of Kericho, Bomet and Uasin Gishu districts. Various potato varieties that do well in our country are the Kerr's Pink, Ngure, Nyayo, Tana, Tigoni, Desiree and Golof (F.A.O., 2006).

1.1.3 Uses and economic importance of potato

Potato tuber is a source of food for a large percentage of the world's population. It is less fattening than other foods in the diet and is a good source of iron. It is used either while fresh or in processed forms. The tubers contain approximately 75 – 80% water, 16 – 20% carbohydrates, 2.5 – 3.2% crude protein, 1.2 - 2.2% mineral water, 0.6% crude fiber (Mwangi, 2003). The protein component is found in the inner layers of the skin (periderm or cork cells). In addition, potato has essential amino acids such as Lysine, Vitamin C1, B1, and B2, and minerals like potassium and phosphorus. The indigestibility of ungelatinized potato starch precludes the consumption of potatoes, as a major food item in the raw state and the presence of protease inhibitors that are considerably denatured by heat is an additional reason for cooking.

Various methods of cooking are used to prepare potato: they are either boiled, fried, roasted or baked. In rural areas, potato is primarily consumed in mashed foods and stews. A small but significant portion of the starch in potatoes is resistant to enzymatic digestion

in the stomach and small intestines and thus reaches the large intestine intact. This resistant starch has healthy benefits such as providing bulk, offering protection against colon cancer, improving glucose tolerance and insulin sensitivity (Cummings *et al.*, 1996; Hylla *et al.*, 1998).

The main dry matter component of the potato tuber is starch which acts as a good source of vitamin C, especially in areas where the common sources of this vitamin are not available. The tubers turn green when exposed to sun for long hours and these should not be consumed because they are deadly poisonous if much is consumed. The poisonous substance is the alkaloid solanine, which is made in all green tissue of the plant. In Central Kenya, it is a staple food where local dishes include potatoes mixed with meat, vegetables and other types of food. In cities, potato is heavily consumed as chips and crisps (Mwangi, 2003). In developed countries potato is mainly consumed in processed forms such as French fries, crisps, potato powder, potato flakes, potato flour, and canned potatoes among others.

In addition to human consumption potato is used in feeding livestock especially the vines. The plant is useful in production industry as its starch is used in sizing and surface coating of papers in the paper industry, in sizing of cotton and in finishing sewing thread and cloth in the textile industry while the white potato is used in the fermentation of vodka. There is also potato dextrins used as adhesives in book-binding (Burton, 1989).

1.1.4 Constraints of potato production in Kenya

Various abiotic and biotic factors such as pests and diseases, agronomic activities and climate usually affect the production of potatoes negatively in most countries. A research

conducted by Kenya Agricultural Research Institute and partners in Kenya, Uganda and South Africa showed that production of potatoes is in many cases dominated by intensive, low input, small-scale agriculture. Low yields have been attributed to near-continuous potato production on the same land, which leads to increased incidence of diseases and pests and decline in soil fertility. Production of potatoes in many cases is negatively affected by factors such as lack of healthy seed tubers, reduction in soil fertility and poor agronomic practices (Kinyae *et. al.*, 1994). Production of potatoes has stagnated over the last 10 years (Figure1) though acreage under the crop has been on the increase.

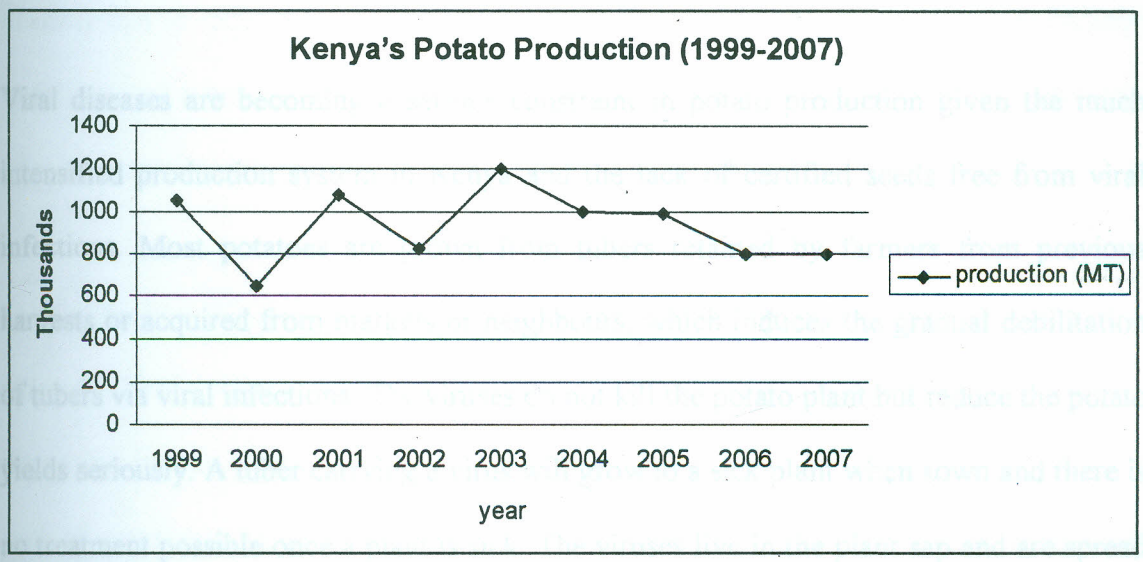

Figure 1: Kenya's Potato Production (1999-2007) (Source: Ministry of Agriculture, 2007)

1.1.5 Diseases and pests of potatoes

Among the common diseases that limit the production of potato is the Late blight of potatoes caused by *Phytophthora infestans* and bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi. Late blight is a disease of potatoes that rarely affects

tubers instead highly affects the leaves where by they develop brown patches or the whole leaf just dries. It therefore kills a potato crop due to interference with photosynthesis processes. Late blight can be controlled through the combined use of resistant varieties and prudent application of fungicides. It has been reported as the most widespread and destructive disease affecting potato production. Symptoms of late blight infection include lesions on the leaves and stems, white mycelia on the back and eventually the plant dies up. The development of late blight resistant varieties has been a focus of the Kenya Potato Programme under the direction of KARI, leading to the release of the Tigoni and Asante varieties in 1996 (CIP, 2006).

Viral diseases are becoming a serious constraint in potato production given the much intensified production system in Kenya and the lack of certified seeds free from viral infections. Most potatoes are grown from tubers retained by farmers from previous harvests or acquired from markets or neighbours, which induces the gradual debilitation of tubers via viral infections. The viruses do not kill the potato plant but reduce the potato yields seriously. A tuber carrying a virus will grow to a sick plant when sown and there is no treatment possible once a plant is sick. The viruses live in the plant sap and are spread from one plant to the other by aphids and other insects that suck sap from potato plants. The best way to minimize the effect of viruses is to plant clean seeds. Symptoms of viral infections include leaf roll, lighter green patches on leaves, stems and leaves pointing upwards, small plants, bunchness of leaves, crinkling of leaf edges and malformed tubers (Gildemacher *et al.*, 2007).

In most cases, the commercially sold tubers are frequently infected with viruses. Viral infection by PVX (potato virus x) reportedly decreased yields in the variety Kenya Baraka by 21% and the variety Roslin Eburu by 10%, potato leaf roll virus (PLRV) was reported to decrease yields of Kenya Baraka by 68% and Roslin Eburu by 35% (Khurana and Garg, 2003). Healthy potato plants are usually characterized by being big, having many thick stems, dark green leaves, and many large and well shaped tubers and do not show viral or bacterial symptoms.

Potato tuber moth (PTM) (*Phthorimaea operculella*) is becoming a greater hazard possibly due to reduced fallow periods and more intensified rotations. It causes damage to foliage and tubers in the field but has especially high potential to destroy potatoes in storage, sometimes to nearly total loss (Lutaladio *et al.*, 1995).

Lack of healthy, high yielding planting materials is a major potato production constraint (Kinyua, 2004). Spread of bacterial wilt is enhanced by diminishing land size and increasing human population that prevents the application of known cultural practices such as practicing crop rotation in control of this disease. Yield losses due to bacterial wilt increase with decreasing soil fertility. A research (Berga *et al.*, 2001a) showed that by incorporating organic manure of *Sesbania sesban* and *Leucaena diversfolia* in fields that were highly inoculated with *Ralstonia solanacearum*, the yields increased significantly compared to the controls where fields were treated with inorganic fertilizers.

The high incidence of potato diseases and pests has been attributed to continuous potato production on the same piece of land. This increases the chances of infection by soil borne pathogens such as *Ralstonia solanacearum* (Smith) Yabuuchi that remains active in the soil for more than two years even in the absence of the plant hosts. Bacterial wilt ranks as the second most important potato disease after late blight that is caused by *Phytophthora infestans.* These two diseases have led to reduction in potato yields in most potato producing countries (Ajanga, 1993). In Kenya, the current production of the crop cannot cope with the ever-increasing human demand and this is partly due to the infection of the crop by bacterial wilt.

Bacterial wilt is a disease whose remedy has not yet been identified yet it has continued to be reported in all potato producing areas both in Kenya and other countries. Farmers are advised to use several integrated measures to reduce the spread of the disease to the uninfected areas. The disease causes great losses because after a plant is infected, it starts to wither without recovery and finally dies. In addition even the small potato tuber that may have formed undergoes rotting after a very short time. The control measures currently in practice are crop rotation, use of disease free seed tubers, removing volunteer potatoes and other susceptible plants, uprooting and burning diseased plants. However these measures have not completely solved the problem of the occurrence of bacterial wilt. The bacterium infects more than 200 plant species especially in tropical and subtropical areas (Mwangi, 2003). It is this high number of hosts that makes crop rotation unsuccessful in managing the disease. The problems associated with this disease calls for urgent action in search of a solution. Plants have been known to contain active principles

against a wide range of plant and animal pathogens and have been traditionally used to manage diseases. It is therefore necessary that plants known to contain such principles are evaluated for a possibility of managing bacterial wilt in potatoes.

1.2 PROBLEM STATEMENT

Bacterial wilt caused by *Ralstonia solanacearum* has continued to be a big menace in most potato producing parts of Kenya and worldwide where it causes drastic poor potato yields. At an international level bacterial wilt is responsible for losses amounting to hundreds of millions of dollars. Farmers from some parts of world abandoned potato growing long ago because of the losses resulting from the severity of the disease (Roger and Knoxfield, 1995).

In Kenya, potato is mainly grown in central parts where the population is very high and this has exerted pressure on the land resources. Continuous cultivation with little or no application of fertilizer has led to a decline in soil fertility (Kihanda, 1996) and in addition no crop rotation is practiced due to high land fragmentation. These have contributed to increased BW incidence and severity (Berga *et al.*, 2001a). Low soil fertility and bacterial wilt are among the most important constraints limiting potato crop production (Muriithi 2000; Muriithi and Irungu, 2004).

Surveys conducted in all major potato producing areas of Nakuru, Bomet, Nyandarua and Nyeri districts have shown that bacterial wilt contributes significantly to the reduction in the crop yield (Otipa *et al.*, 2003). Worse still is the fact that no chemical in

the market is available to eradicate the disease and the methods currently used to reduce the spread of the disease like crop rotation and uprooting of infected plants among others do not solve the problem fully. In addition the use of poor quality seeds has led to contamination of most potato fields by the pathogen causing a general increase in bacterial wilt incidence. This causes low yields and reduced shelf life in stored tubers (Ajanga, 1993). The disease therefore poses a major threat to food security in Kenya and other parts of the world.

1.3 JUSTIFICATION

Irish potato is one of the most important sources of income and employment in the rural areas (Olanya *et al.*, 2006). The annual potato acreage in Kenya is about 100,000 hectares which is distributed among approximately 500,000 small holder farmers. These farmers own, on average two hectares of land and thus increased productivity is the only viable option to enhance production (Ogola *et al.*, 2002), given that production increase through land expansion is limited. To achieve the productivity, diseases like bacterial wilt should be controlled. The control measures against *Ralstonia solanacearum*, which includes crop rotation, planting in disease free lands and the use of disease free seed tubers, have proved not to be fully effective in controlling the bacterial wilt of potato. This is partly due to their impracticability for reasons such as poverty among community members, land fragmentation and ignorance of the farmers (French, 1996).

In line with the EUREPGAP protocol whose guidelines aims at producing products that are safe, environmental friendly, socially acceptable and which are of high quality, it is

therefore imperative that an alternative control method to synthetic chemicals must be sought. Locally available plant resources with medicinal properties to control plant diseases and boost soil nutrition and by far being not hazardous environmentally make them good products as far as successful biocontrol agents are concerned. Previous studies have shown that *Ocimum gratissimum, Brassica oleracea* var. *botrytis* and *Ipomoea batatas* have antimicrobial properties against variety of bacteria and fungi. This research is therefore intended to document the effects of plant extracts from these species on the growth and disease-causing ability of *Ralstonia solanacearum*. This will provide potential sources of widely sought solution to the losses incurred in potato production due to bacterial wilt infection.

1.4 OBJECTIVES

1.4.1 Main objective

The general objective of this research is to investigate the effect of extracts from *Brassica oleracea* var. *botrytis, Ipomoea batatas* and *Ocimum gratissimum* on the growth and disease-causing capability of *Ralstonia solanacearum*.

1.4.2 Specific objectives

1. To determine the effect of plant extracts from *Ocimum gratissimum, Ipomoea batatas* and *Brassica oleracea* var. *botrytis* and their concentration on the *in vitro* growth and development of *Ralstonia solanacearum* colonies.

2. To establish whether extracts from *Ocimum gratissimum, Ipomoea batatas* and *Brassica oleracea* var. *botrytis* suppress the development of bacterial wilt in potatoes under green house conditions.
3. To evaluate the effect of the extracts of *Brassica oleracea* var. *botrytis, Ipomoea batatas* and *Ocimum gratissimum* on potato growth parameters.

1.5 HYPOTHESES

1. Extracts from *Ipomoea batatas*, *Ocimum gratissimum* and *Brassica oleracea* var. *botrytis* does not inhibit the *in vitro* growth and development of *Ralstonia solanacearum* colonies.
2. Extracts from *Ipomoea batatas, Brassica oleracea* var. *botrytis* and *Ocimum gratissimum* does not lower the development of bacterial wilt symptoms under green house conditions.
3. Addition of *Ocimum gratissimum*, *Ipomoea batatas* and *Brassica oleracea* var. *botrytis* extracts have no effects on the growth of *Solanum tuberosum.*

CHAPTER TWO: LITERATURE REVIEW

2.1 BACTERIAL WILT OCCURRENCE

This is one of the most destructive diseases known to attack plants. The disease has a very wide host range. On potato, the disease is also known as brown rot, southern wilt, sore eye or Jammy eye and is a major limiting factor to potato crop production in most countries. Other hosts include economically important crops such as tobacco, pepper, banana, beans, tomato and egg plant (Mwangi, 2003).

Gunawan *et al.* (1987; 1999) reported an extensive number of hosts from more than 50 families of weeds that were affected by the bacterium. Thorn apple and nightshade are two common weed hosts that are attacked by the disease (Roger and Knoxfield, 1995). The bacterial wilt occurs even in unexpected areas. For instance the importance of indigenous vegetation has been clearly established when potato wilted in a field considered to have virgin soil, that was never previously planted with a solanaceous crops (Jackson and Gonzalez 1979; Martin, 1979).

The spread of the causative agent has been mostly favoured by dissemination in latently infected planting materials. The disease is the second most important constraint to potato production in tropical and subtropical regions of the world. Breeding programmes have not been successful in developing varieties with stable resistance to the disease (Ateka *et al.*, 1999). Research on resistance to the disease started decades ago. One of them was carried out to identify resistance of 51 different cultivars of potato in 1978 and 1979 in Georgia. They were evaluated for reaction to infection by *R. solanacearum* under high

disease pressure and it was found out that most of them were highly susceptible to BW (Jaworski *et al.*, 1980)

Bacterial wilt has spread to most potato producing countries. Its occurrence in Australia and in the Southeastern United States has resulted in concentration of bacterial wilt research in these areas. This is the case in Latin America where the disease has spread to all potato producing countries except Ecuador. The disease is also common in Asia, particularly in the midhills of the Himalayas in India, Nepal, Bhutan and Pakistan (Priou *et al.*, 1999).

In East and Southern Asia, this bacterial disease is common in Indonesia, the Philippines, Southern Vietnam, Laos, Japan and Southern China. In the early 1990s bacterial wilt became a serious threat to potato production in European countries including Belgium, England, France, Spain, Italy and Portugal. It has also been reported in Russia. Other areas where the disease is also a constraint in potato production are the Central, Eastern and Southern Africa. It has been reported in countries such as Uganda, Ethiopia, Kenya, Madagascar, Rwanda, Burundi, Nigeria and Cameroon (Priou *et al.*, 1999).

In Kenya, it was first reported in 1940s and since then it has spread to most potato growing areas. A study carried out to assess the extent of bacterial wilt in the vicinity of the National Potato Research Center (NPRC), Tigoni, showed very high incidence of the disease. The average infection rated above 50% while in some farms the infection was 70% (KARI, 1994).

A survey of bacterial wilt incidence by Ateka *et al.*(2001) was conducted in three potato producing Districts in Kenya namely Nyeri, Nyandarua and Meru. The survey was carried out in 30 randomly selected farms in each district. The incidence of bacterial wilt was highest (18.8%) in Nyeri district, intermediate (16.7%) in Meru and lowest (10.4%) in Nyandarua.

A study carried out by Otipa *et al.* (1998) in all major potato producing areas of Nakuru, Bomet, Nyandarua, Meru and Nyeri districts showed that bacterial wilt continues to negatively affect the crop production and shelf life of harvested tubers. An average incidence of the disease ranged between 30 – 70% (Mwangi, 2003).

Nitrogen and phosphorus are the soil major nutrients limiting crop production (Kihanda, 1996; Rao *et al.*, 1998) and their levels influence disease development. Reduced nitrogen can increase the susceptibility of potato to bacterial wilt and its severity and effective control of BW can be achieved through increased N-inputs (Prior *et al.*, 1993; Berga *et al.*, 2001b).

2.2 CAUSATIVE AGENT

Ralstonia solanacearum (Smith) Yabuuchi which is the causative agent of BW was first reported in Kenya by Natrass and is not clear whether the bacterium is indigenous or is an introduced exotic (Mwangi, 2003). It is a gram-negative rod measuring 0.5 – 0.7 by 1.5 – 2.5 µm, motile with a single polar flagellum. The bacterium is also aerobic, oxidase and catalase positive. It accumulates poly-beta-hydroxyl butyrate (PHB). Its colonies are non-fluorescent on complex media and most strains of this species reduces nitrate to nitrite.

2.3 CLASSIFICATION OF RALSTONIA SOLANACEARUM

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Betaproteobacteria
Order	Burkholderiales
Family	Ralstoniaceae
Genus	*Ralstonia*
Species	*R. solanacearum*

Ralstonia solanacearum is a very complex bacterial species that is pathogenic to several plant species. The species is divided into biovars. Buddenhagen *et al.* (1962) distinguished three races on the basis of pathogenicity.

(a) Race 1: Affects tobacco, potatoes, tomatoes, diploid bananas and other Solanaceae crops and weeds; with high optimum growth temperature of 35 – 37 °C. It is known as Solanaceae race.

(b) Race 2: Affects the triploid bananas and *Heliconia* spp with high optimum temperature of 35 – 37°C. It is called Musa race.

(c) Race 3: Also called potato race grows well at lower temperature of 27°C (Gunawan and Smith, 1987).

The *Solanacearum* species were divided into five different biovars based on how the bacterial isolate utilize and/or oxidize three hexose alcohols and three disaccharides.

Table 1: Classification of *R. solanacearum* into biovars

Biovar	Utilization of disaccharides	Oxidation of alcohols
1	-	+
2	+	-
3	+	+
4	-	+
5	+	+*

Key

+ = Positive, - = Negative, +*= Positive for manittol only (Source: Momol *et al.*, 2000).

The race and biovar classification do not correspond, except that race 3 is generally equivalent to biovar 2. Race 3 (biovar 2) is adapted to cooler temperatures and is the one that causes brown rot of potatoes (Hayward, 1991; Hayward, 1964).

2.4 HOST RANGE

Ralstonia solanacearum as a species has an extremely wide host range but different pathogenic races within the species may show more restricted host ranges. Over 200 crop species, especially tropical and subtropical, are susceptible to one or more races of *R. solanacearum*. Worldwide, the most important hosts are *Lycopersicon esculentum* (tomato), *Solanum tuberosum* (potato), *Musa* spp. (banana), *Solanum melongena, Arbergine* spp. and *Heliconia* spp. Other host crops include *Anthurium* spp., *Arachis hypogea, Capsicum annuum, Gossypium* spp., *Ricinus communis* and *Zingiber officinales* (ginger). Many weeds are also hosts of the pathogen and therefore increase the potential

of the pathogen to build up inoculums. The development of bacterial wilt associated with weed species was recorded in more than 50 weeds that grow well in potato field in Pangalengan, Lembang and Garut in Indonesia (Gunawan *et al.*, 1999). Wild hosts include *Solanum nigrum, Urtica dioica* and *Portulaca oleracea.*

2.5 SYMPTOMS OF BACTERIAL WILT

The symptoms of this disease in an infected potato plant are depicted both in the above ground parts and in the tuber. The above ground symptoms of bacterial wilt include wilting, stunting and sometimes yellowing of the foliage. When the cortex is peeled the browning of vascular bundles is seen clearly.

The symptoms can also first occur as initial wilting of only part of the stems of a plant or even one side of a leaf or stem. Sometimes the disease develops and the entire plant wilts quickly without yellowing (Plate 1).

Plate1: A wilted potato plant among non-wilted ones (Source: Priou *et al.*, 1999).

Temperatures between 25°C and 37°C and wetness of soil generally favour the rapid development of the disease (Mwangi, 2003).

External symptoms of the disease on the tubers are seen at harvest when infection is severe and bacterial ooze collects at tubers eye or on the end of stolon, causing soil to adhere to the secretions. Cut tuber shows brownish discoloration of the vascular ring (Plate 2) and gentle squeezing forces pus like slime out of the ring or it may exude naturally.

Plate 2: BW infected tubers showing colored eyes and ring (Source: Priou *et al.*, 1999).

At advanced stage of the disease development, the whole tuber may disintegrate completely releasing very bad smell (Priou *et al.*, 1999).

2.6 DISEASE CYCLE IN POTATO

Inoculums may be from infected potatoes, which include seed tubers, harvest leftovers and infected plants or infested soil or both. The pathogens can survive in soil and in the

rooting system and rhizosphere of many hosts such as weeds, other host crops and potato volunteers. The pathogen is mainly spread by movement of infected seed tubers. The spread can also be accomplished through contaminated surface water used for irrigation and that used in cultivation (Priou *et al.*, 1999).

The bacteria enter the potato roots or tubers through facilitation by wounds made by tools during post emergence cultivation and by nematodes, and insects in the soil (Priou *et al.*, 1999).

2.7 CONTROL OF BACTERIAL WILT OF POTATO

Bacterial wilt is a major problem to farmers of potatoes worldwide particularly because its management is difficult (Ateka *et al.*, 1999). The only control method is to use clean seeds and fields that have not yet become contaminated by the disease. This is because the disease stays in the soil for a very long time infecting any susceptible crops planted. Integrated management approaches are usually advocated to reduce the disease spread but these approaches are based on appropriate combinations of control measures that suit specific circumstances. The measures, which have individual practical, technological and economic limitations, include planting disease free tubers, planting in non infested soils, crop rotation, field sanitation, soil fumigation and use of resistant varieties (Priou *et al.*, 1999; Ateka *et al.*, 1999).

2.7.1 Planting disease free tubers

Though the use of disease free tubers is viewed as the most important of all control measures, the availability of such materials to most farmers has remained a challenge in Kenya. Infected seed tubers are the main means of dissemination in many areas. In cool conditions such as tropical elevations above 2500m, infected but symptomless plants may

harbour the bacterial and transmit to the progeny tubers as latent infection leading to severe disease outbreaks when grown at warmer locations. For seed production, only bacteria free seed tubers originating from disease free areas must be used (Priou *et al.*, 1999; Ateka *et al.*, 1999). Selection of potato seed in the storage before planting improves the quality of seeds and the use of Diffuse Light Storage (DLS) results in strong seeds and vigorous sprouts (Gunawan *et al.*, 1999).

2.7.2 Crop rotation

This is important for maintaining soil fertility and preventing the build up of soil borne diseases. One planting of potatoes per plot in every four seasons is the recommended rotation practice (Durr and Lorenzi, 1980). After a bacterial wilt infected crop, potato and other susceptible hosts must not be planted for at least two years as this will prevent proliferation of the inoculum (Priou *et al.*, 1999). Rotation with cereals or gramineous pastures can be implemented to eliminate soil inoculums. This in most cases is not practical because of the intensive fragmentation of land due to increase in human population. For this reason, farmers tend to continuously plant potatoes on the same plot. A study carried out (French, 1996) reported that rotation with maize and beans achieved good control of bacterial wilt of potato in Honduras. It was also reported by Elphinsone and Aley (1992) that rotation to control bacterial wilt of potato has been in practice in many countries. In Rwanda, one crop rotation with any of the five principal crops (maize, sorghum, climbing bean and soybeans) grown there greatly reduced wilt incidence (Gunawan *et al.*, 1999).

Various experiments were conducted by Berga and others between 1995-1999 at Kachwekana Agricultural Research and Development Centre (Uganda) which involved one and two season rotation to control bacterial wilt. Pulses, cereals, root crops and vegetables commonly grown in the area were included in different sets of rotation experiments. Each time prior to planting, the field was planted with a bacterial wilt susceptible potato variety and artificial inoculation was also done to achieve uniform distribution of *Ralstonia solanacearum*. Though the rotations had positive effects, finger millet and sweet potatoes was found to reduce the wilt more effectively (below 13% wilt incidence) (Berga *et al.*, 2001a).

2.7.3 Field sanitation

Crop sanitation and cultivation measures are aimed at avoiding and limiting the survival of pathogen and its dissemination. These measures are commonly used to manage other potato diseases and pests and include: removal of infected potato haulms, weeding, rouging volunteer potato plants, rouging wilted potato plants, removal of rotted tubers among others (Priou *et al.*, 1999).

Removal of potato haulms is whereby after harvest of a bacterial wilt infested crop, potato haulms, must be removed from the field and buried deep, down slope and far from irrigation canals. Alternatively the potato haulms can be burned (Priou *et al.*, 1999). This would ensure that bacteria within the haulms cannot reach crop plants growing on the same plot. Removal of rotted tubers from the field is also a measure advocated in control of bacterial wilt spread. The rotted tubers should be buried deep or burned. Another recommended measure is to weed before planting potatoes since weeds and other crops harbours the pathogen. Volunteer potato plants also enhance the survival of *R.*

solanacearum and thus must be removed in the subsequent crop soon after their emergence (Priou *et al.*, 1999). If the incidence of bacterial wilt in a field is low the wilted potato must be removed as soon as they are observed to avoid contamination of healthy neighbouring plants. Tools used in farming should be decontaminated to prevent movement of soil from an infested to a disease free field. The decontamination process can be accomplished by washing with water and calcium hypochloride or other available bactericide or sterilized by flame. Farmers should ensure that no water flows from an infested field to adjacent fields. In infested areas, irrigation by well water is preferred over surface water from rivers or irrigation canals (Ateka *et al.*, 1999).

Nematodes are known to open paths for the bacterium to enter the plant through root injuries. Thus it is important that they be controlled to avoid their interaction with *R. solanacearum*. Major nematode control components are soil fumigation, rotation with cereals, and application of high quantities of organic amendments. These are some of the measures that are effective means of ensuring that bacterial wilt do not spread to non-infested areas. The production of seed potatoes should be restricted to regions without bacterial wilt incidences (Priou *et al.*, 1999).

2.7.4 Resistant varieties.

Although many potato varieties have been found to have some degree of resistance to bacterial wilt, they still transmit latent infection to their progeny tubers. It is therefore advisable that use of moderately resistant varieties must be coupled with a program that provides bacterial wilt free seed tubers. Most small-scale farmers are ignorant of such varieties and tend to plant any seed tuber available known to yield well or accessible in

the market. Breeding programs have been initiated in many parts of the world but acceptable varieties with good resistance to bacterial wilt are yet to be identified (Tung *et al.*, 1990). Cruza 148 of East Africa and Achat in Brazil are two varieties with some degree of resistance to the disease (Priou *et al.*, 1999).

2.7.5 Soil fumigation

Soil fumigation is the application of chemicals into the soil with intention of killing weed seeds, plant pathogens, nematodes, and insects in the soil. Fumigants are generally applied as liquid formulations after which true fumigants volatilize and form gases. They include 1, 3- dichloropropene, 1, 3- dichloropropene + chloropicrin, methyl isothiocyanate compounds, methyl bromide and methyl bromide + chloropicrin among others. It is an expensive exercise and mostly carried out only in developed countries The combination of crop rotation and soil fumigation is a sound approach used by farmers in controlling bacterial wilt (Ateka *et al.*, 1999)..

2.8 USE OF IPOMOEA BATATAS, OCIMUM AND BRASSICA SPECIES IN DISEASE MANAGEMENT.

Green plants have been shown to represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin *et al.*, 1985; Hostettman and Wolfender, 1997). Reports are also available on the use of active agents from higher plants in place of chemical fungicides that are non phytotoxic, more systemic and easily biodegradable (Fawcett and Spencer, 1970).

Momol and Pradhanang (1998) carried out preliminary *in vitro* and greenhouse experiment using essential oils of thyme, palmarosa, and lemon grass. The results showed that they had significant efficacy against *R. solanacearum* and against several soil borne fungi of tomato.

2.8.1 Ocimum gratissimum

Ocimum gratissimum (Plate 3) is one of the wild species of the genus *Ocimum* in the family Labiaceae. It is a herbaceous plant commonly found in tropical areas.

Plate 3: *Ocimum gratissimum*

The important relatives with similar properties include *O. basilicum* (sweet basil), *O. canum* (hairy basil), and *O. sanctum* (holy basil). It's commonly known as basil and its leaves and those of relatives are used as medicine. The seeds are also used medicinally in India and Southeast Asia (Farnsworth and Bunyapraphatsara, 1992).

Basil contains a strong scented volatile oil composed primarily of terpenoids. It also has what are known as chemotypes. The exact components of basil oil vary widely, being affected not only by the chemotypes but also by factors such as the time of the day of harvest. This may account for some of the variability in scientific research and reports of medicinal efficacy of basil from culture to culture. Its dried leaves are known to contain 0.20-1% essential oil and also some compounds such as safrole, rutin, caffeic acid and tryptophan. Its essential oils, seeds, leaves, flowers and roots are used as medicines (Farnsworth and Bunyapraphatsara, 1992).

The essential oil has been shown *in vitro* to have antibacterial activity against *Staphylococcus aureus, Salmonella enteritidis* and *Escherichia coli* (Mbata and Saikia, 2005). It has also been shown to have antiseptic activity against *Proteus vulgaris, Bacillus subtilis* and *Salmonella paratyph*. In addition, it has been depicted to have antifungal activities against *Candida albicans, Penicillium notatum* and *Microsporeum gypseum* (Nguyen, 1993).

2.8.2 Brassica oleracea

Brassica oleracea var. *botrytis* (Plate 4) originated in the Northeast Mediterranean. It is a cultivar group within *Brassica oleracea* in the family Brassicaceae. It is an annual plant

that reproduces by seed and typically only the head (the white curd) is eaten while the stalk and surrounding thick green leaves are discarded. Cauliflower and broccoli are the same species and have very similar structure, though cauliflower replaces the green flower buds with white inflorescence meristem (Kirkegaard, 2005).

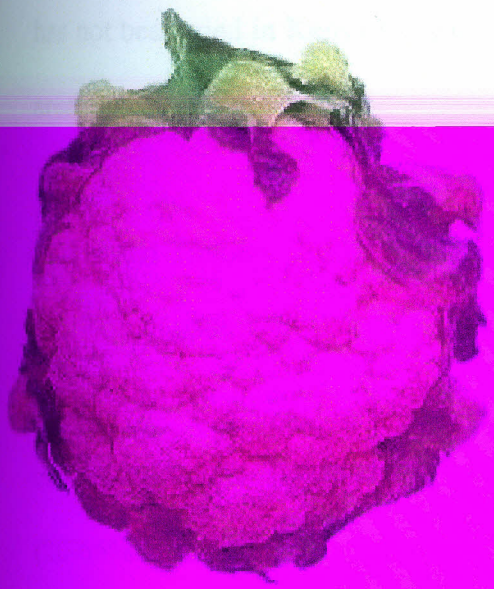

Plate 4: The white curd of cauliflower (Source: Kirkegaard, 2005)

An Australian Center for International Agricultural Research has recently discovered a method they call biofumigation (Kirkegaard, 2005). This is the process of incorporating residues of certain varieties of brassicaceous crops which has been shown to reduce the levels of several soil borne pathogens including bacterial wilt pathogen and root knot nematode. This practice has been shown to reduce incidence of bacterial wilt and involve naturally occurring chemicals being released from *Brassica* crops. The chemicals are released when cell wall of fresh plants are broken down. Chopping plants finely is the best possible option. In Philippines, farmers use a rotary hoe to chop leaves and mix them into the soil (Kirkegaard, 2005).

In one of the experiments which was conducted at Mayjayjay in 1998, tissues of cabbage, broccoli, cauliflower and radish reduced wilt incidence at six weeks in tomatoes (from 21% to 2.8-8.4%). In the same experiment, root knot nematode numbers were reduced by all brassicas and sweet potatoes (57 - 87% reduction) (Kirkegaard, 2005). This method has not been tried in Kenya to deal with the problem of bacterial wilt in potato producing areas (Nganga, 2006).

Larkin and Timothy (2003), evaluated the effect of various brassica crops in controlling soil borne pathogens of potato in culture, greenhouse tests and in field trials on commercial potato farms. They used *Brassica* crops like yellow mustard, rape, turnip and canola. All the *Brassica* crops they used reduced inoculums levels of *Rhizoctonia solani* (20-65% reduction). Radish, rape and white mustard decreased subsequent potato seedling disease by 30-85% in greenhouse tests. In on farm trials at sites with substantial disease problems, white mustard, rape and canola grown as a green manure rotation crop reduced powdery scab by 15-40% and black scurf by 50-85% and white mustard decreased common scab by 25% in the subsequent potato crop relative to a standard oats rotation crop. They concluded that brassica crops have potential for use in the control of powdery scab and other soil borne disease problems.

Onkar and others (2006) evaluated the effects of essential oils extracted from B*rassica rapa* seeds on suppression of *Rhizoctonia solani* growth *in vitro* and in field soils. The *in vitro* growth was completely inhibited at a concentration of 50µ/l. In the field test, snap beans were used as indicator plants and in 24h after treatment, saprophytic substrate

colonization was drastically reduced to 45% at 150 µl/kg soil concentration. The study was extended to evaluate effects of soil amendments, which showed that amending soil with plants of *Brassica* species can reduce the disease incidence and inoculums level of *Macrophomina phaseolina* (Tassi) Goid, *Pythium ultimum* Trow and *Fusarium oxysporium* Schl (Kimber and McGregor, 1995).

Further, Larkin and Timothy (2007) evaluated the effect of *Brassica* green manure on a variety of soil borne pathogens. They tested the effect of rape seed, turnip, Indian mustard and white mustard. All of them inhibited the growth of *Rhizoctonia solani, Phytophthora erythroseptica, Pythium ultimum, Sclerotinia sclerotiorum* and *Fusarium sambucinum*. Indian mustard resulted in nearly complete inhibition (80-100%). Seed treatment with *Brassica* species seed meal to control *Rhizoctonia solani* in cabbage has been reported (Chung *et al.*, 2002). The mechanism involved in the disease control or suppression of a pathogen is considered to be the production of allyl isothiocyanate by the tissues of *Brassica* spp. (Olivier *et al.*, 1999).

2.8.3 Ipomoea batatas

Ipomoea batatas (Plate 5) is a tuberous-rooted perennial usually grown as an annual, top herbaceous, stems forming a running vine usually prostrate and slender with milky juice. It is cultivated mainly for the tuber which is used as a vegetable. The leafy tops are also eaten as vegetables mainly in Malaysia and other parts of the world and are greatly esteemed as feed for farm animals in most parts of the world. Sweet potato is native to the tropical parts of the Americas and today they are cultivated throughout tropical and warm temperate regions wherever there is sufficient water to support their growth.

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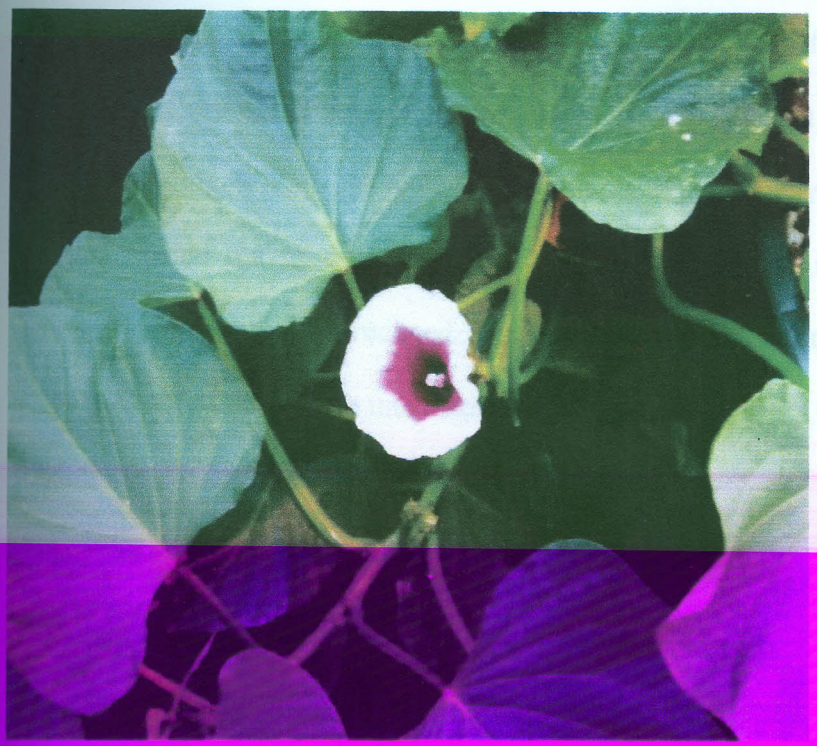


Plate 5: Sweet potato plant (Source: Ng'ang'a, 2006)

The tuberous edible roots are good for diabetics as studies on animals have revealed that it helps to stabilize blood sugar levels and to lower insulin resistance. Various ethno botanical uses have been reported and include: the aerial parts are used as galactogogue, the leaves are used to treat diabetes, hookworm, hemorrhage and abscesses, and the tuber is used to treat asthma. Traditionally the plant has been used as remedy for some ailments; the leaf decoction is used as remedy for tumors of the mouth and throat (Graham *et al.*, 2000). According to Duke and Wain (1981) the plant is used in remedy for asthma, burns, diarrhea, stomach distress, nausea and fever.

together with *Brassica* crops and tobacco. Many were shown to reduce the incidence of the disease significantly. Kirkegaard and others (2005) also indicated that fresh chopped leaves of sweet potato significantly reduced the populations of bacterial wilt pathogen in sealed laboratory containers. They evaluated *Brassica* crops whereby mustard caused more rapid suppression due to its high content of 2-propenyl isothiocyanate (ITC). A research carried out to evaluate the effect of crop rotation in reducing bacterial wilt showed that sweet potato reduced wilt more in comparison to other crops like maize, onions and carrots that were under test (Berga *et al.*, 2001a)

CHAPTER THREE: MATERIALS AND METHODS

3.1 PLANT MATERIALS

The leaves of *Ocimum gratissimum* and vines of *Ipomoea batatas* variety SPK 004 were collected from Maseno region. Maseno is in Western Kenya on latitude 0°1'N - 0°12'S and longitude 34°25'E - 34°47'S. The area is about 1500m above sea level and it receives an annual rainfall of 1750mm with a bimodal pattern of distribution. The mean temperature is 28.7 °C. The existing soils that are classified as acrisols are deep reddish brown, friable clay with the pH ranging between 4.5-5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5 mg/kg respectively (Netondo, 1999). Leaves of *Brassica oleracea* var.*botrytis* from Nyeri region. Nyeri district is one of the districts in Central province and forms parts of Kenya Eastern highlands. It covers an area of 3266$Km^2$and is situated between longitude 36° and 38 °East and between the equator and latitudes 0 °38' South. The region lies between 1800-2200M above sea level and the temperatures ranges between 10°C and 30°C and receives rainfall of between 500-1500mm per annum depending on the altitude. Geology of most sections consists of volcanic rock which gives rise to rich red volcanic soils that are rich in organic matter (Ministry of Energy, 2010). The authenticity of the three plants was confirmed at the East African Herbarium.

3.2 LABORATORY EXPERIMENT

3.2.1 Preparation of plant extracts

The plucked leaves of the plants were washed thoroughly 2 to 3 times with running tap water and once with sterile distilled water. They were cut into small pieces and dried under shade until they were completely dry (Okigbo and Ogbonnaya, 2006). They were then ground into fine powder at Kenya Sugar Research Foundation in Kisumu in readiness for solvent extraction. Cold extraction of the powdered plant materials was done sequentially with organic solvents of varying polarities – Ethyl acetate and Methanol. The percentage yield of the extracts was calculated by dividing the weight of the extract by the weight of raw material and multiplying by one hundred (Table 2).

3.2.1.1 Extraction of *Ocimum gratissimum*

Extraction was done by soaking two hundred grams of the milled plant leaf materials in one thousand milliliters ethyl acetate solvent contained in five thousand milliliters Erlenmeyer flasks. The mixture was then allowed to stand for four days in the corked Erlenmeyer flask with occasional shaking. It was then decanted and the liquid component was poured in a beaker. This was then filtered using Whatman No. 1 filter paper with the help of a suction pump (Model 7049-05, Cole Parmer Instrument Company, Chicago) and concentrated using a rotary evaporator (BUCHI model) at 70°C. The ethyl acetate extract obtained was put in a vial bottle ready for bioassay studies.

Seven hundred and fifty milliliters of methanol was added to the leaf material in the Erlenmeyer flask and left for four days with occasional shaking. The liquid portion was

then filtered using Whatman no.1 filter paper. The filtrate was then concentrated *in vacuo* in a round-bottomed flask using rotary evaporator at 60°C. The extract was then weighed and kept in a vial bottle at 4°C ready for bioassay studies (David, 1989; Junaid, *et al.*, 2006).

Two kilograms of fresh leaves of *Ocimum gratissimum* were subjected to steam distillation using Soxhlet apparatus to generate essential oils. The oil obtained was then stored at 0°C until needed (Aureli, 1992).

3.2.1.2 Extraction of *Ipomoea batatas*

Three hundred grams of the powdered leaf material of *Ipomoea batatas* was weighed and poured in a five thousand mililitres flat bottomed Erlenmeyer flask. One thousand and two hundred mililitres of ethyl acetate solvent was added and the flask was placed in an orbital shaker for three hours. The shaker was set at 100 revolutions per minute. The mixture was then left to stand for 24 hours. The liquid part was then poured in a beaker and filtered. The filtrate was then concentrated using a rotary evaporator at 70 °C and the extract obtained was weighed and kept in vial bottles.

One thousand and two hundred mililitres of methanol was added to the plant residues in the flask. The mixture was again placed on an orbital shaker for three hours and then left to stand for 24 hours after which the liquid component was poured in a beaker and filtered. The filtrate was then concentrated using a rotary evaporator at 60 °C. The extract obtained was weighed and kept in vial bottles ready for bioassay tests (David, 1989).

3.2.1.3 Extraction of Brassica oleracea var.botrytis

Three hundred grams of the milled plant leaf material of *Brassica oleracea* var.*botrytis* was weighed and added into a five thousand milliliters flat-bottomed Erlenmeyer flask. One thousand and one hundred milliliters of ethyl acetate solvent was added in the same flask and the mixture was left for four days with shaking being done twice daily. The mixture was decanted after which the liquid portion was filtered using Whatman no. 1 filter paper and a suction pump. The filtrate was then concentrated using rotary evaporator at 70 °C. The extract was then weighed and kept in vial bottles.

One thousand and five hundred milliliters of methanol was added to the plant residues and the mixture was left for four days with shaking being done twice daily. It was then left to decant after which the liquid component was poured in a beaker and then filtered. The filtrate was then concentrated *in vacuo* and the extract obtained was weighed and kept in vial bottles in readiness for bioassay tests (David, 1989; Llorach, *et al.*, 2003).

3.2.2 Bacterial cultures

Tubers infected with bacterial wilt were obtained from a test plot at the National Agricultural Research Laboratories (NARL) fields, Nairobi. The tubers were cleaned under running water to remove adhering soil, and then air-dried. They were then cleaned using 97% ethanol to remove any microorganism on its surface. The skin at the end of the stolon was removed using a clean and a disinfected scalpel to make vascular tissues visible. The cut tuber was left for about five minutes and one mililitre of bacterial ooze from the vascular bundles was picked using a sterilized toothpick and put in a test tube. One milliliter of sterile distilled water was added in test tube and shaken well for the bacteria to diffuse uniformly (Priou *et al.*, 1999).

The bacterial suspension was serially (1:1) diluted 10 times and 0.5 ml of the final dilute was spread on nutrient agar in Petri dishes. The plates were then incubated for 48 hours at 28°C. Fluidal, pearly white, flat and irregular colonies were expected to form.

Pathogenicity test was done to confirm the identity of the bacteria where Koch's postulates were performed with *Solanum tuberosum* var. *Tigoni* 381381 as the host. After a 24 hour period without water, one side of some potato roots were injured one centimetre from the stem and approximately twenty mililitres of an aqueous suspension of *Ralstonia solanacearum* of 1×10^7 cfu/ml was poured around the base of the stem. Wilting of the potato plants began to occur five days after inoculation. After the symptoms were exhibited, vascular flow test was run by cutting a piece of potato stem (5cm long) and suspending it in clear water in a glass container. The cut stem was held with a clip to keep it in a vertical position and within few minutes smoke like threads streamed downwards from the cut stem (Priou *et al.*, 1999).

3.2.3 Antibacterial assay

Glass Petri dishes, test tubes, glass rods, cotton swabs, paper discs, forceps were wrapped in a foil paper and together with the medium they were sterilized by autoclaving at 121°C for 15 minutes and kept in a refrigerator until required. The susceptibility of *R. solanacearum* to the plant extracts was done using the method described (Barry, *et al.*, 1979; Souza, *et al.*, 2005). Zero point four milligrams of each solid extract and the essential oils were weighed and dissolved in one milliliter of Dimethyl sulphoxide (DMSO) to make a concentration of 0.4mg/ml. These were serially diluted to give test solutions of concentrations 0.2mg/ml, 0.1mg/ml, 0.05mg/ml and 0.025mg/ml.

Nutrient agar medium was prepared by suspending 28 grams of powder in one liter of distilled water followed by boiling and autoclaving. The sterile agar medium (15 ml) was dispensed in 90 mm diameter Petri dishes and left to solidify. Inoculation was done by rubbing a sterile cotton swab containing the pathogen on the surface of solidified agar (Linnette *et al.*, 1974). Paper discs (5mm diameter) were prepared by soaking in the test solutions (in DMSO) followed by drying. The extract-impregnated discs were picked by sterilized forceps and placed on the surface of an inoculated solidified Nutrient agar.

The plates were incubated at 28°C for 48 hours and the presence of zones of inhibition around the discs was interpreted as an indication of antibacterial activity. Plates set with discs dipped in pure DMSO without plant extracts served as controls. All tests were done in quad replicates and the plates laid down in randomized complete block design for statistical purposes. The antibacterial activity was recorded as the width (in mm) of clear zones of inhibition surrounding the diffusion discs after 48 hours as described by (Reiner, 1982; Baker *et al.*, 1983; Deans and Ritchie, 1987).

3.3 GREENHOUSE EXPERIMENT

3.3.1 Extraction of plant compounds

125g, 250g and 500g of the milled plant materials were weighed and put in separate containers. 5000ml of sterile distilled water was added to each of the materials to prepare test solutions of 10%, 5% and 2.5%. This was left for three days for any water-soluble active component to dissolve with stirring done twice a day to enhance the interaction of water and the plant materials. The macerate was first filtered through a muslin cloth and

the extract was preserved aseptically in bottles in a freezer at 0°C until use (Gupta *et al.*, 1996).

3.3.2 Experimental site and treatments

The experiment was set up in the greenhouse at Maseno University Botanic Garden. The minimum and maximum temperatures inside the greenhouse were 27±4 °C and 34±4 °C respectively with a relative humidity of 37±6%.

The soil which was used as the rooting medium was collected from Maseno University Botanic Garden fields and sterilized by solarisation for fourteen days (Heald and Stapleton, 1990). The soil was then mixed with sterilized quartz sand (3:1) prior to potting to improve aeration and infiltration and then fertilizer (N: P: K-20:20:20) diluted at 0.5% in water was applied. The soil was potted in 20cm diameter pots which were placed on saucers to ensure extracts and water are retained and that water does not flow from one pot to another. A single, medium sized clean potato tuber of variety Tigoni CIP 381381.13 obtained from National Potato Research Centre, Tigoni was planted in each pot and watering was done daily to support the potato growth. The variety was chosen for its high susceptibility to bacterial wilt (Ateka *et al.*, 2001). The potatoes were ready for inoculation ten days after planting (Plate 6).

Plate 6: Potato plants at the age of inoculation and addition of extracts

Using a sterilized scalpel, a cube measuring one centimeter by one centimeter of nutrient agar in Petri dishes containing colonies of bacteria was scooped and dipped in 500 ml of distilled water to make a concentration of approximately 1×10^{7}cfu/ml. In the rhizosphere of each plant some soil was scooped to expose some of the roots. Using a disinfected knife the roots were injured on one side and 10 ml of the bacterial suspension was added along that zone in each pot using a hypodermic needle and a 10 ml-syringe. The soil was then placed back to cover the roots as before (Bashan, 1986).

The experimental units consisted of 12 treatments (three plant extracts and four concentrations). The experimental layout was randomized complete block design with three replications. Application of the treatment began one hour after the inoculation

(Plate 7) and involved addition of 250ml of each extract type. The application of the treatment were repeated on weekly basis for three times, that is 17th day, 24th day, and 31st day after sowing.

Plate 7: Addition of plant extracts to the rooting medium of potato plant

The treatments were as follows:

$T_{1(10)}$ Pots sprayed with 10% *Brassica oleracea* var.*botrytis* extracts.

$T_{1(5)}$ Pots sprayed with 5% *Brassica oleracea* var.*botrytis* extracts.

$T_{1(2.5)}$ Pots sprayed with 2.5% *Brassica oleracea* var.*botrytis* extracts.

$T_{1(0)}$ Pots sprayed with tap water (control plants)

$T_{2(10)}$ Pots sprayed with 10% *Ocimum gratissimum* extracts.

$T_{2(5)}$ Pots sprayed with 5% *Ocimum gratissimum* extracts.

$T_{2(2.5)}$ Pots sprayed with 2.5% *Ocimum gratissimum* extracts.

$T_{2(0)}$ Pots sprayed with tap water (control plants).

$T_{3(10)}$ Pots sprayed with 10% *Ipomoea batatas* extracts.

$T_{3(5)}$ Pots sprayed with 5% *Ipomoea batatas* extracts.

$T_{3(2.5)}$ Pots sprayed with 2.5% *Ipomoea batatas* extracts.

$T_{3(0)}$ Pots sprayed with tap water (control plants).

The recording of wilt incidences began five days after the inoculation day and was carried out twice a week on the following scale: 1 = no symptoms, 2 = wilting of up to 50% of the leaves, 3 = wilting of up to 75% of the leaves, 4 = wilting of up to 99% of the leaves, 5 = complete wilting of the plant (Hayward, 1991). Abnormal symptoms on the potato plants were observed and recorded throughout the experiment.

3.3.3 Plant height measurement

The height of the plants was considered as the distance from the base of the stem to the growing shoot apex. The initial plants heights of potatoes were taken one hour before the application of treatments began. The final height of any plant that completely wilted before the end of the experiment was measured in centimetres immediately using a meter rule. Four days after the application of the last treatment, the height of the remaining plants' were measured using a meter rule and all were recorded in terms of centimeters (Momol *et al.*, 2000). The increase in height was calculated by subtracting initial height measurement from final height measurement.

3.3.4 Plant Biomass Determination

Determination of the fresh and dry weights of the potato plants involved destructive measurements. On the 35th day after planting, the potato plants were carefully uprooted after loosening the soil and rinsed using tap water. The root masses that were embedded in the soil were carefully removed by soaking the rooting media in water and sieving all the root segments. They were dried using tissue paper and then separated into shoot and root segments. The fresh weight of each plant roots and shoots were measured in grams using an electronic balance. To determine the plant dry weight, the samples were oven-dried at 70°C for forty eight hours to obtain a constant weight (Luvaha, 2006). An electronic weighing balance (Denver Instruments Model-31000) was used to obtain weight measures.

3.4 DATA PRESENTATION AND ANALYSIS

The data obtained was presented using bar graphs, line graphs and tables. Data on antimicrobial activity of the different plant extracts, disease development indices and potato parameters in the laboratory and greenhouse were subjected to ANOVA using SAS version 9.1 (SAS Institute, 2005) and effects declared significant at 5% level. Separation of means was done only for those parameters where the ANOVA was significant, using Least Significant Difference at 5% level of significance [LSD5%]. Linear correlation was done where necessary to compare the relationship between variables (Steel and Torrie, 1980).

CHAPTER FOUR: RESULTS

4.1 *RALSTONIA SOLANACEARUM* DERIVED FROM INFECTED POTATO TUBER

The serially diluted bacterial suspension was spread on nutrient agar in Petri dishes which were then incubated for 48 hours at 28°C. Fluidal, pearly white, flat and irregular shaped colonies were the morphological characteristics depicted by the colonies formed.

Pathogenicity test was carried out using Koch's postulates whereby the wilt bacteria inoculums was inoculated into *Solanum tuberosum* var. *Tigoni* 381381. Wilting of the potato plants began to occur five days after the inoculation. After the symptoms were exhibited, vascular flow test was run by cutting a 5cm long piece of potato stem and suspending it in clear water in a glass container. The cut stem was held with a clip to keep it in a vertical position and within few minutes smoke like threads streamed downwards from the cut stem turning the initially clear water into a milky suspension.

4.2 PLANT EXTRACTS YIELD

The powdered plants' leaf materials of *Ipomoea batatas, Ocimum gratissimum* and *Brassica oleracea* var. *botrytis* were extracted sequentially using organic solvents of varying polarities (ethyl acetate and methanol) and the yields were weighed and recorded (Table 2). Steam distillation of two kilograms of fresh leaves of *Ocimum gratissimum* yielded 16 ml of greenish yellow essential oils.

Table 2: Weight of raw material and yield of plant extracts.

Plant	Raw material (g)	Solvent used	Yield of plant extract (g)
Ocimum gratissimum	200	EtOAc	10.93
		MeOH	08.7
Ipomoea batatas	300	EtOAc	10.0
		MeOH	32.4
Brassica oleracea	300	EtOAc	12.6
		MeOH	47.3

Key: EtOAc = Ethyl acetate

MeOH = Methanol

4.3 ANTIBACTERIAL ACTIVITY OF DIFFERENT PLANT EXTRACTS

Extracts from leaves of *Ocimum gratissimum, Ipomoea batatas* and B*rassica oleracea* var. *botrytis* (ethyl acetate and methanol) and essential oils of *Ocimum gratissimum* were subjected to antibacterial bioassays (see methodology). Measurement (mm) of diameters of zones of inhibitions was taken. The plant extracts exhibited varying activity against growth of *Ralstonia solanacearum* at different concentrations.

4.3.1 Effect of essential oils of O*cimum gratissimum* on *R. solanacearum*

The inhibitory activity of essential oils against the wilt bacteria are shown in figure 2. Different concentrations (0.4, 0.2, 0.1, 0.05 and 0.025 mg/ml) of the oils dissolved in DMSO were put under test. The best results were observed with the concentration of

0.4mg/ml giving an inhibition zone mean measurement of 10.12mm (Plate 8) and the lowest was 2.75mm as a result of treatment with 0.025mg/ml (Figure 2). There was a high significant difference ($p<0.0001$) among the treatments with the different concentrations of essential oils (Appendix 8A).

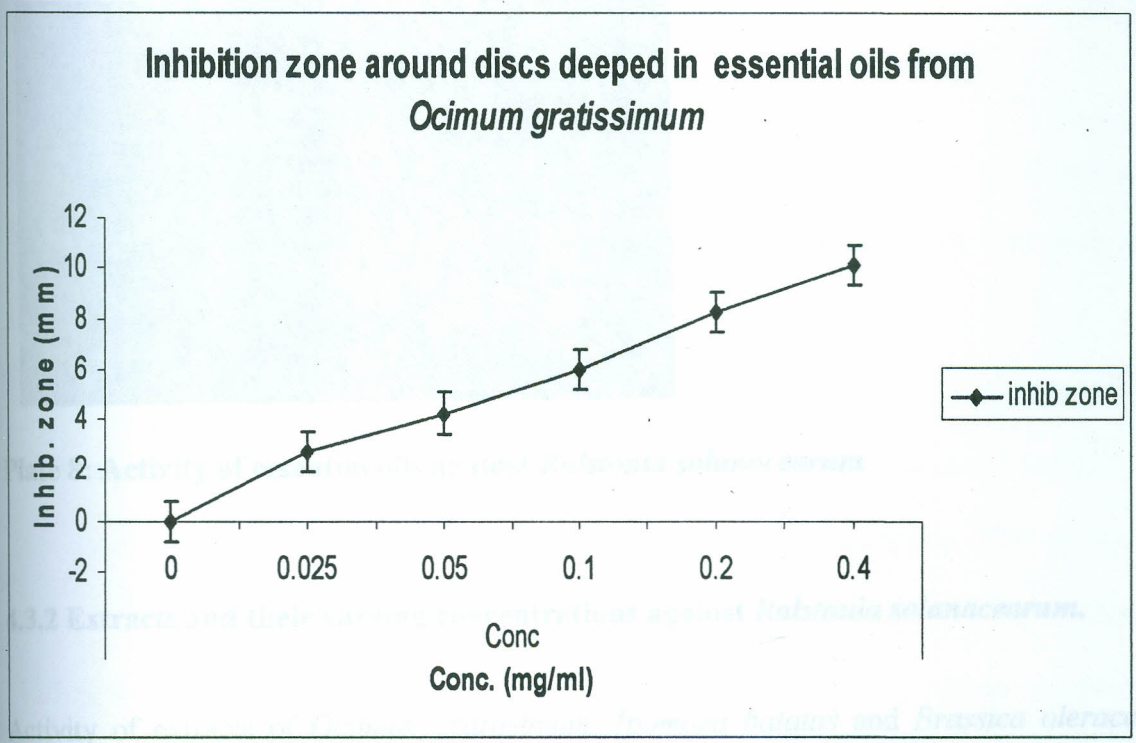


Figure 2: Activity of essential oils against *Ralstonia solanacearum*. Error bars represent standard error (0.3486) of means calculated from four individuals per replicate

Plate 8: Activity of essential oils against *Ralstonia solanacearum*

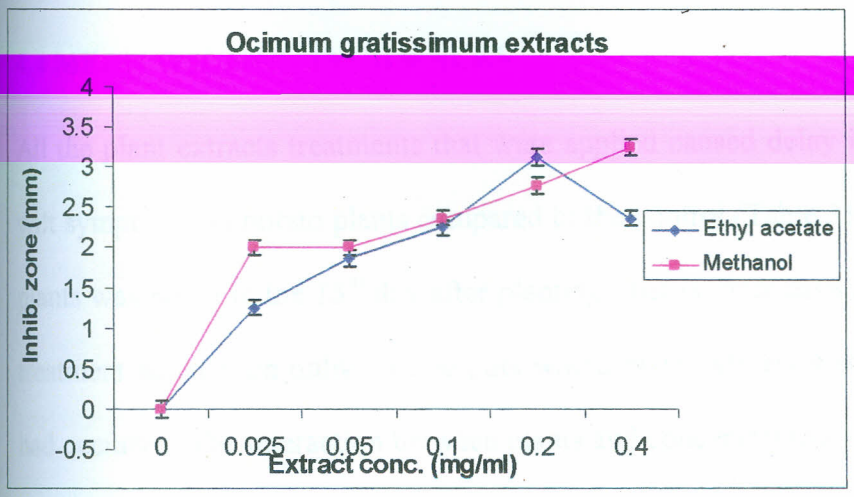
4.3.2 Extracts and their varying concentrations against *Ralstonia solanacearum.*

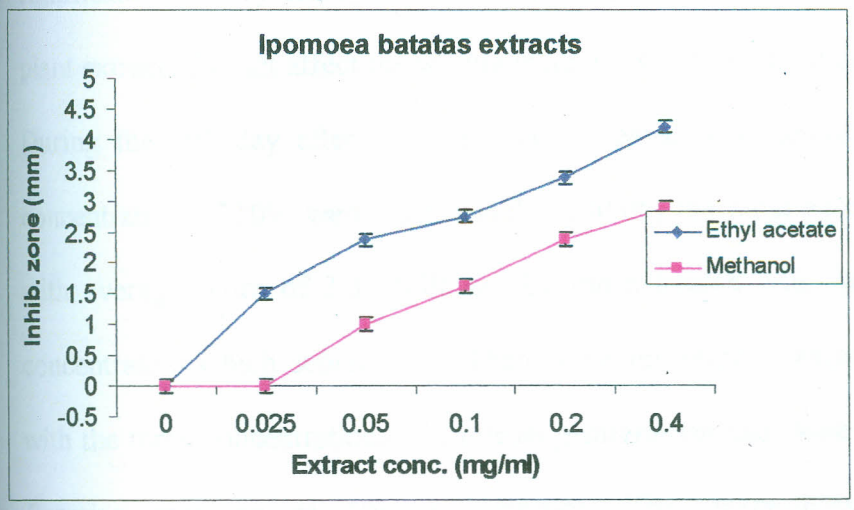
Activity of extracts of *Ocimum grattisimum, Ipomoea batatas* and *Brassica oleracea* using ethyl acetate and methanol solvents are shown in figure 3. The best results were observed with ethyl acetate extracts of *Ipomoea batatas* at concentration of 0.4mg/ml giving mean inhibition zone of 4.2mm followed by ethyl acetate extract of *Brassica oleracea* at concentration of 0.05mg/ml that was 4.12mm. Methanol extract of *Ipomoea batatas* at concentration of 0.025mg/ml were inactive against *Ralstonia solanacearum*.

The methanol extracts of *Ocimum gratissimum* at concentration of 0.025 and 0.05mg/ml exhibited similar activity where inhibition zone measured 2mm but the activity then increased as the concentration increased. Activity of ethyl acetate *Ocimum* extracts against wilt bacteria increased as concentration increased up to 0.2mg/ml and then declined when concentration was increased to 0.4mg/ml. At concentration 0.1mg/ml both the methanol and ethyl acetate extracts of *Ocimum* were not significantly ($P>0.05$) different in their inhibitory activity against the wilt bacteria (Figure 3).

The activity of both methanol and ethyl acetate extracts of *Ipomoea batatas* against wilt bacteria increased as their concentration increased. However best activity was observed with ethyl acetate extracts compared to methanol extracts (Figure 3)

Inhibitory activity of methanol extracts of *Brassica oleracea* increased as concentration increased but was lower than those of ethyl acetate up to 0.2mg/ml. The optimum concentrations of ethyl acetate extracts of *Brassica oleracea* were shown to be 0.025 and 0.05mg/ml but reduced with increasing concentrations (Figure 3). The interactions among the three plants extracts, their concentrations and the different solvents used were highly significant ($p<0.0001$) (Appendix 8B). This means that the different plant extracts derived using different solvents affected the size of inhibition zone around bacterial colonies at different concentrations.





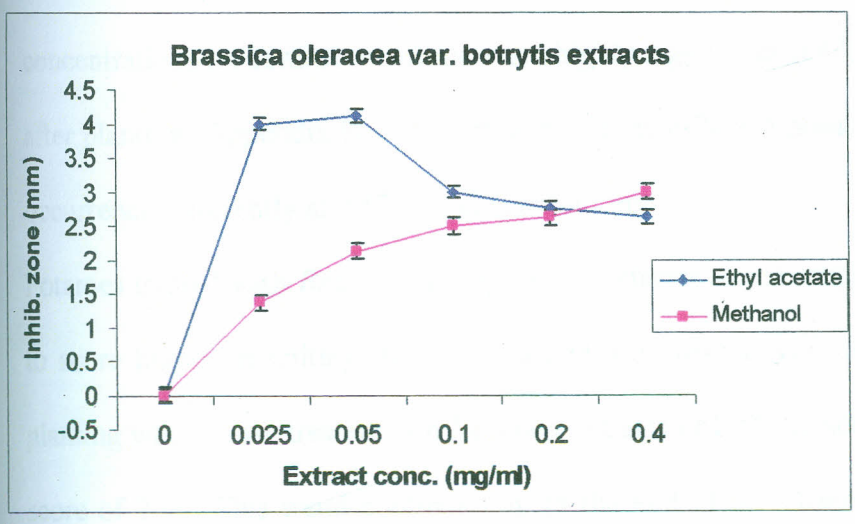


Figure 3: Zones of inhibition around wilt bacteria colonies as a result of different treatments. Error bars represent standard error (0.1161) of group means calculated from four individuals per replicate

4.4 MEAN WILTING INCIDENCES

All the plant extracts treatments that were applied caused delay in the onset of bacterial wilt symptoms on potato plants compared to the control (Table 3). Wilting of the control plants was noted in the 15th day after planting, that is, four days after commencement of treatment application unlike in the pots where plant extracts were added and no wilting had occurred. The interaction between plants and concentrations effect on wilting was not significant ($p<0.05$) during this 15th day (Appendix 8C). This means that the different plant extracts did not affect the wilting incidences differently at different concentrations.

During the 19th day after planting, plants treated with *Brassica oleracea* extracts at concentration of 10% were significantly ($p<0.05$) the most susceptible to bacterial wilt with average score of 3.33 followed by those treated with *Ipomoea batatas* of 10% concentration which scored 2.67. There were no wilting symptoms in potatoes treated with the three concentrations of *Ocimum gratissimum* and those of *Ipomoea batatas* and *Brassica oleracea* of 5% concentration. The interactions of plants and their concentrations on control of wilting were highly significant ($p<0.001$) during the 19th day after planting (Appendix 8C). This means that the different plant extracts affected wilting occurrence differently at different concentrations.

Potatoes treated with *Brassica* and *Ipomoea* extracts at concentrations of 10% continued to score highly in wilting, that is 4.00 and 3.00 respectively during the 23rd day after planting while those treated with *Ipomoea* extracts of 2.5% concentration followed with a score of 2.00. This trend continued up to the end of the observation period and potato plants treated with *Brassica* at 5% and *Ocimum* at 10% showed to be highly effective in managing the wilting disease with only a score of 1.33 and *Ipomoea* and *Ocimum* extracts

of 5% concentration with a score of 1.67 meaning that only less than 50% of wilting had occurred. Potatoes treated with *Brassica* extracts of 10% concentration and the controls had completely wilted by the end of observation period (Table 3). The interactions of plants extracts and their concentrations on wilting from the 23rd day to the 35th day after planting were highly significant [($p<0.0001$) (Appendix 8C)]. This means that the plant extracts affected the occurrence of wilting symptoms differently at different concentrations.

Table 3: Mean wilting indices

Treatment	15 DAP	19 DAP	23 DAP	27DAP	31DAP)	35 DAP
Brassica oleracea 10%	1.00b	3.33ab	4.00b	4.00b	4.00b	5.00a
Brassica oleracea 5%	1.00b	1.00c	1.00f	1.00d	1.00e	1.33d
Brassica oleracea 2.5%	1.00b	1.33c	1.33e	1.33d	2.00d	2.33c
Ipomoea batatas 10%	1.00b	2.67b	3.00c	3.00c	3.00c	3.33b
Ipomoea batatas 5%	1.00b	1.00c	1.00f	1.00d	1.67d	1.67cd
Ipomoea batatas 2.5%	1.00b	1.67c	2.00d	2.67c	3.33c	3.33b
Ocimum gratissimum10%	1.00b	1.00c	1.00f	1.00d	1.00e	1.33d
Ocimum gratissimum 5%	1.00b	1.00c	1.00f	1.00d	1.00e	1.67cd
Ocimum gratissimum 2.5%	1.00b	1.00c	1.00f	1.33d	2.00d	2.00cd
Control	1.67a	3.67a	5.00a	5.00a	5.00a	5.00a
LSD(5%)	0.3132	0.7308	0.3132	0.5323	0.4304	0.8545
CV%	17.12	24.11	8.98	14.55	10.46	18.45

Means followed by the same letter in the same column are not significantly different.

Key: DAP = Days after planting

The disease indices were:

1 = no symptoms

2 = wilting of up to 50% of leaves

3 = wilting of up to 75% of leaves

4 = wilting of up to 99% of leaves

5 = complete wilting of the plant

An abnormal feature was noted on leaf tips of potato plants treated with *Brassica* extract of 10% concentration whereby partial drying had occurred (Plate 9).

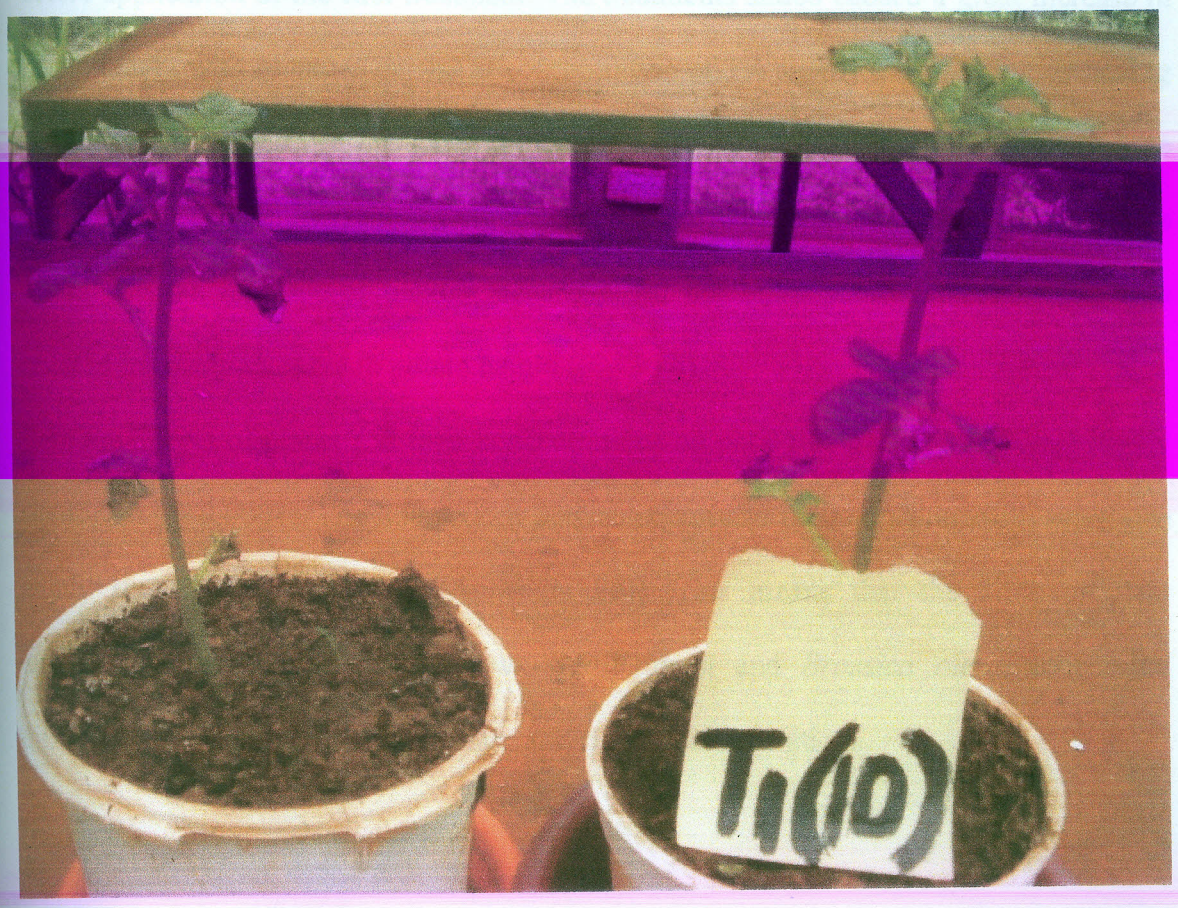


Plate 9: Potato plants treated with *Brassica oleracea* extracts of 10 % concentration depicting dry leaf tips from 13th day.

4.5 PLANT EXTRACTS ON POTATO PLANTS' PARAMETERS

The various treatments used in the study had varying effects on the different potato parameters as shown in figures 4, 5 and 7.

4.5.1 Increase in potato plant height

This was calculated as the difference between the final potato height and their height just before application of the first treatment. The treatments added caused a great increase in potato height with *Ipomoea* extract at concentration of 5% significantly ($p<0.001$) having the highest effect resulting to 64.6cm increase and *Brassica* extracts at 10% having the lowest with 21.33cm increase but significantly high ($p<0.05$) compared to control plants where only an increase of 6.33cm occurred. All the other plants concentrations also resulted into high increase in potato height (Figure 4).

Both the effects of plants and their concentrations on potato height increase were highly significant ($p<0.0001$). The interactions between plant and their concentrations were also highly significant [($p<0.0001$) (Appendix 8d)]. This means that the different plant extracts of *Ipomoea batatas, Ocimum gratissimum* and *Brassica oleracea* affected differently the increase in potato height at different concentrations.

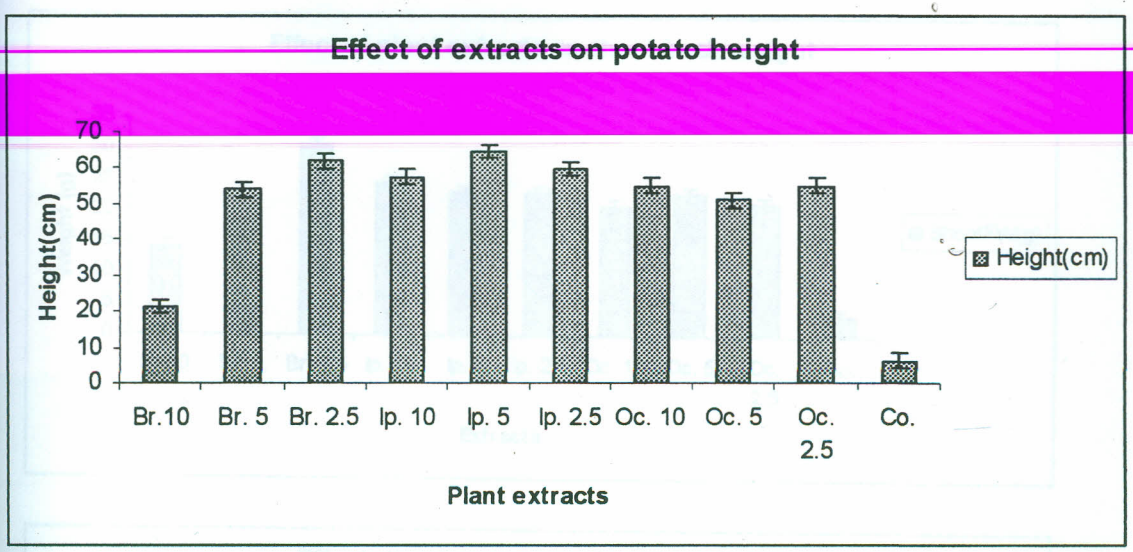
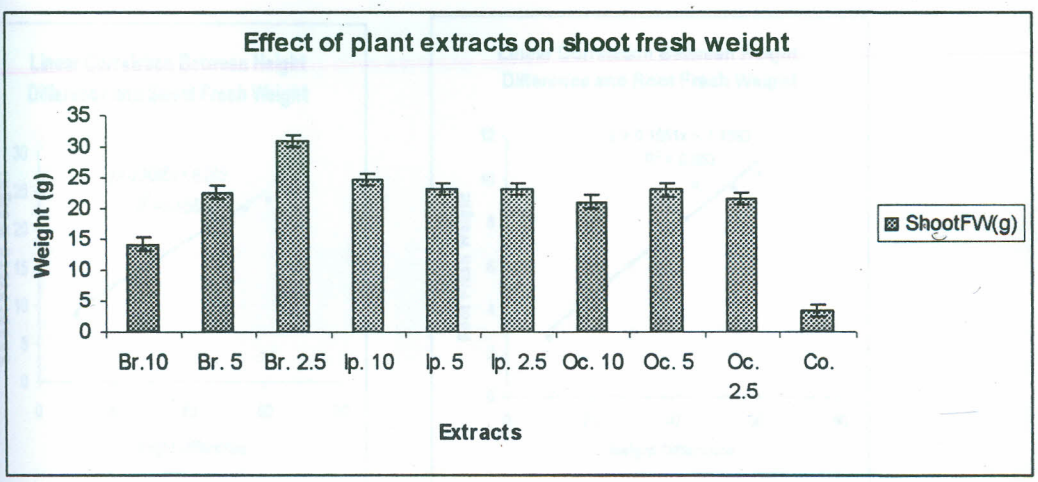


Figure 4: Increase in height of potato plants treated with varying plant extracts to control bacterial wilt. Error bars represent standard error of means calculated from one individual per replicate. Br = Brassica, Ip = Ipomoea, Oc. = Ocimum, Co = Control.

4.5.2 Effect of extracts on potato fresh weight

The 2.5% *Brassica* extract significantly ($p<0.001$) increased the fresh weight of both the shoots and roots of potato plants that weighed 30.97g and 14.43g respectively. All the other treatments except 10% *Brassica* extract portrayed appreciable effect on both the shoot and the root fresh weight that ranged between 21.57g – 24.60g and 8.5g – 10.83g respectively. *Brassica* extracts at 10% had the lowest effect on potato fresh weight with shoots measuring 14.20g and roots 4.67g but significantly high compared to control which only caused an increase of 3.37g for shoot and 1.67g for root (Figure 5). The interactions between plant and the concentrations were highly significant [($p<0.0001$) (Appendix 8d)] on both the root and shoot fresh weights. This means that the different plant extracts affected the fresh weight of potato plants differently at different concentrations.



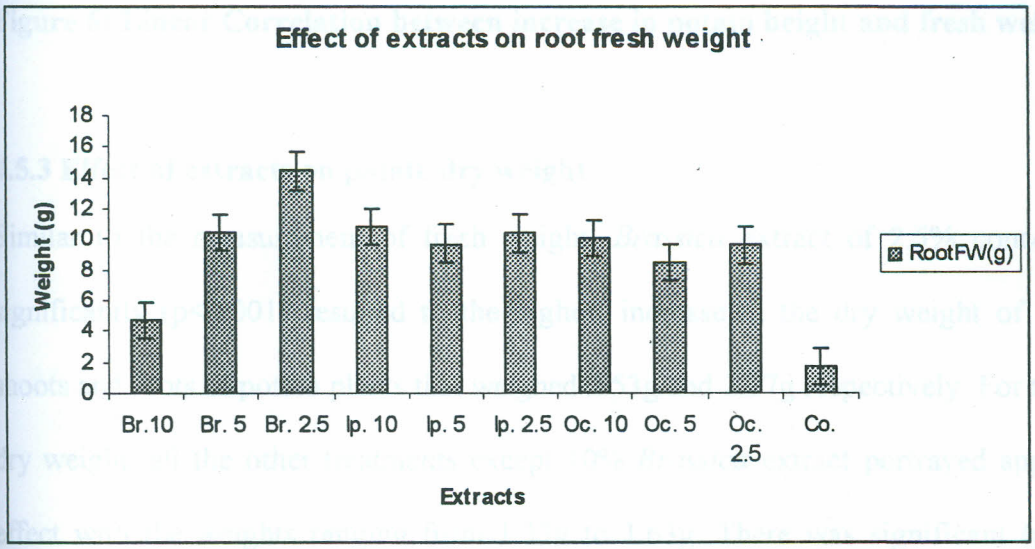


Figure 5: Potato plants fresh weight after treatment with extracts from different plants to control bacterial wilt. Error bars represent standard error of means calculated from one individual per replicate. Br = Brassica, Ip = Ipomoea, Oc. = Ocimum, Co = Control.

There was a highly significant ($p<0.001$) and strong ($R^2=0.958$, $R^2=0.953$) positive correlation between the potato increase in height and shoot and root fresh weights respectively as shown in figure 6. This means that as the height of potatoes increased the fresh weight of both the shoot and roots increased.

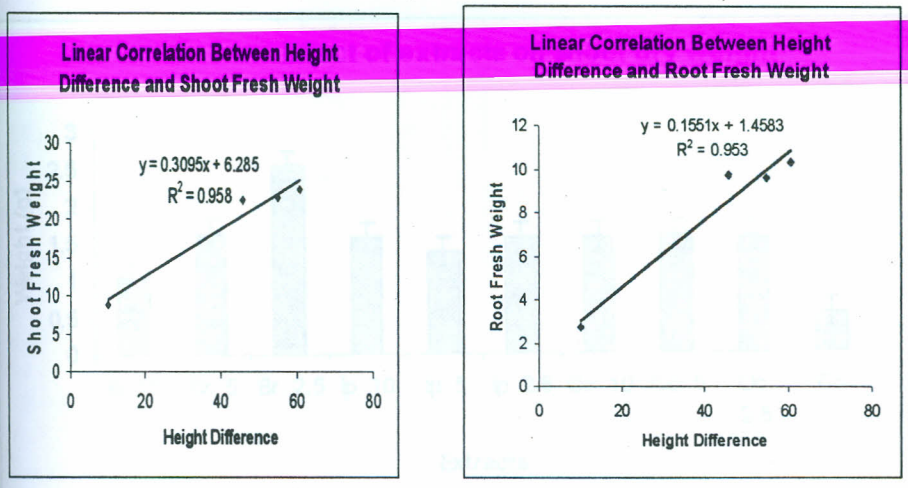
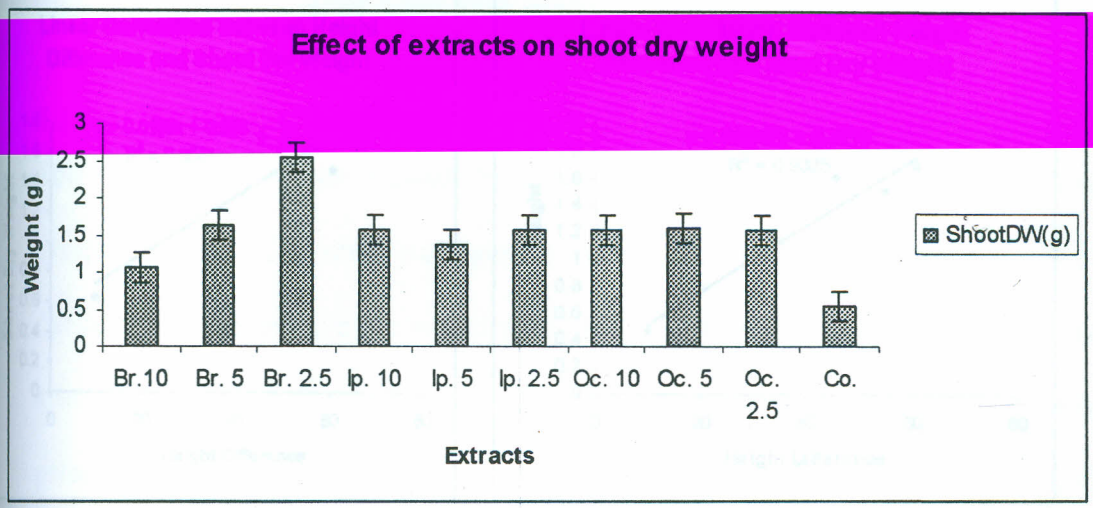


Figure 6: Linear Correlation between increase in potato height and fresh weight.

4.5.3 Effect of extracts on potato dry weight

Similar to the measurement of fresh weight, *Brassica* extract of 2.5% concentration significantly ($p<0.001$) resulted to the highest increase in the dry weight of both the shoots and roots of potato plants that weighed 2.53g and 2.27g respectively. For the shoot dry weight, all the other treatments except 10% *Brassica* extract portrayed appreciable effect with the weights ranging from 1.37g to 1.63g. There was significant ($p<0.001$) variation in the effects of other concentrations on the root dry weight (Figure 7). The interactions of plants and their concentrations were significant [($p<0.001$) (Appendix 8d)] on both the root and shoot dry weights. This means that the effects of different plant extracts on dry weight of potato plants were different at varying concentrations.



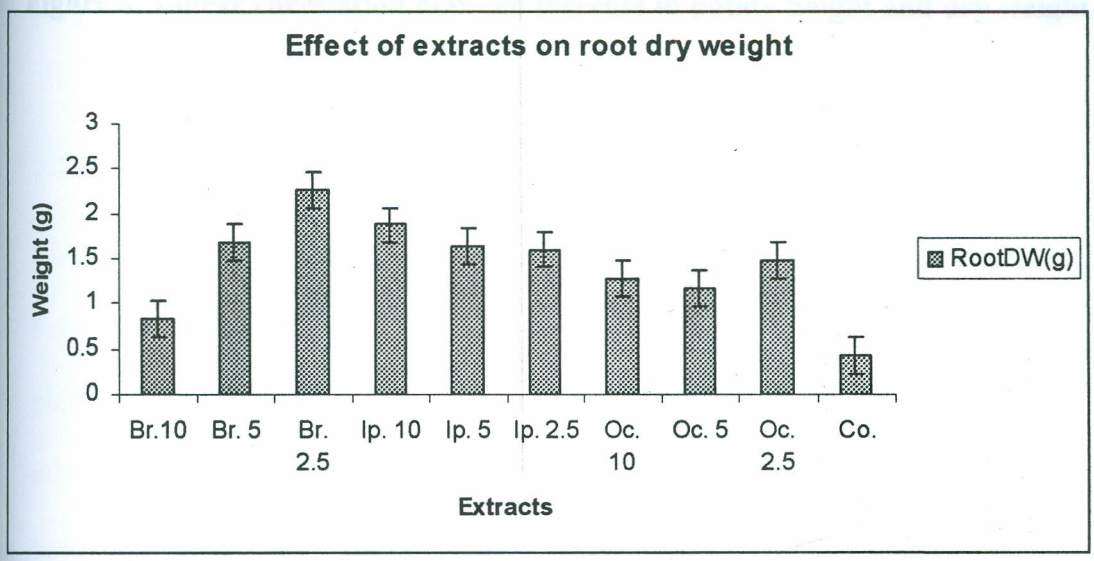
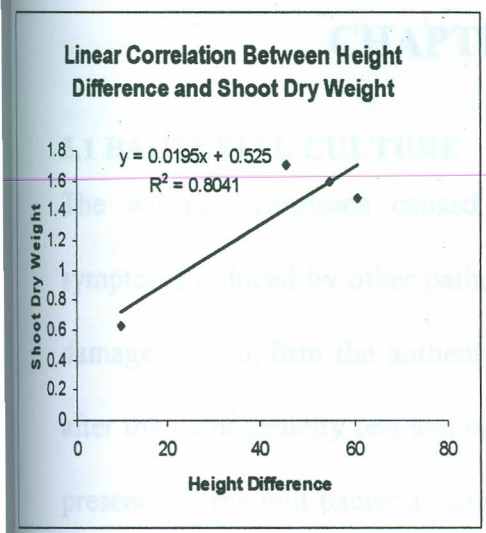


Figure 7: Potato plants dry weight after treatment with extracts from different plants to control bacterial wilt. Error bars represent standard error of means calculated from one individual per replicate. Br = Brassica, Ip = Ipomoea, Oc. = Ocimum, Co = Control.

There was a highly significant ($p<0.001$) and strong ($R^2=0.804$, $R^2=0.932$) positive correlation between the potato increase in height and shoot and root dry weights respectively as shown in figure 8. This means that as the height of potatoes increased the dry weight of both their shoots and roots increased.



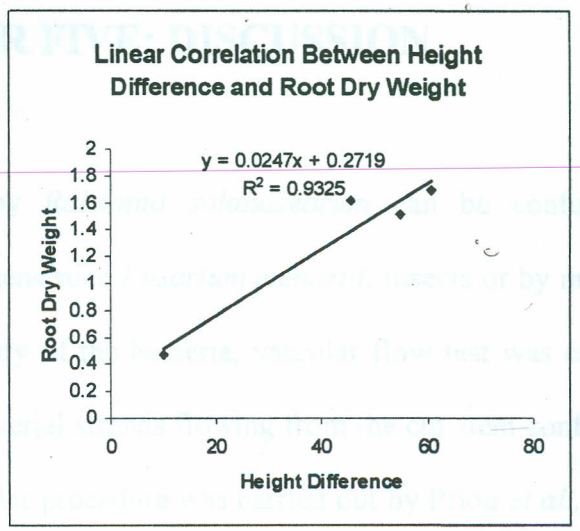


Figure 8: Linear correlation between increase in potato height and dry weight.

CHAPTER FIVE: DISCUSSION

5.1 BACTERIAL CULTURE

The wilting symptoms caused by *Ralstonia solanacearum* can be confused with symptoms induced by other pathogens such *Fusarium eumartii*, insects or by mechanical damage. To confirm the authenticity of the bacteria, vascular flow test was carried out after the pathogenicity test and bacterial strands flowing from the cut stem confirmed the presence of the wilt bacterial. Similar procedure was carried out by Priou *et al.*, 1999 to confirm the identity of *Ralstonia solanacearum*.

5.2 EFFECT OF ESSENTIAL OILS ON BACTERIAL GROWTH

The results from this study showed that essential oils from *Ocimum gratissimum* were very effective in combating the *in vitro* growth of bacterium colonies compared to the plant extracts obtained using organic solvents. A study carried out to analyze essential oils of *Ocimum gratissimum* by Gas Chromatography (Masada, 1976) showed that they contain diverse compounds such as thymol, eugenol and d-limoneme among others. Another study carried out (Matasyoh, *et al.*, 2007) using leaves of *Ocimum gratissimum* from Meru region showed that the essential oils contain compounds like eugenol, methyl eugenol, cis-ocimene and trans-ocimene. When tested against both gram positive and gram negative bacteria and also *Candida albicans*, the essential oils had pronounced antibacterial and antifungal activities on all the microbes used. The compounds identified could be responsible for the inhibitory activity of the oils against *R. solanacearum*. The study has also demonstrated that essential oils were significantly effective at higher concentrations with the highest used (0.4mg/ml) in the study showing the highest

inhibitory effect. Similar trend was shown in a study (Mbata and Saikia, 2005) while evaluating the antibacterial activity of the *Ocimum* oils against *Listeria monocytogenes.*

5.3 EFFECT OF PLANT EXTRACTS ON *IN VITRO* GROWTH OF BACTERIA

This study has demonstrated that compounds extracted from the three plants using the two organic solvents vary in their efficiency in inhibiting bacterial growth. The two solvents have different polar abilities with methanol having higher polarity and thus they tend to dissolve different compounds from the plant materials dipped in them. Polar solvents dissolve polar compounds best and non polar solvents dissolve non polar compounds best (Siddhuraju *et al.*, 2003). Similar to the activity of most drugs, their effectiveness were best at different concentrations.

5.3.1 *Ocimum gratissimum* extracts

The results showed that the effectiveness of ethyl acetate extracts of this plant were gradually increasing as their concentration increased. However this trend changed with concentration above 0.2mg/ml where the activity decreased with increase in concentration. Though there was the gradual increase in the inhibitory activity, it remained less effective compared to the methanol extracts except for the 0.2mg/ml. The significantly higher activity of methanol extracts compared to ethyl acetate extracts could be an indication that methanol extract contained more antibacterial principles. This could probably mean that the anti microbial principles of *Ocimum* against wilt bacteria are polar compounds. The findings are similar to the findings of a study (Junaid, *et al.*, 2006) while examining the methanol and hexane extracts of *Ocimum gratissimum* against

Escherichia coli, found higher inhibition in methanol extracts and concluded that the active components of the plant could be highly polar.

5.3.2 *Ipomoea batatas* extracts

The significantly high activity of ethyl acetate extract for all concentrations used compared to methanol extract could be an indication that ethyl acetate extract contained more anti microbial principles against *R. solanacearum* than methanol extracts. This could probably mean that the active compounds of this plant against the bacteria are less polar compounds. Shahidul (2006) reported that sweet potato leaves contains large amounts of polyphenolics such as anthocyanins and phenolics acids. When ethanol and water were used in their extractions, higher polyphenols concentration was found with ethanolic extracts. The compounds extracted using the solvent may be similar to those released when the vines were used in a biofumigation study (Kirkegaard, 2005) to control bacterial wilt where the effectiveness of the vines in controlling bacterial wilt increased with increase in the amount used.

5.3.3 *Brassica oleracea* var. *botrytis* extracts

The significantly high activity of ethyl acetate extract at lower concentrations of 0.025, 0.05 and 0.1mg/ml compared to methanol extract was an indication that anti microbial principles of ethyl acetate extract were highly effective against *R. solanacearum* in lower concentrations. However the activity decreased with increase in concentrations for ethyl acetate extract. This could mean that the less polar active compounds of this plant interact best with the bacteria at low concentrations. In addition the activity of methanol extract increased gradually with increase its concentration surpassing the effectiveness of ethyl

acetate. This could mean that the polar compounds were more effective at higher concentrations. Walters and Kirkegaard (2009) reported the release of isothiocyanates (ITCs) from *Brassica* species like cauliflower that suppressed the bacterial wilt of potatoes when used as green manure and that the effectiveness increased as materials incorporated increased. Larkin and Griffins (2007) also reported the release of surfur compounds from brassica species that suppresses soil borne potato diseases when chopped leaves are incorporated in potato fields.

5.4 EFFECT OF AQUEOUS EXTRACTS ON OCCURRENCE OF WILTING

Bacteria are known to grow and multiply very fast, millions in a day provided there are adequate feeding materials and conducive environment in terms of temperature, moisture and pH (Hayward, 1991). Extracts were added to the growing medium of the potato after the inoculation to ensure adequate interaction with the bacteria. *R. solanacearum* is known to survive well in soil and get into the plant through injuries which may be as a result of nematodes intrusions, injuries by farming tools (Ateka, 1999). Thus to ensure that the bacterium enters the potato plant some injuries were inflicted on the roots using a disinfected scalpel. The study has demonstrated that onset of wilting of the potato plants were determined by both the plant species extract and their concentrations. Control plants depicted the symptoms very early (during first observation) compared to the treated pots where wilting was observed in second observation.

Aqueous extract of *Ocimum* at the three concentrations were highly effective in controlling the bacterial wilt symptoms and the effectiveness was high in higher concentrations. By the end of the study period all the potato plants treated with *Ocimum* extracts depicted less than

50% wilting indices. The results corresponded with those obtained from the in *vitro* study using the organic solvents where polar compounds were more effective.

The results also showed that aqueous extracts of *Ipomoea batatas* and *Brassica oleracea* were highly effective at middle concentration (5% concentration). The results did not correspond with the *in vitro* study where activity of the probably polar compounds increased with concentration. This could be due to the difference in polarity of water and methanol resulting to extraction of different plant principles.

5.5 EFFECT OF PLANT EXTRACTS ON POTATO PARAMETERS

Morphologically plant growth is perceived as an increase in plant size as indicated by parameters such as plant height, fresh weight and dry weight (Vessey, 2003).

5.5.1 Effect of plant extracts and their concentrations on increase in plant height

The study showed that each aqueous plant extract that was used had a positive effect on increase in plant height. Initial plant height was taken one hour before the application of the first treatment and final height was taken four days after the completion of application of extracts, that is 39th day after planting unless the plant wilted before the end of experiment. The positive effect of plant extracts on increase in height could be explained by the fact that the plant materials that were added in the growth medium of potato plants provided essential compounds necessary for the plant growth. A study (Ng'ang'a, 2006) in evaluation of the effect of mustard plants in control of bacterial wilt concluded that the plant were more effective while used as green manure and stated that other green manures that may work as well included those of cabbage stems and leaves and sweet potato vines.

The delay on the onset of the wilting symptoms by the treatments also gave the plants an advantage of having more time to grow over the control plants that wilted very fast. There is a possibility that too much glucosinolates were present in 10% *Brassica* extracts resulting to stunted growth and fast wilting. Haramoto and Gallandt (2005) reported that all Brassicaceae plants contains glucosinolates that are hydrolyzed to toxic breakdown products such as isothiocyanates and ionic cyanates among others which have phytotoxic effects leading to reduced vigor of established plants.

5.5.2 Effect of plant extracts and their concentrations on potato fresh weight

The different plant extracts increased fresh weight of potato plants significantly as compared to the controls. The depicted increase of the fresh weight of potato plant shoots where treatments were subjected could be explained by the fact that there was a significant increase in the potato plants' height. There was no significant variation in the effect of extracts on potato fresh weight except those subjected to treatments *Brassica* 10% and 2.5%. This means that probably the different concentrations provided almost similar amounts of the required compounds that had an effect on the potato fresh weight. The significant effect of extracts on plant root weight could be explained by the fact that as plant increases in shoot growth, roots tend to increase in length to absorb more minerals for growth and also for support. The interaction of plants and their concentrations were highly significant for both the root and shoot fresh weight. The partial drying of the leaves that occurred in potatoes treated with *Brassica* extracts of 10% probably as a result of phytotoxicity of the extract could be the cause of its low effect on their fresh weight.

5.5.3 Effect of plant extracts and their concentration on dry weight

Aqueous extract of *Brassica oleracea* var. *botrytis* at concentration of 2.5% had highly significant effect on the dry weight of the plant probably due to the lower concentrations of glucosinolates thus capable of facilitating growth and not causing any phytotoxic effects on the potato plants. The phytotoxic effects of high concentrations of glucosinolates from *Brassica* plants were reported by Haramoto and Gallandt (2005). The other plant extracts also had appreciable effect on the dry weight of the potato plants. This could be attributed to the presence of essential compounds for growth in the plant extracts. The interaction of plants and their concentrations were significant for shoot dry weight.

There was a significant variation on the effect of the different plant concentrations on the dry weight of potato roots. This could be as a result of the interactions between plants and their concentrations whereby different plant extracts affected the dry weight of the potato plants differently at different concentrations.

CHAPTER SIX: CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 CONCLUSIONS

The following are the major conclusions that were drawn from this study:

- The inhibitory activity of essential oils of *Ocimum gratissimum* against the growth of *R. solanacearum* colonies was shown to increase as its concentration increased. Thus higher concentrations of the oils could result to higher inhibitory activity.
- The differences in the inhibitory effect of various plant extracts derived using the different organic solvents evaluated in this study demonstrated that the antibacterial principles in them differed in terms of quantity and quality. Ethyl acetate extracts of *Brassica oleracea* was highly effective at low concentrations and the efficiency decreased with increase in concentrations. The inhibitory activity of Ethyl acetate and methanol extracts of *Ipomoea batatas* were shown to increase as their concentrations increased and thus more activity could be exhibited when higher concentrations are used.
- Aqueous extracts of the three plants tested controlled the multiplication of the bacteria within potato plants resulting to delay in the onset and development of wilting symptoms. *Ocimum* extracts exhibited high effectiveness in its three concentrations used while *Brassica* and *Ipomoea* extracts exhibited highest activity at 5% concentrations.

- Addition of the plant extracts to the potted potato plants significantly increased their growth which was exhibited by high increase in both potato height and weight.

6.2 RECOMMENDATIONS

- The plant extracts tested under this study contain antibacterial compounds and thus may be utilized as phytobactericide to control the pathogenic bacteria on irish potato.
- Aqueous extracts of the three plants are effective in the control of bacterial wilt symptoms in potatoes. However Brassica aqueous extracts should be dispensed at low concentrations.
- The aqueous extracts of the three plants significantly promoted the morphological growth of potato plants. This could lead to increased high potato productivity when applied to potato fields.

6.3 SUGGESTIONS FOR FURTHER RESEARCH

- Phytochemical analysis of the methanol and ethyl acetate extracts should be carried out to ascertain the specific chemical compounds that are active against the bacterium.
- The mode of action of the plant extracts used in this experiment against *Ralstonia solanacearum* needs to be studied and higher concentrations of extracts need to be investigated to ascertain the optimum concentration necessary for inhibitory activity against the bacterial growth.

- There is need to conduct the experiment under field conditions especially in areas where potatoes are grown to ascertain whether the cool temperatures could enhance delay of the onset of symptoms of wilting while treatments are applied.
- Both wild and cultivated relatives of the plants under test should be tried out since they may be having higher concentrations of the active compounds.
- Research should be carried out to determine the effectiveness of the three plants under test in the control of the disease while used as green manure. This would aid in determining whether to encourage farmers to use these plants as source of organic manures while growing potatoes to increase their production.

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