EFFICACY OF PHOSPHORIC ACID AND STINGING NETTLE EXTRACT IN THE MANAGEMENT OF LATE BLIGHT OF POTATO

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DECLARATION

I declare that this is my original work and has not been presented for an award of a degree in any other university.

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DEDICATION

This work is dedicated to God for giving me grace and strength to complete both course and research work. Also to my dear parents, John Njogu and Esther Njeri, Brothers James, Stephen and Samuel for their love, support and encouragement.

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ABSTRACT

Late blight of potato caused by *P. infestans* is the major disease constraint to potato production in Kenya. Knowledge on sensitivity of late blight pathogen (*Phytopthora infestans*) to metalaxyl and different fungicide and evaluation of alternative fungicides is important for effective management of late blight. Studies were carried out at two sites in Kenya to evaluate the efficacy of phosphoric acid and stinging nettle compared to other commonly used fungicides, Ridomil and Dithane M45 in the management of potato late blight. *Invitro* studies were also carried out to evaluate the sensitivity of *P. infestans* from the major potato growing regions to different levels of metalaxyl, Ridomil, Dithane M45, phosphite and stinging nettle extract. The experiments in both locations were conducted in 4.5x 3 m plot with 6 rows and 10 tubers per row in a randomized complete block design laid down as a split plot and replicated three times.

The effects of these fungicides alone or in combination on epidemic development, lesion growth rate and number of lesions were measured. No fungicide sprayed alone or in combination completely arrested epidemic development under the environmental conditions of these experiments. However, alternating Ridomil with Phosphite fungicide had the most suppressive effect with relative area under disease progress curve (R.AUDPC) and percentage disease severity of 4.8 and 5.5 in Tigoni and Marimba locations respectively. The mechanism of effect included suppression of disease progress and lesion expansion. Phosphoric acid when alternated with Ridomil resulted into the least numbers and size of lesions. It was also observed that phosphoric acid alone resulted to significantly higher yield compared to Ridomil in both locations. Plot treated with stinging nettle extract differed significantly with respect to (R.AUDPC) and percentage disease severity with other fungicide tested apart from Dithane M45, however both stinging nettle and Dithane M45 differed significantly compared with control in terms of disease control and increase in yields.

Sensitivity to metalaxyl and other different fungicides was determined by culturing *P. infestans* isolates on 15% V8 medium amended with 0, 5 and 100 ppm metalaxyl and 20ppm of Ridomil, 25ppm of Dithane M45, 20ppm of Phosphite and 1ml of stinging nettle extract in 1ml of water. Sensitivity was determined by measuring the colony diameter at 14 and 21 days, counting the numbers of spores and determining the dry weight of the mycelium. The colony diameter reduction of isolates which were collected from Tigoni research station of 41.7 and 80.3mm in 5 and 100ppm respectively did not differ significantly with the colony diameter of isolates from Njambini which was 42.2 and 80.2mm in 5 and 100ppm respectively, however both Tigoni and Njambini location differed significantly from colony diameter of those isolates which were collected from Marimba of 41.7 and 74.1mm in 5 and 100 ppm metalaxyl respectively. The result of the experiment indicated that there was intermediate resistance of *P. infestans* metalaxyl in the entire region

All the fungicides tested differed significantly in their effect on pathogen colony diameter, number of spores and weight of mycelium. The pathogen was more sensitive to phosphite and least sensitive to stinging nettle extract. These studies demonstrate that incorporating alternative fungicides like phosphate may be beneficial in the management of potato late blight as well as avoiding development of metalaxyl resistance. Further studies should be carried out to determine the chemical responsible in stinging nettle, application rate and frequency of phosphate and stinging nettle extract in the management of late blight.

CHAPTER ONE:

INTRODUCTION

1.1 Potato production and importance

Potato is a vegetable crop of major economic importance world wide. It is the fourth world most cultivated food crop after wheat (*Triticum aestivum*), Rice (*Oryza sativa*) and Maize (*Zea mays*) (Hawkes, 1990, KARI, 2006; Anon, 2007). It is the world's most important tuber crop with annual production of 300 million tones with estimated production area of 18.9 million hectares (FAOSTAT, 2004; Hansen *et al.*, 2004). There is an increasing popularity of the potato in developing countries due to its high nutritional values, palatability, simplicity of its propagative amplification, ease of cooking and convenience (Were, 1996; Alisdair *et al.*, 2001).

In Kenya potato production is estimated at 3.5 million tonnes over an area of 94,848/ ha making the crop the second most valuable staple food crop after maize (Guyton et *al.*, 1994; MOA / GTZ, 1998). The crop plays an important role in national food and nutritional security, poverty alleviation, income generation and provides employment in production to consumption continuum because of its short maturity period and ability to grow throughout the year (Kabira *et al.*, 2006). Due to emerging urban markets, the crop is increasingly becoming an attractive cash crop and a source of rural employment and income for smallholders. Potato production is concentrated in the densely populated highlands where altitude ranges from 1500 to 2500 m using intensive, low-input agriculture. (Hunt 1980; Kabira 1990; MOA, 2006), while its consumption in Kenya is higher in urban population where all income groups consume potatoes (FAO, 1989, KARI, 2006). Yields are variable by locality and management practices and the average potato production is 0.55 kilograms per capiata (Theisen, 2007). Due to its short growth cycle, the crop is very suited for intensive cultivation especially in high altitude areas where one can get two crops as compared to one crop of maize. Potatoes are thus a cash crop with high potential for poverty reduction in such areas.

1.2 Problem statement & Justification

Potato in Kenya remains predominantely a crop of smaller holders, many of them women, although some larger-scale growers have specialized in commercial production to meet the demands of urban areas, where the potato has become a very popular food item. However, production in Kenya has continued to decline over the years. There has been a decline in the area harvested countrywide: 128,484 ha in 2004 to 120,421 ha in 2005 (FAOSTAT, 2001). Recently, the average plot size of farms planted with potatoes is less than 0.5 ha (Machangi, 2003). An average yield of 12-15 tonnes / ha for high yielding varieties and 4-6 tonnes / ha for low yielding varieties has been maintained (MOA, 2000; MOA/JICA, 2002). Due to its short growth cycle, the potato crop is well suited for intensive cultivation especially in high altitude areas.

The highlands, as a result of their favourable weather conditions, are densely populated, farms are small, and agricultural productivity is challenged to meet the demands of a growing population. Because of its suitability to highland conditions and culture, the crop has high potential for poverty reduction, food security and employment creation. For these reasons and for the achievement of the country's food security, the government and other institutions are promoting potato production. However, potato production and profitability are low in Kenya due to low use of inputs, intensive and continuous cultivation, declining soil fertility, poor crop husbandry especially disease and pest management, and lack of adequate certified seeds within economic reach of small-scale poor farmers (MOA, 2005; Kabira *et al.*, 2006).

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary is a significant production constraint causing yield losses of 30 - 75%, (Olanya *et al.*, 2001). Management of late blight remains very costly and difficult for most poor farmers. Control is often inadequate due to limited fungicide applications and use of cultivars with low to moderate resistance to late blight. Studies have shown a high development of metalaxyl resistance and possibility of oospore production of *P. infestans* in Kenya. Any further build up of resistance against metalaxyl represents a real threat to potato production as farmers only affords 2-3 applications. Since *P. infestans* mating type in Kenya is still US-1, it is anticipated that cultivar resistance and alternative fungicides would be a suitable strategy for disease control, and effective use of fungicide will be an important step in increasing late blight control, saving costs and reducing adverse environmental effects. Applications of product like phosphonate have also been shown to be effective against oomycetes (Fenn and Coffey, 1984, 1985; Forster *et al.*, 1998; Johnson *et al.*, 2000) and *Chenopodium ambrosioides* and *Lantana camara.* Also use of stinging nettle (*Urtica dioica*) extract as a treatment against late blight has been reported in Sweden, however no studies have been done to indicate their effectiveness in Kenya.

1.3 Objectives

The overal objective of this study was to assess the potential role of alternative fungicides in management of late blight of potato and to determine variation in metalaxyl resistance among *P. infestans* isolates.

The specific objectives were:

1) To determine the variation in metalaxyl resistance, and *in vitro* sensitivity of late blight pathogen (*P. infestans)* to phosphoric acid and stinging nettle extract in the major potato-growing areas.

2) To Determine the efficacy of phosphoric acid and stinging nettle extract in controlling potato foliar and tuber late blight

1.4 Hypotheses

1. There is regional variation of *P. infestans* sensitivity to metalaxyl and other fungicides in Kenya to neccesitate a regional approach to deployment of resistant genotypes and fungicide applications.
2. Phosphorus acid and stinging nettle extract as alternative cheaper fungicides could control late blight effectively in Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Potato crop

Potato (*Solanum tuberosum* L.) is an annual plant of the solanaceae, or nightshade family, commonly grown for its starchy tuber. The crop has its origins in the Andes Mountains of Peru and Bolivia from where it spread throughout the whole world (Hawkes, 1994). It was introduced to Kenya 80 years ago. Since then the crop has expanded in its cultivated area, total production and usage; it contributes a great deal to human diet in crop growing areas and in large towns (Guyton *et al*., 1994, Walingo, 2000). In Kenya, potato is the second most valuable staple food crop after the cereal grains (MoA, 2003).

The potato tuber is an enlarged portion of an underground stem, rhizome or stolon of the potato plant. Most tubers are found at the end of the stolon. Botanically, the tuber is a stem with the "eye", as true leaf scar. It is a storage organ for nutrients manufactured during the growth cycle of the plant. The proximate composition and nutrient composition depends on growing season-temperature, rainfall, soil and nutrient elements, area grown, cultural practices, maturity, method of harvesting and genetic make-up (Smith, 1977, Burton, 1989). Potato requires deep, well-drained fertile soils with a pH of 5.5-6.0. A cool growing season with an average daily temperature of 15-18 ^{0}C is ideal. Temperatures above 21 ^{0}C have adverse effects on growth of potatoes. It requires appropriate agronomic, disease and pest control and storage management practices for production of healthy seeds.

2.1.1 Potato production and its utilization in Kenya

Potato is grown mainly in the cool high altitude areas with well-distributed rainfall. The most suitable elevation is between 1,500 and 3,000 meters above sea level (MOA /JICA, 2002, Kabira *et al., 2006*, KARI, 2007), where it has higher production potential than maize and other cereals (Hunt 1980; Kabira 1990; MoA, 2006). The main potato growing areas are found in Central, Eastern and Rift valley provinces. Central provinces produce more than 53 percent, while Eastern and Rift valley provinces produce a combined total of 44 percent. The main growing region in Eastern province is Meru district in areas around the slopes of Mount Kenya. In central province, nearly all the districts produce some potatoes with Nyandarua district that lies along the Aberdare mountain range being the largest and most diversified potato producing area. In the Rift valley, potatoes are grown in Mau escarpment region in Dundori, Mau Narok, Molo and in the western highlands of Kericho, Bomet and Uasin Gishu districts. (MOA / GTZ, 1998; MOA, 2005).

Potatoes are mainly consumed at the commercial level as French fries chips and crisps served in restaurants and take away facilities in Nairobi and other major towns in Kenya. In homes, especially within the producing areas, potatoes are consumed daily for both mid-day and evening meals; they are made into stews and marshes which form the staple foods for most people in these areas. In local dishes, potatoes are mixed with vegetables such as carrots, cabbages, and meat and made into stews, which are eaten with ugali, chapati or rice. Potatoes are also used in traditional dishes in which they are mashed together with maize and beans or peas and other pulses into which some varieties of green vegetables may be added. This mode of consuming potatoes is duplicated in urban homes of families whose origin are from potato growing areas (MOA / GTZ, 1998). It s importance is shown in providing diet for a large cross-section of the Kenyan population (Guyton *et al.*, 1994). Common potato varieties in Kenya are include Dutch Robyjn, Kenya Karibu (kp90172.3), Kenya Sifa (720097), Kenya Faulu (KP90142.7), Kenya Mavuno (KP90131.10) Asante (381381.20), Tigoni (381381.13), Kerr's pink, Anett, Desiree, Kenya Baraka, Roslin Eburu (B 53), Kenya Dhamana, Kenya changuo (ECAPAPA, 2006).

2.2. Late blight

Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary has been a devastating disease for over one hundred and fifty years. The losses caused by late blight consist of yield reduction attributed to premature foliage death, destruction of foliage, tuber rots in the field and storage, and excessive costs associated with fungicide use for disease control (Guenthner *et al.*, 2001; Fry, 2007; Wustman, 2007).

Evidence over the last 30 years indicated a major change in the population of *P. infestans* worldwide with emergence of more aggressive and fungicide resistant strains (Fry *et al.*, 2008). Prior to the 1980's, most of the *P. infestans* population consisted A1 (US-1 genotype), with A1 and A2 (US-8 genotype) mating types confined exclusively to central Mexico. The recent genetic changes in *P. infestans* and corresponding increase in virulence has facilitated the spread of the US-8 displacing US-1 in North and South America, Europe, and other parts of the world (Fry, 2008). The significance of the occurrence of both mating types in some locations is the ability of the fungus to propagate through sexual recombination and potential of production of oospores. This has necessitated the introduction of new fungicides or fungicides with different chemistries in order to effectively combat late blight on foliage and tubers (Stein and Kirk, 2003, Andreu *et al.*, 2006; Latorse *et al.*, 2007). The occurrence of both mating types has also increased the survival and inoculum potential of oospores with tremendous epidemic implications. Although late blight occurs mainly on potato and tomato, infection of wild hosts belonging to the *Solanum* spp. such as black nightshade (*Solanum nigrum* L.), hairy night shade (*Solanum sarrachoides* L.), common morning glory (*Pharbitis purpurea* L) by *P. infestans* are common (Dandurand *et al.*, 2005, Flier *et al.*, 2003, Olanya *et al.*, 2005, 2009), however, tuber blight occurs on potato tubers only.

2.2.1 Biology of late blight pathogen

Late blight pathogens belongs to class Oomycetes and are fungi because they form filaments similar to the ones that fungi make (Waggoner and Speer, 1995). In reality, oomycetes are fungal-like organisms that differ from fungi in that their cell walls do not contain chitin but a mixture of cellulosic compounds and glycan. Another difference is the nuclei in the cells that form the filaments; each have two sets of genetic information in oomycetes instead of just one set as in fungi (Waggoner and Speer, 1995).

Phytopthora infestans is an extremely prolific pathogen and can produce as many as 300,000 sporangia per lesion every 3–5 day on a susceptible cultivar under conducive weather conditions (Mayton *et al.*, 2001a). The majority of sporangia are produced in the lower half of the leaf canopy and are deposited or washed to the soil surface and depending on environmental conditions can infect tubers in soil (Mayton *et al.*, 2001b). Conversely, many of the sporangia produced in the upper half of the leaf canopy are aerially dispersed, and can travel long distances and initiate foliar and tuber infections in fields some distance away from the initial infection foci (Aylor *et al.*, 2001). Late blight infected tubers are often accompanied by soft rot caused by *Erwinia carotovora* which can lead to complete loss of the crop in storage (Perombelon, 1980). Tubers from fields that apparently have no foliar disease may be infected with *P. infestans* during storage if stored together with infected tubers. Symptom caused by the disease includes water soaked lesions on the foliage, which turn brown when dry and black when wet. The spots can also occur on the tips of the stems, which turn black and die. On the underside of the leaf the fungus produces a white mouldy growth seen more clearly at the edges of the spots (Chycoski and Punja, 1996). Late blight is found nearly in all areas where potatoes are grown but it's more severe in humid high rainfall areas. Even the dry production areas are not free of the diseases (Lashomb and Casagrade, 1981; Agrios, 1988; Chycoski and Punja 1996).

2.2.2 Transmission of *P. infestans* and survival potential on blighted tubers

The aerial transport of *P. infestans* deserves considerably more investigation than it has received in dispersal of foliar late blight. Van der Zaag (1997) demonstrated that sporangia of late blight pathogen can be transported by at least 11 km through the air. Crosier and Minogue (1981) demonstrated that sporangia could survive several hours in unsaturated atmospheres at a variety of temperatures (Minogue and Fry, 1981). Andrivon (1995) has indicated reported that sporangia of the new lineages can be carried on ground spray equipment, because the first late blight they had seen was in the wheel rows of the sprayer that came from an affected field into a previously unaffected field

The removal and deposition of sporangia of *P. infestans* from foliage and stems onto tubers in soils often initiates tuber infection process. Once tubers are infected, transmission of *P. infestans* from diseased tubers to non-diseased tubers is one of the pathways for late blight spread (Kirk *et al.*, 1999). It has been documented that the spread of *P. infestans* from infected tubers to healthy tubers in soil is approximately 1.3 cm within the same plant, depending on the suitability of temperature (10-18°C) and moisture conditions in the soil. At 75 % field capacity, pathogen spread from diseased or inoculated tubers to healthy tubers in neighboring plants have been recorded over a distance of 60 cm, giving a rise to infection of daughter tubers from seed (Fairclough *et al.*, 1993).

The transmission of *P. infestans* from infected tubers to healthy tubers during storage or seed handling, particularly where seed cutting is a practice may also pose considerable risk for tuber blight spread (Lambert *et al.*, 1998). Once tubers are infected, the rate of spread or development of blight in tissues of potato tubers depends on storage temperatures (Kirk *et al.*, 2001). Other than localized spread of *P. infestans*, long distance spread of late blight on latently infected seed tubers across regions, national and international boundaries have been suspected but no concrete evidence is available.

Regardless of the mechanism of late blight spread on tubers, the epidemiological significance of *P. infestans* transmission on tubers is based on the possible dispersal of pathogen strains as well as their survival and inoculum potential. The survival of *P. infestans* in infected tubers either in storage or in the field until the following season has been well documented (Gigot *et al.*, 2009). It has been hypothesized that the survival potential is dependent on pathogen aggressiveness.

In situations where poor quality seed is used, tubers could easily be degraded and deposit pathogen structures in soil in the field. Research results on the formation of oospore of *P. infestans* in potato tubers has been provided (Levin *et al.*, 2001) making it more likely that pathogen structures could easily be deposited in soil. Although only a limited number of infected tubers have been shown to have oospores (Levin *et al.*, 2001), the potential for pathogen survival is exacerbated, given the fact that soil-borne oospores have already been noted to occur in certain potato production regions such as the Toluca Valley of Mexico (Fernandez-Pavia *et al.*, 2004).

2.2.3 Control of foliar and tuber late blight

2.2.3.1 Resistance

In developing countries, cultivars with high resistance and low cost fungicides are important (Grunwald *et al.*, 2002, Wastie, 1991; El-Bedewy *et al.*, 2001). Two types of resistance have been recognized, qualitative resistance due to R-genes, which confer resistance to *P. infestans* isolates with the corresponding virulence genes and quantitative resistance due to minor genes (Vanderplank, 1963). 'Horizontal' resistance is durable than 'vertical' resistance (Bradshaw *et al.*, 2004; Wastie 1991; and Umaerus, 1994). The division between these two resistances is an over simplification and often there is a continuum in resistance. So far *P. infestans* in Kenya has been shown to be US-1 (Vega-Sanchez *et al.*, 2000), with high level of metalaxyl resistance and low numbers of oöspores (Hohl, 2000). Despite development of resistant cultivars in Kenya in most cases based on CIP's population A with major gene resistance and Population B with minor gene resistance (KARI 2000; Landeo *et al.*, 1995, 2000, 2001) most varieties grown have low to moderate resistance (Haverkort, 1990; Nyankanga *et al.*, 2004). Negative correlations have been observed between AUDPC and tuber yield for cultivars with major gene resistance, but no correlations for cultivars with minor gene resistance in Kenya (Olanya *et al.*, 2006).

2.2.3.2 Chemical control

The companion fungicide in Ridomil prepacks, such as Bravo or Mancozeb, is a protectant fungicide and have been reported to be effective for controlling late blight. However late blight strains, including US-8 and US-11, have been reported to be resisrance to Ridomil.The availability of effective fungicides has enabled the continued use of susceptible cultivars. As a result, massive amounts of fungicide are used in those environments in which late blight is problematic, typically in rain-fed production systems. In the USA in 2001 alone, more than 2000 tons of fungicides were used to suppress the disease (Anon, 2004).

Control of foliage blight by fungicide applications, resistance and vine kill using mechanical or chemical desiccants before harvest are the most common methods for reducing tuber infection (Cooke and Little, 2001; Miller *et al.*, 2002). However; good control of foliar blight does not necessarily mean effective control of tuber blight (Nyankanga *et al.*, 2007). As a result there have been efforts to directly control tuber blight by use of fungicides. Foliage application of contact, systemic and curative fungicidal compounds have been shown to be effective in minimizing foliar blight and destruction of sporangia and therefore, indirectly reducing the levels of tuber infection by *P. infestans* (Stein and Kirk, 2003, Latorse *et al.*, 2007). Application of Fluazinam fungicide prevents tuber infection by inhibiting sporangia production and indirect germination (Nærstad *et al.*, 2007).

Different trials have shown that phosphonates or phosphates can suppress tuber and foliar late blight (Cooke and Little, 2001; Lobato *et al.*, 2007; Mayton *et al.*, 2008; Miller *et al.*, 2006). When applied to foliage, phosphonates can move systemically to the roots and developing tubers. The mode of action of the phosphonate fungicides is thought to be both direct and indirect. Direct effects include inhibition of mycelial growth, reduction or alteration of membrane metabolism and phosphorylation reactions in the pathogen, as well as suppression of sporulation and germination. Indirect effects of chemicals such as phosphonates are documented to be activation of plant defense responses (Andreu *et al.*, 2006). Foliar application of aminobutyric acid and fosetyl-aluminium, applied to foliage at early stages of crop growth, has been shown to increase the resistance of potato foliage to late blight and tubers when assessed at harvest (Andreu *et al.*, 2006).

Other fungicides such as fentin hydroxide and fentin acetate have been reported to reduce both tuber and stem infection (Bain and Edmonds, 2001). Fentin fungicides inhibit tuber infection by suppressing sporulation and release and germination of zoospores (Schwinn and Margot, 1991). Foliar applications of zoxamide have exhibited foliar and tuber protection against *P. infestans* in potatoes (Kirk *et al.*, 2003; Olsen 2002). Zoxamide functions by arresting nuclear division and destroying the microtubule cytoskeleton of oomycete pathogens (Young and Slawecki, 2001). Application of fungicides immediately after harvest but before storage has been shown to reduce tuber infection. Chlorothalonil, mancozeb and zineb has been shown to reduce tuber infection when applied at harvesting and on seed pieces (Kirk *et al.*, 1999). Disinfestants such as chlorine dioxide and mixtures of hydrogen peroxide and peroxyacetic acid (HPPA) are often used in the potato industry and have been evaluated for control of potato diseases in storage with variable results. Olsen *et al.*, (2003) reported limited efficacy with chlorine dioxide applications using the recommended rates and application methods. Hydrogen peroxide and peroxyacetic acid mixtures provided inconsistent control of potato diseases when applied to prevent disease development at harvest (Kimes, 2002). In post-harvest application studies, zoxamide and phosphite significantly reduced late blight and pink rot (*Phytophthora erythroseptica*) incidence and severity when applied immediately after inoculation with the pathogen. Phosphite was effective at reducing late blight development at all time intervals up to 6 h post-inoculation. Zoxamide appeared to have good post-harvest disease control if applied soon after inoculation (Miller *et al*., 2006). The authors concluded that phosphites are highly effective post-harvest management tools for controlling late blight and pink rot. Zoxamide was less effective as the interval between inoculation and treatment increased. Other authors have similarly found phosphates to be effective against *P. infestans* when applied during post-harvest (Lobato *et al*., 2007).

For a few years after its initial release, the fungicide metalaxyl/mefenoxam was so effective that late blight became much less problematic. Unfortunately, resistance was selected in pathogen populations all over the world, and the efficacy of this fungicide declined noticeably. Thus, potato late blight remains as one of the most important and most costly plant diseases. For both economic and environmental reasons, improvements in the efficiency of fungicide use via forecasts are being sought (Westerdijk and Schepers, 2006). Low cost measures have been tried by some farmers to control late blight, including the application of stinging nettle (*Urtica dioica*) (KARI, 2000).

2.2.3.3 Cultural practices

Late blight had been reported to develop during periods of high humidity even in the absence of rainfall; sprinkler irrigation provides ideal conditions for late blight development (Minogue, 1981) The initial sources of late blight inoculum are likely to be infected plants in cull piles, volunteer potato plants infected the previous year that have survived in the field, and infected seed tubers. Although weather conditions are beyond our control, field selection and carefully managing irrigation practices have been used in reducing the extent of periods favorable for disease development (Andrivon, 1995). It has been reported that excessive nitrogen applications promote heavy vine growth extending the period during which relative humidity within the canopy remains above 90 percent, a level favoring spore production and leaf infection there fore developing a nitrogen management plan that promotes optimum plant growth and yields, without stimulating excessive vine growth had been used as a cultural practice (Andrivon, 1995). Also during planting seed lots should be kept separately to avoid mixing uninfected lots with seed lots potentially-infected with late blight. Eliminating cull potatoes early in the season is critical because these potatoes could potentially carry the late blight fungus. Cull piles should not be allowed to build up in the field.

2.2.3.4 Integrated management

The new form of the late blight fungus, the "A2 mating type", resulting in many new strains of the fungus which are more aggressive has been reported and may have overcomed genetic resistance in some potato cultivars or be resistant to some fungicides (Minogue, 1981). Strains have been found that differ in their response to the fungicide metalaxyl. Integrated management must be adopted for effective control by producers, large and small, including organic farmers, home gardeners and other specialized growers (Minogue, 1981). Fungicides cannot be used alone for effective control of late blight, and should be used as one tool in an integrated management strategy. Cultural practices are the first line of defense, and forecasting techniques and proper application technology are essential for efficient, targeted applications of fungicides. Fungicide should be used as protectants, because use of fungicide to eradicate the disease after it is well established promotes the selection and spread of new resistance.

2.3 Phosphorus and phosphorous acid

Phosphorous acid releases the propionate ion ($HPO3^{2-}$), also called phosphite, upon disassociation. Phosphonate is easily taken up and translocated inside the plant. Phosphorous acid and its related compounds are often referred to as phosphonate, Phosphate, and phosphonic acid (Cohen and Coffey, 1986; McGrath, 2004; Wilcox, 2005.). Phosphorous acid does not get converted into Phosphate, which is the primary source of P for plants (Ouimette and Coffey, 1989b). In contrast, some soil bacteria are capable of transforming phosphonate into phosphate. However, this process is so slow that it is of no practical relevance (McDonald *et al.*, 2001). Up to date, no plant enzymes have been described that could oxidize phosphonate into phosphate. This explains why phosphonate is stable in plants and doesn't get converted into phosphate (Smillie *et al.*, 1989).

2.3.1 Phosphorous Acid and control of oomycetes

It has been documented that phosphorous acid is able to control diseases caused by organisms that belong to the Oomycota (Datnoff *et al.*, 2003; Förster *et al.*, (1998). Different trials have shown that phosphonates or phosphates can suppress tuber and foliar late blight (Cooke and Little, 2001; Lobato *et al.*, 2007; Mayton *et al.*, 2008; Miller *et al.*, 2006). The mode of action of the phosphonate fungicides is thought to be both direct and indirect (Guest and Bompeix, 1990). Direct effects include inhibition of mycelia growth, reduction or alteration of membrane metabolism and phosphorylation reactions in the pathogen (McGrath,2004). Sporulation and germination of *Phytophthora* species have also been shown to be suppressed by these fungicides (Cohen and Coffey, 1986). Indirect effects of phosphonates are thought to be through the activation of plant defense responses (Ouimette and Coffey, 1989a; Guest and Bompeix, 1990; Andreu *et al.*, 2006). Ability of phosphorous acid to control oomycetes for long periods of time appears to be its chemical stability in the plant (Smillie *et al.*, 1989). Phosphorous acid does not convert into phosphate and is not easily metabolized (Ouimette and Coffey, 1989b).

Plant species may differ in uptake and translocation of phosphonate (Cook and Little, 2001), and there is great variation in sensitivity of individual *P. infestans* isolates (Bashan *et al.*, 1990; Cohen and Bower, 1984) to phosphonate compounds, which may negatively impact the effectiveness of phosphonate. In most cases, research has been done with foliar applications of phosphorous acid. The compound gets translocated in the plant to the roots and is therefore effective against oomycetes that affect roots. Phosphorous acid has been shown to be effective when applied as a root drench against *P. cinnamomi*, *P. nicotianae*, and *P. palmivora* in lupin, tobacco, and papaya, respectively (Smillie *et al.*, 1989). The rot of *Persea indica* L. and pepper, was tested both as curative and preventive method of control (Ouimette and Coffey, 1989a). Even though sensitivity of each of the *Phytophthora spp.* used in their experiments in the laboratory was variable, there was little difference in the ability of different phosphonate compounds to control the stem rot of pepper, as a curative or a preventive agent in pots. (Ouimette and Coffey, 1989a).

Fosetyl-Al is a systemic fungicide that is often used against root pathogens because it is mobile in the plant and gets transferred to the roots (Cohen and Coffey, 1986). Cooke and Little (2001) found that foliar application of fosetyl-Al did not reduce tuber blight on potato caused by *P. infestans*, while foliar sprays with partially neutralized phosphonate reduced the number of tubers that developed symptoms after inoculation with the pathogen. Potassium phosphonate negatively affected mycelia growth more than propionates that had alkyl groups, but some exceptions were noted (Ouimette and Coffey, 1989a). Potassium phosphite was shown to be effective for control of strawberry leather rot caused by *P. cactorum* (Rebollar-Alviter *et al.*, 2005).

Phosphonate have been found to be effective when applied to potato foliage against *P. infestans* and *P. erytrhoseptica* which cause pink rot but not against *Pythium ultimum* (causal agent of Pythium leak) (Johnson *et al.*, 2004; Fenn and Coffey, 1984). Phosphorous acid also appears very effective against downy mildew on grapes (Wilcox, 200), and against *Phytophthora* root and crown rot on tomato and green pepper in hydroponics culture (Förster *et al.*, 1998). For control of oomycetes on turf grass, Riverdale Magellan (a mixture of phosphorous acid compounds) and Chipco Signature (Aluminum tris [O-ethyl phosphonate]) were found to be equally effective against Pythium blight development on perennial rye grass (*Lolium perenne*; Datnoff *et al.*, 2003). Similarly, different commercial formulations of phosphorous acid suppressed Pythium blight on rough blue grass (*Poa trivialis*) during the 2001-2002 season (Datnoff *et al.*, 2005, 2007). The existence of *Phytophthora* spp. resistant against phosphonate has been reported (Brown *et al.*, 2005; Griffith *et al.*, 1993; Nelson *et al.*, 2004 ;).

2.4 The use of Stinging Nettle (*Urtica dioica)* in the management of plant pathogen

Stinging neettle *(Urtica dioica*) is a herbaceous perennial flowering plant, native to Europe, Asia, Northern Africa and North America. (Riehemann, *et al*,. 1999) The plants have stinging hairs (trichomes), whose tips come off when touched, transforming the hair into a needle that will inject a cocktail of irritants: acetylcholine, histamine, 5-HT and possibly formic acid (Riehemann , *et al*,.1999). This mix of poisons causes a sting or paresthesia from which the species derives its common name. Stinging plants grow up to 1-2 m tall. It has very distinctively yellow, widely spreading roots. The soft green leaves are 3-15 cm long, with a strongly serrated margin, a cordate base and an acuminate tip. The taxonomy of stinging nettles has been confused, however, there are at least five clear subspecies, classified as separate species: *U. dioica* subsp. *dioica*, *U. dioica* subsp. *afghanica*.), *U. dioica* subsp. *gansuensis*, *U. dioica* subsp. *gracilis U. dioica* subsp. *holosericea*. (Riehemann, *et al*,. 1999).

Stinging nettle herb and flowers contain chemicals which include flavonoids, chlorophylls a and b, chlorophyll degradation products and carotenoids , vitamins C, B and K, triterpenes and sterols, mineral salts including silica, potassium salts, nitrates, boron, other ubiquitous plant substances like formic, acetic, citric and other acids are present (Schottner *et al.,* 1997). The stinging trichomes in particular contain amines including histamine, serotonin, and choline. The roots have been found to contain 4 different polysaccharides extracted by methanol that inhibit cell proliferation. Polar extracts of the root also contain the lignins (+)-neoolivil, (-)-secoisolariciresinol, dehydrodiconiferyl alcohol, isolariciresinol, pinoresinol, and 3, 4-divanillyl-tetrahydrofuran (Schottner *et al*., 1997).

The dose of stinging nettle which has been used in plant diseases is 8-12 g of dried herbal drug or 40-60 g fresh herb or 120-300g freshly pressed juice (Randal, *et al*., 2000), Stinging nettle extact has been found to be effective against stem canker of tea caused by *Phompsis thea* and had been reported to have 50% control compared to the negative control. (Oniango *et al.*, 2005). In Kenya, low cost measures used by farmers to control late blight, includes the application of a mixture made of stinging nettle and Omo (commercial brand of laundry detergent) (KARI, 2000). This treatment is apparently not a common practice in Kenya, but the use of stinging nettle (*Urticaria dioica*) as a treatment against late blight has been reported in Sweden (Vershney, 2003).

CHAPTER THREE

FUNGICIDE RESISTANCE OF *P. INFESTANS* AND ITS SENSITIVITY TO PHOSPHORIC ACID AND STINGING NETTLE

3.1 Abstract

Late blight is a significant disease of solanaceous crops worldwide. Knowledge on sensitivity of lateblight pathogen (*Phytopthora infestans*) to metalaxyl and different fungicide is important for effective management of late blight. *Invitro* studies were carried out to evaluate the sensitivity of *P. infestans* to different levels of Metalaxyl Ridomil, Dithane M45, phosphate and stinging nettle. Sensitivity to metalaxyl and other different fungicides was determined by growing *P. infestans* isolates on 15% V8 medium amended with 0, 5 and 100 ppm metalaxyl and 20ppm of Ridomil, 25ppm of Dithane M45, 20ppm of Phosphite and 1ml of stinging nettle extract in 1ml of water. The pathogen isolates were from the leaves collected from different potato growing areas of Kenya. Sensitivity was determined by measuring the colony diameter at 14 and 21 days, counting the numbers of spores and determining the weight of the mycelium.

The result of the experiment indicated that there was intermediate resistance of *P. infestans* metalaxyl in all the region as the growth was recorded as 57.8% in 5ppm and 19.8 in 100ppm in Tigoni station and 58.3 in 5ppm and 19.7 in 100ppm in Njambini while in Meru region the growth was 52.9 in 5ppm and 25.9 in 100ppm metalaxyl relative to the control. Njambini and Tigoni regions did not differ significantly interm of resistance; however both regions differed significantly with Meru region in terms of weight of mycelium and number of spores with Meru location having the highest number of spores and weight compared to both Tigoni and Njambini locations.

All the fungicides tested differed significantly in their effect on pathogen colony diameter, number of spores and weight of mycelium. The reduction of colony diameters of isolates collected from Tigoni of 41.7 and 80.3 in 5 and 100ppm metalaxyl did not differ significantly with that from Njambini sifnificantly of 42.2mm and 80.2mm in 5 and 100ppm respectively. The pathogen was more sensitive to phosphite and least sensitive to stinging nettle powder.

3.2 Introduction.

The management of late blight in Kenya remains a major production challenge because of the continuous potato cultivation which ensures abundant *P. infestans* inoculum for disease development throughout the year (Olanya *et al.*, 2001; Nyankanga *et al.*, 2007). Foliar blight and tuber blight have been frequently reported to occur and cause considerable losses in the tropical highlands (Nyankanga *et al.*, 2007). Attempts to control late blight are almost entirely through the use of protectant or systemic fungicides with little regard to application strategy in addition to other disease management options (Nyankanga *et al.*, 2004, Nyankanga *et al.*, 2008; Ojiambo et al., 2001).

Metalaxyl is very effective against sensitive strains and when applied early can suppress an epidemic (Fry *et al.*, 1979). The immediate effect on epidemic progress is achieved through suppression of lesion expansion (Bruck *et al.*, 1980) and possibly by prevention of latent infections. Effects that also contribute to suppression of established epidemics are reduced sporulation from lesions, and prevention of sporangium germination and subsequent infection (Bruck *et al.*, 1980). However, against resistant strains of *P. infestans*, metalaxyl alone has little or no detectable effect on epidemic development (Goodwin *et al.*, 1996).

Until recently, populations of the late blight fungus, *P. infestans* world wide contained only the A1 mating type. Considerable evidence suggests that about 20 years ago new populations of the fungus containing both A1 and A2 mating types is spreading around the world (Fry *et al.*, 1992). These isolates are rapidly replacing the original lineages in many regions (Fry *et al.*, 1993). They carry the potential to complete their sexual life cycle, which includes formation of oospores that enable the fungus to survive for long, and appear to have higher levels of virulence and high frequencies of resistance against the widely used fungicide metalaxyl (Deahl *et al.*, 1995; Goodwin *et al.*, 1996).

Except for one report of the occurrence of the A2 mating type in an Egyptian population (Shaw *et al.*, 1985), there are no definite indications of occurrences of new biotypes in Africa. However, the increased occurrence of devastating late blight epidemics, coupled with reduced fungicide sensitivity (Fry *et al.*, 1993) suggest the possible existence of new *P. infestans* populations in the continent. A premix of Ridomil (a.i. metalaxyl and Mancozeb) and Dithane M-45 (a.i. Mancozeb) have been widely used by potato growers for the control of late blight in the highland tropics (Ojiambo *et al.*, 2001; Nyankanga *et al.*, 2004). In other situations, systemic fungicides have been used for blight control in excess of four times per cropping cycle (Nyankanga *et al.*, 2004).

In Kenya, there are no evidences for the presence of new lineages or genotypes of the pathogen and the *P. infestans* population consists of US-1 genotype, A1 mating type (Vega-Sanchez *et al.*, 2000; Olanya *et al.*, 2001). However, isolates of *P. infestans* collected from potato fields in Kenya have shown a high level of metalaxyl insensitivity in areas where growers routinely and excessively use the systemic fungicide for blight control on potato and tomato hosts (Hohl, 2000, Ojiambo *et al.*, 2001). Metalaxyl insensitivity has also been reported among some isolates in production regions with similar agroecological conditions and production techniques as those used in the Kenyan highlands (Mukalazi *et al.*, 2001). Fungicidal compounds with systemic mode of actions as well as chemicals with protective properties have often been used by potato farmers for late blight control in various application sequences, rates or combinations. Strategies to reduce the potential destructive effects of late blight on potato production must be developed. To do this it would be helpful to acquire more knowledge on the resistance of this fungus to metalaxyl in Kenya. Also studies on sensitivity of this pathogen to different fungicide may result in more effective late blight management and contribute to better tuber yield. This study was conducted to test the occurrence of pathogen resistance to metalaxyl, and evaluating the sensitivity of late blight pathogen to Ridomil, Dithane M45, Phosphate and stinging nettle extract compared to the untreated check on the solid media.

3.3 Materials and methods

3.3.1 Collection of leaf samples

Potato leaf samples infected with late blight were collected from three major potato-growing areas, Meru Central, Njabini in Nyandarua District and Tigoni in Kiambu District. The leaf samples were collected randomly from 10 farmers fields within a radius of one kilometer between the farms in each district apart from Tigoni research centre where the leaves were collected from the field experiment. In each farm 20 leaf samples each containing10 leaflets with single late blight lesion were collected along a transect (Forbes et al. 1997). The leaflets were kept in iced box until taken to the laboratory where they were maintained at 5^0C for 1-2 days before isolation.

3.3.2 Isolation of the *P. infestans*.

Sporulating lesions on leaf tissue were washed in fresh water and placed in water agar plates in humid chambers (inverted petridish with water agar) with the leafs abaxial side up. Then these boxes were incubated at 15-18^0 C for 1-2 days and when sporuration appeared, small pieces of infected tissue from the sporurating border of the lesion were cut and placed under potato slices in empty petri dishes and washed potato were incubated at 15-18^0 C for 1 week until there was abundant sporuration on the upper side of potatoes slice. The fungus was then transferred on to selective V8 based media (V8 juice 100ml, $CaCO_3$ 1g, B-sistosterol 0.05g, Agar 15g) by touching the surface of the mycelia with a sterile needle and placing pieces of mycelia on the selective media surface.

3.3.3 Production of *P. infestans* Inoculum.

Sporulating lesion on the leaf tissue from the field were washed in sterile distilled water and placed in a humid chamber (inverted petridish with water agar) with abaxial side up. The plates were then incubated at 15-18^0 C for one week when sporuration occurred.

Tubers from Tigoni potato variety which tubers are known to be susceptible to late blight were washed in sterile distilled water and then allowed to dry. The tubers were dowsed in alcohol and then flamed off. Using sterile forceps to hold the tubers, 0.5cm thick tuber slices were cut using sterile scapel and placed in sterile plates. Small pieces of infected potato leaf tissues from the sporurating border of the lesion were cut and placed under the potato slices in empty plates. The Petri dishes were incubated at 15-18^{0}C at 90% RH for 1 week until abundant sporulating occurred on the upper surface of potato slices, then the

P. infestans was sub cultured in a V8 based media to produce pure cultures which were used in subsequent experiments. Isolates were assessed for growth on V8 agar (V8 juice 100ml, $CaC0_3$ 1g, B-sistosterol 0.05g, Agar 15g) amended with different levels of metalaxyl and then Phosphite, stinging nettle extract, Dithane M45 and Ridomil after the media was autoclaved and cooled to between 35 and 40 ^{O}C.

3.3.4 *In vitro* Screening for sensitivity of *P. infestans* isolates to metalaxyl, phosphoric acid and stinging nettle extract in solid media

In vitro screening was done on V8 selective media whose ingredients included V8 juice 100ml, $CaCO_3$ 1g, B-sistosterol 0.05g, and Agar 15 g. V8 Juice and distilled water were mixed to bring up to 1litre, $CaCO_3$ and glycerol were added and mixed well. Then agar was added and the mixture was autoclaved at a temperature of 70^{0}C and 15psi for 20 minutes. After cooling, antibiotics which included penicillin, tetramycin and streptomycin were added each at the rate of 20ppm in 100ml of media. After cooling the media to a temperature of 40 ^{O}C in a laminar flow hood and then the media was amended with two different concentration of Metalaxyl (5pm and100ppm) and different fungicides using one concentration of each fungicide (20ppm of Ridomil, 25ppm of Dithane M45, 20ppm of phosphite and 1ml of stinging nettle extract in 1ml of water) at the same temperature of 40 ^{O}C. Control consisted of the selective media without any fungicide. Each treatment was replicated three times in a complete randomized design.

Fifteen ml of medium which had been amended with different fungicides was dispensed into 8.5 cm –diameter plastic petri dishes. A 0.5mm-diameter agar disk was taken using a sterilized cork borer from an actively growing colony of *P. infestans* grown on V8 based media and was placed with the fungal side downward in the centre of each plate. After 14 and 21 days of incubation, radial growth was determined by measuring colony diameter at two points on isolates using a divider which was then fitted into a ruler and the average measurement of all the isolates taken. The spores from each isolates were harvested and counted using a haemocytometer and average spores in each treatment was recorded. After 21 days the mycelium of each isolate from each treatment was harvested. During harvesting 10 ml of distilled water was added in each petridish and then the mycelium was scrapped from the V8 based media using a sterile slide and sieved through cheese cloth and then dried in an oven at controlled temperature of 70 ^{O}C to obtain a dry weight.

Based on the results of radial growth, sporulation and mycelial dry weight, isolates were classified as susceptible, moderately resistant, or resistant depending on sensitivity to metalaxyl following the procedures by the International Potato Center (Forbes, 1997). Susceptible isolates were those isolates with less than 40% growth relative to control on both concentrations of metalaxyl (5 and 100ppm) while intermediate were those with growth greater than 40% at 5 ppm but less than 40% at 100 ppm of metalaxyl and resistant growth greater than 40% on both concentrations of metalaxyl .

3.3.5 Data analysis.

All data was subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (Lawes Agricultural Trust Rothamsted Experimental station 2006,Version 9) and difference among the treatment means were compared using the Fisher s protected LSD test at 5% probability level.

3.4. Results

3.4.1. Sensitivity of *P. infestans* isolates to different concentration of metalaxyl

Differences in mean growth rates of colony diameter for the different metalaxyl concentrations were highly significant (*$P \leq 0.05$*). The addition of metalaxyl to culture medium significantly reduced growth rate of the *P. infestans* isolate collected from the two regions and Tigoni Research Station (Table 3.1). The reduction of colony diameter of isolates collected from Tigoni of 41.7 mm and 80.3 mm in 5 and 100ppm did not differ significantly with that from Tigoni of 42.2mm and 80.2mm in 5 and 100ppm respectively while this differe significantly from colony diameter of those isolates collected from Marimba of 41.7mm and 74.1mm in 5 and 100 ppm metalaxyl respectively (Table 3.1) For all the regions. *P. infestans* isolates was not considered resistant but intermediate in resistance to metalaxyl.The result indicated that all the tested rates differed significantly (*$P \leq 0.05$*) amongest themselves and the control.

There was a significant difference (*$P \leq 0.05$*) in the weight of mycelium and numbers of spores produced after amending the media with different concentrations of metalaxyl. However there was no significant difference between Tigoni and Njambini location while the two locations differed significantly (*$P \leq 0.05$*) with Meru location (Table 3.2). Metalaxyl concentration of 100 ppm resulted in the lowest number of spores of 34.6, 21.7 and 76.5 in Tigoni, Njambini and Marimba respectively, as well as the biomass of 0.56g, 0.4g and 0.67g in Tigoni, Njambini and Marimba respectively.

Media amended with metalaxyl at 5ppm did not differ significantly in term of number of spores with control plates in Tigoni location, however the two differed significantly with 100ppm in the same location (Table 3.2) while in Njambini the two tested rates differed significantly between themselves and the control. In meru location the rates of 5ppm differed significantly with the control, however this did not differ significantly with the higher concentration of 100 ppm (Table 3.2).

3.4.2 Sensitivity of *P. infestans* isolates to different fungicides

There was a significant difference in late blight pathogen sensitivity due to different fungicides in all regions as shown by mean colony diameters measured at 14 and 21 days, among the different fungicide tested (Table 3.3). Phosphoric resulted in the least diameter size and this differed significantly with other tested fungicide. There was no significant difference in colony diameter in plates which were amended with either stinging nettle or Dithane M45 after seven days, however after 14 days the two fungicides differed significantly with Dithane M45 having less colony diameter compared to stinging nettle extract. The highest colony diameter was recorded in untreated plates in all the readings which differed significantly with all the fungicide tested (Table 3.3).

Table 3.1. Mean colony diameters (mm) of *P. infestans* isolates collected from different region measured at 14 and 21 days after incubation at different metalaxyl concentrations

Location	Metalaxyl concentration (ppm)	14 days	21 days	Mean	% colony reduction
Tigoni	0	32.6	70.6	52.6	0.0
	5	20.3	40.5	30.4	42.2
	100	10.3	10.5	10.4	80.2
	LSD($P \leq 0.05$) Treatment	4.8	6.2		
	CV (%)	19.9	32.8		
Njambini	0	29.2	71.5	50.3	0.0
	5	19.1	39.6	29.3	41.7
	100	9.3	10.5	9.9	80.3
	LSD($P \leq 0.05$) Treatment	4.8	5.2		
	CV (%)	24.6	27.5		
Marimba	0	55.5	76.5	66.0	0.0
	5	23.3	46.5	34.9	47.1
	100	14.3	19.9	17.1	74.1
	LSD($P \leq 0.05$) Treatment	5.9	6.2		
	CV (%)	31.8	24.9		

Table 3.2. Mean mycelia weight (g) and number of spores of *P. infestans* isolates collected from different regions measured at 14 and 21 days after incubation at different metalaxyl concentrations

Locations	Metalaxyl concentration (ppm)	Mean mycelia weight (g)	Number of spores
Tigoni	0	0.96	116.0
	5	0.85	96.0
	100	0.56	34.6
	LSD($P \leq 0.05$) Treatment	0.15	24.0
	CV (%)	29.3	27.9
Njambini	0	0.93	126.0
	5	0.73	92.4
	100	0.4	56.7
	LSD($P \leq 0.05$) Treatment	0.16	21.7
	CV (%)	35.7	16.8
Marimba (Meru)	0	1.12	178.0
	5	0.91	116.4
	100	0.67	76.5
	LSD($P \leq 0.05$) Treatment	0.13	46.6
	CV (%)	23.7	29.4

Table 3.3 Mean colony diameter (mm) of *P. infestans* isolates Collected from different locations measured at 14 and 21 days after incubation from different fungicides, phosphoric acid and stinging nettle

Location	Treatments	14 Days	21 days	Mean diameter	Percentage reduction
Tigoni	Ridomil	9.6	12.3	10.5	80.5
	Phosphite	4.4	6.8	5.7	89.4
	Dithane M45	19.6	39.1	29.4	45.3
	Stinging nettle	20.3	51.9	36.6	31.8
	Control	53.7	74.7	53.7	0.0
	LSD($P \leq 0.05$) Treatment	5.1	5.2		
	CV (%)		29.6		
Njambini	Ridomil	10.3	12.5	11.4	79.4
	Phosphite	4.9	7.7	6.3	88.6
	Dithane M45	20.3	39.1	29.7	46.2
	Stinging nettle	20.3	48.9	34.6	37.3
	Control	42.6	67.7	55.2	0.0
	LSD($P \leq 0.05$) Treatment	4.6	4.8		
	CV (%)		19.3		
Marimba	Ridomil	12.9	18.3	15.6	76.1
	Phosphite	5.4	7.9	6.7	89.7
	Dithane M45	25.3	45.4	35.3	45.9
	Stinging nettle	23.8	59.7	41.7	36.1
	Control	55.5	75.7	65.3	0.0
	LSD($P \leq 0.05$) Treatment	5.8	6.2		
	CV (%)		30.1		

Different fungicides resulted to a significant difference ($P \leq 0.05$) on both the weight of mycelium and the number of spores. Phosphate resulted in the least weight of mycelium and the numbers of spores and this was observed in all the regions and it did not differ significantly with the Ridomil in Njambini and Tigoni though the two differed significantly (Table 3.4). Stinging nettle extract and Dithane M45 differed significantly with other pathogens in all the regions but the two did not differ significantly apart from Tigoni region.

The result indicated that control plates resulted with the highest number of spores which differed significantly with other fungicides ($P \leq 0.05$). Among the fungicides tested Dithane M45 produced the highest number of spores in all the location while Phosphate resulted with the least number of spores (Table 3.4). Spore production was observed to differ significantly across the different region with isolates from Meru region producing the highest number of spores.

Table 3.4 Mean mycelia weight (g) and number of spores of *P. infestans* isolates collected from different region measured at 14 and 21 days after incubation at different fungicides, phosphoric acid and stinging nettle

Location	Treatments	Mycelia weight (g)	Number of spores
Tigoni	Ridomil	0.41	12.0
	Phosphite	0.35	8.0
	Dithane M45	0.96	52.0
	Stinging nettle	0.73	40.0
	Control	1.1	124.0
	LSD($P \leq 0.05$) Treatment	0.12	5.6
	CV (%)	27.9	17.2
Njambini	Ridomil	0.38	22.0
	Phosphite	0.34	9.0
	Dithane M45	0.74	62.0
	Stinging nettle	0.69	48.0
	Control	0.85	142.0
	Lsd ($P \leq 0.05$) Treat	0.15	11.6
	Cv %	37.7	26.5
Marimba (Meru)	Ridomil	0.5	46.0
	Phosphite	0.37	12.4
	Dithane M45	0.85	69.0
	Stinging nettle	0.78	62.0
	Control	0.97	166.0
	Lsd ($P \leq 0.05$) Treat	0.12	19.8
	Cv %	27.2	11.4

3.5 Discussion

The result of this study revealed the intermediate metalaxyl resistance by the *P. infestans* isolate isolated from leaf samples collected from Njambini, Meru and Tigoni Research station. Mean colony diameter, number of spores and dry mycelia weight were reduced in all the isolates by different treatment compared to control. All the measured parameter were significantly reduced using phosphoric acids, although other fungicide also proved effective in reducing the number of spores, colony diameter and mycelia weight. These results are in agreement with Jonson *et al* (1994) who found that phosphoric acid reduced lesion development and sporuration when applied as a foliar spray in the field than other fungicides. In this country there is only moderate Ridomil (56% Metalaxyl and 7.5% Mancozeb) application to control late blight, largely due to economic restriction. In other parts of the world, resistance to metalaxyl and other related compounds has developed rapidly in populations of *P. infestans* after the use of metalaxyl (Davidse *et al.*, 1983; Deahl *et al.*, 1995).A greater frequency of resistance to metalaxyl occurs among the new biotypes having both A1 and A2 mating types (Fry *et al.*, 1993; Erwin and Ribeiro, 1996). Low metalaxyl dosage also tend to result in high frequencies of metalaxyl-resistant *P. infestans* isolates (Davidse *et al.*, 1981; Dowley and O'Sollivan, 1981; Davidse *et al.*, 1983) which might be the case in Kenya.

The presence of intermediate level of metalaxyl-resistant *P. infestans* in these locations is not necessarily a strong indicator of the presence of the A2 mating type as there is no genetic correlation between resistance and mating type and this agree with the finding that resistance to phenylamide became established in A1 populations before the appearance of the A2 type (Gisi and Cohen, 1996). Thus, the metalaxyl-intermediate resistant isolates in Meru, Njambini and Tigoni locations may still be of the A1 mating type. However, the level of intermediate resistance to metalaxyl suggests that metalaxyl use should be planned carefully, as it could increase management costs (Bradshaw and Vaughan,1996). Since Ridomil contains both Mancozeb and Metalaxyl, it is recommended to use Ridomil sparingly, and partially substitute it with other alternative fungicides. The results of these experiments provide information essential for the development of effective disease control strategies. The presence of *P. infestans* isolate with intermediate resistance requires that integrated management rather than depending on one fungicide on late blight management strategies be adopted. This is supported by the fact that the pathogen was able to grow for over 50% in media amended with 5ppm metalaxyl and this also suggests that use of under dose may result in pathogen developing resistance to metalaxyl. None of the fungicide tested was effective in total control of the pathogen however result indicated that phosphoric acid and stinging nettle might have some useful effect in controlling late blight pathogen. Certainly, to minimize further development of fungicide resistant strains, application of fungicides should be combined with the use of host resistance.

CHAPTER 4

EFFICACY OF PHOSPHORIC ACID, STINGING NETTLE AND OTHER FUNGICIDES IN THE MANAGEMENT OF LATE BLIGHT OF POTATO

4.1 Abstract

Late blight of potato caused by *P. infestans* is the major disease constraint to potato production in Kenya. Studies were carried out at two sites, KARI Tigoni and Marimba (Meru) in Kenya to evaluate the efficacy of phosphoric acid and stinging nettle in the management of potato late blight. Other fungicides, Ridomil and Dithane M45 were used for comparison.

The effects of these fungicides alone or in combination on epidemic development, lesion growth rate and number of lesions were measured. No fungicide sprayed alone or in combination completely arrested epidemic development under the environmental conditions of these experiments. However, alternating Ridomil with phosphate fungicide had the most suppressive effect with relative area under disease progress curve (R.AUDPC) and percentage disease severity among the tested chemicals of 4.8 and 5.5 in Tigoni and Marimba locations respectively. The probable mechanism of effect included suppression of disease progress and lesion expansion. It was also observed that phosphoric acid alone resulted in significantly higher yield of 24 t/ha in Tigoni variety during 2009 season compared to Ridomil with 22.4 t/ha while in Desiree variety phosphoric acid had a yield of 15.6 t/ha and Ridomil resulted to 14.4 t/ha in Tigoni location. Plot treated with stinging nettle extract differed significantly with other fungicide tested apart from Dithane M45; however both stinging nettle and Dithane M45 differed significantly compared with the control in terms of disease control and in yields.

4.2 Introduction

Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary is one of the most devastating disease of potato worldwide (Erwin and Ribeiro, 1996). The pathogen infects foliage and tubers resulting in tuber yield loss due to premature death, tuber rot in the field and storage. Management of potato late blight remains a major challenge, especially among small-scale farmers in the tropical highlands of Kenya. Attempts to control late blight are almost entirely through use of fungicides on cultivars with low to moderate levels of resistance (Haverkort, 1990). For most poor farmers, there is inadequate control of the disease, resulting in heavy losses and, in some cases, complete crop loss. It is estimated that approximately 30 to 60% of the crop is lost due to late blight annually in Kenya (Njuguna *et al.*, 1998). Fungicide application strategies (timing, frequency and rates) are important for control of potato late blight.

Most farmers rely on intensive use of fungicides which leads to high environmental and production costs. Integrated crop management offers an alternative to reduce the use of fungicides. Certain chemical compounds are capable of naturally increasing plant defense mechanisms (Gozzo 2003, 2004). These compounds could be used as part of integrated pest management. In a previous study done it was reported that amino butyric acid (BABA) and fosetyl–Al, foliarly applied at early stages of crop growth, can increase the resistance of potato foliage and tubers to late blight (Andreu *et al*,. 2006). Phi in general can stimulate plant defense responses and are also active against oomycetes in vivo (Guest and Grant, 1991).

Metalaxyl and mefenoxam have been effective in the past in controlling late blight, (Mulrooney, 1982, Torres, *et al.,* 1985, Wicks., *et al.*, 2000), but metalaxyl resistant populations of *P. infestans* now predominate in the world. (Derie, and Inglis, 2001, Miller, *et al. 1997*. Miller, and Johnson, 2000). Control by metalaxyl and mefenoxam products is no longer effective against this pathogen in some fields, and alternative control measures are needed.

Phosphoric acid has been reported to inhibit sporulation and phosphoric acid may have some activity after infection (Howard, *et al.*, 1996, Schwinn, and Margot, 1991). Both phosphoric acids and fosetyl- AL are effective in controlling disease caused by oomycetes, (Barrett, *et al.*, 2003, Coffey, 1987, Fŏrster, *et al.*, 1998, Ouimette, Coffey, 1989b) the mode of action is direct antifungal activity of phosphonate toward mycelia growth (Fenn, and Coffey. 1989) and, perhaps, indirect stimulation of host defences (Dunstan, *et al.*, 1990, Guest and Bompeix, 1990, Guest., and Grant, 1991).

These compounds are systemic in both basipetal and acropetal direction (Cohen, Coffey, 1986), and this systemic property permits their use as a foliar spray control of root rot caused by *Phytopthora spp.* (Coffey, 1987) and other oomycetes and appears to be stable in plants for several weeks (Quimette., Coffey, 1989a, Smillie, Grant., and Guest, 1989) and may offer long term protection in potato Leaf and tubers. Phosphoric acid applied to potato foliage reduced incidence of inoculated tubers that became infected with *P.infestans* in Ireland (Cooke, Little, 1996, 2001). There was no published result on effect of stinging nettle on late blight pathogen though stinging nettle extract has been reported to be effective against *Phomopsis thea* of tea (Oniango, 2005). Fungicides are most effective in late blight management when applications are made before infection (Bruck, .*et al.*, 1981, Schwinn, and Margot, 1991, Stevenson, 1993), because most fungicides have protective modes of action and are not effective after *P. infestans* enters plant tissues (Bruck, *et al.*, 1981, Schwinn,, and Margot, 1991). Furthermore a protectant fungicide application may not thoroughly cover all foliage within a canopy, leaving sections of the canopy unprotected. For example, lower canopy leavess may not initially receive sufficient fungicide when application is made by knapsack or even airplane after closure between rows (Hamm, and Clough, 1999). The objective of this study was to determine the efficacy of phosphorus acid and stinging nettle in controlling late blight alone or when incorporated with the convectional fungicides Ridomil and Dithane M45.

4.3 Materials and methods

4.3.1 Experimental layout and design

The study was carried out at the National Potato Research Centre (NPRC), Tigoni and Marimba, Meru. The two sites have different climatic conditions of (temperature and rainfall) and late blight pressure (Ojiambo *et al.*, 2001). Late blight is often present at these locations and disease development was initiated by natural inoculum present in the field. Treatments were laid out in a randomized complete block design with a split plot arrangement and replicated three times. The main plot consisted of the fungicide spray treatment while the subplots consisted of the two potato varieties (Tigoni and Desiree), Each main plots were 4.5 x.3.0 m while the subplots were 2.25 x 3.0 m and potatoes were planted in furrow at a spacing of 0.75m in between rows and 0.3 m within rows. The distance between blocks was 2 m wide while a gap of 1.5 m separated the subplots. Two outer rows acted as guard rows while disease severity was recorded in two middle rows and a border of one meter width surrounded each potato plot.

A standard agronomic inter-row spacing of 0.75 m and intra-row spacing of 0.3 m for this region were used for all trials. In all experiments two potato cultivars Desiree, a cultivar susceptible to late blight and grown in many potato production regions for fresh consumption and Tigoni a cultivar resistant to late blight and grown in many potato production regions for making chips. Sprouted certified potato seeds were planted on October 23rd 2008 and November 11th 2008 in Tigoni and Marimba, respectively during the short rainy season and March 13th 2009 and March 28th 2009 in Tigoni and Marimba, respectively during long rains. Diammonium phosphate (DAP) fertilizer was applied at the rate of 500 kg/ha and was incorporated in field plots at planting time during all cropping seasons in both locations. The plots were hilled two times during crop growth in both locations. Water supplement through sprinkler irrigation was utilized on a weekly basis in Tigoni location while no water supplement was used in Marimba location. Pests like aphids were controlled using insecticides Deltamethrin while weeding and other agronomical practices were done as recommended for the both regions. Weather data was collected from Tigoni and Marimba weather stations.

4.3.2 Treatment applications

Seven treatments were applied which included Ridomil (64% Mancozeb, 4% metalaxyl) at the rates of 2.5kg/ha, Dithane M45 (80% mancozeb) at the rates of 2.5kg/ha, phosphite (mono dipotassium phosphoric acid), at the rates of 2.5l/ha, stinging nettle at the rates of 50gm/L (Appendix 3), Dithane M45 alternating with phosphite, Ridomil followed by phosphite in subsequent application and a control without any treatment. Dithane M45 and Ridomil were used as the protective and curative fungicides controls respectively commmonly used for these region. Fungicides were applied at 7-day intervals except ridomil which was applied twice during the crop season. The sprayer was calibrated prior to commencement of fungicide application so as to deliver spray volume of 400 L/ha at a pressure of 3 bars. The potato plants in the untreated plots were sprayed with water. Treatment applications were initiated at the onset of late blight symptoms.

The stinging nettle plants were collected from University of Nairobi farm (Kanyariri) and the plant was harvested during the flowering stage. The plant was prepared by crushing naturally dried whole plants except the roots into a fine powder. The powder was passed through a 5mm mesh sieve to obtain a fine powder, Fifty grams of the fine powder was soaked overnight in 1 liter of water one day before application and then diluted to 20 l water for spraying.

4.3.3 Late blight assessment

Late blight parameters assessed included disease severity, incidence, lesion size, number of lesions and tuber blight. Field plots were assessed for late blight severity by visual rating based on percent leaf area blighted using a scale of 0% to 100% where 0% = no disease; and 100% refers to total foliage damage (Lutaladio *et al.,* 1996), (Table 4.1). Assessment was done in the two inner rows beginning from the time when symptoms were first observed and subsequent assessments were done at 4 day intervals until the potato foliage in the untreated plot were completely destroyed by the disease.

The data on incidence was taken only during disease establishment as the percentage numbers of diseased plants out of the total number of plants per plot and the percentage number of leaves blighted out of total number of leaves per plants. The data was taken at aninterval of four days upto crop physiological maturity.

Table 4.1 Table of scale values, percentage late blight and symptoms used in scoring late blight disease adapted from Lutaladio *et al.*, 1996

CIP scale	Blight %		Symptoms
	Mean	Limit	
1	0		No late blight observable.
2	2.5	Tr<05	Late blight present. Maximum 10 lesions per plant.
3	10	05,<15	Plant look healthy but, lesion are easily seen at a close distance. Maximum foliage area affected lesion corresponds to more than 20 leaflets.
4	25	15<35	Late blight easily seen on most plants. About half foliage area is destroyed.
5	50	35<65	Plot kook green; However all plants are affected. Lower leaves are dead. About half foliage area destroyed.
6	75	65<85	Plot kook green; However all plants are affected. Lower leaves are dead. About 75% foliage of each plant is affected. Leaves of lower half of plants are destroyed.
7	90	85<95	Plot neither predominantly green no brown. Only top leaves are green many stems have large lesions.
8	97.5	95<100	Plot is brown-colored. A few top leaves still have some green areas. Most stems have lesion or are dead.
9	100		All leaves and stems are dead.

The number and size of lesion determined as the diameter of each lesion out of five lesions on five plants per subplot and the measurement was repeated on the same plants after every 4 days until the end of the season. The number of lesions per plant was determined in five plants per subplot.

4.3.3.1 Tuber blight assessment

At physiological maturity, tubers from each of the 10 hills per sub plot were carefully dug separately and diseased tubers were identified visually and their number counted. Tubers with external symptomatic lesions were sliced to confirm the presence of brown discoloration. The number of diseased tubers was calculated as a percentage of the total number of tubers per plot. Twenty tubers per treatment were selected and stored at 5 ^{0}c for one month after which the number of infected tubers was counted.

4.3.4 Determination of effect of fungicide on Potato tuber Yield and yield components.

At physiological maturity, the potato plants were dehaulmed 2 weeks before harvesting. At harvest, tubers from 10 hills in the middle of the two inner rows of each plot were harvested by carefully lifting out the tubers. The tubers were graded according to Kabira *et al.*, (2006) as chatt (<25mm, seed (25-30mm) and ware (>55mm) in diameter. Tubers were also separated into marketable (> 6.4 cm diameter) and unmarketable (< 6.4 cm diameter) and weighed separately. The number of tubers per hill was also recorded and yields converted to tonnes per hactare.

4.3.5 Statistical Data analysis.

The area under disease progress curve was calculated from the disease severity and incidence data as described by Shaner and Finney (1977). All other data apart from lesion expansion rate was subjected to Analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (LAWES Agricultural Trust Rothamsted Experimental station 2006, Version 9) while lesion expansion rate was analysed using repeated measures analysis, and difference among the treatment means were compared using the Fisher s protected LSD test at 5% probability level.

4.4 Results

4.4.1. Effect of fungicides on late blight severity

Late blight was observed within 55 and 48 days after planting in 2008 in NPRC, Tigoni and Marimba locations respectively (Figure 4.1) while in 2009 the disease was observed after 52 and 56 days after planting in Tigoni and Marimba. The disease progressed rapidly in unsprayed plots reaching up to severity of 95% in Desiree cultivar and 38.33% in Tigoni cultivar within 30 days after appearance of the symptom in Marimba (Figure 4.1) and 83.3 % in Desiree and 28.8% in Tigoni cultivar within 36 days after initial detection of disease in Tigoni location (Figure 4.1).

Ridomil followed by Phosphite resulted in significantly *($P \leq 0.05$)* lower late blight severity than Ridomil alone and Dithane M45 followed by phosphite but it did not differ significantly with plots where Phosphite alone was applied (Table.4.2). Also Phosphite (Mono and Dipotasium Phosphorus Acid) applied alone did not differ significantly from Ridomil and Dithane M45 followed by phosphite. The application of Dithane M45 and stinging nettle extract resulted to significantly *($P \leq 0.05$)* lower disease severity than untreated plots, however the disease severity on plots treated with Dithane M45 also differed significantly with those plots treated with stinging nettle extract, with Dithane M45 resulting to lower disease severity of 12.43 % compared to stinging nettle extract which resulted to disease severity of 15.33 both on the susceptible cultiva in Tigoni location (Table 4.2)

There was a significant difference (*$P \leq 0.05$*) in late blight severity between plots treated with phosphite followed by Ridomil and those treated with Ridomil alone with the combination resulting to lower disease severity and R.AUDPC on susceptible cultivar, however the two treatments did not have significant difference on resistant cultivar. Result also indicated that Dithane M45 + Phosphite and Dithane M45 alone treated potato plants did not show any significance difference between themselves on both potato cultivars at Tigoni site while the two treatments showed a significant difference with Dithane M45 having significantly (*$P \leq 0.05$*) higher disease severity and R.AUDPC on susceptible cultivar but no significant difference on resistant cultivar at Marimba site during 2008 (Table 4.2, figure 4. 1, 4.2). Potato plants on untreated plots had higher late blight severity and bigger R.AUDC while Ridomil followed by Phosphite treated potato plants had the least level of disease progress on both site and locations. (Table.4.2). Application of Ridomil followed by Phosphite resulted in a significantly (*$P<0.05$*) lower late blight severity compared to the rest of the treatments (Table 4.2) at both locations.

In 2008 cropping season at Tigoni, there was considerably lower disease levels compared to Marimba (Table 4.3 and Figure 4.1). Also progress of late blight was significantly greater in control plants compared to all other treatment (Figure 4.1). There was significance difference which was observed during this season between the different cultivars on both site with Tigoni cultivars having significantly lower disease severity than Desiree cultivar and this was observed on both sites (Table 4.3), Also two site differed in terms of disease levels and time taken for the plant to be completely destroyed with Meru location having higher disease level and taking shorter time (Table 4.3, figure 4. 1, 4.2, 4.3 and 4.4).

The disease severity was significantly higher in 2009 compared to 2008 in Tigoni location while in Marimba there was no significant different, however in 2008 season it took shorter time for the controlled plots to be wiped by the diseases on both locations.

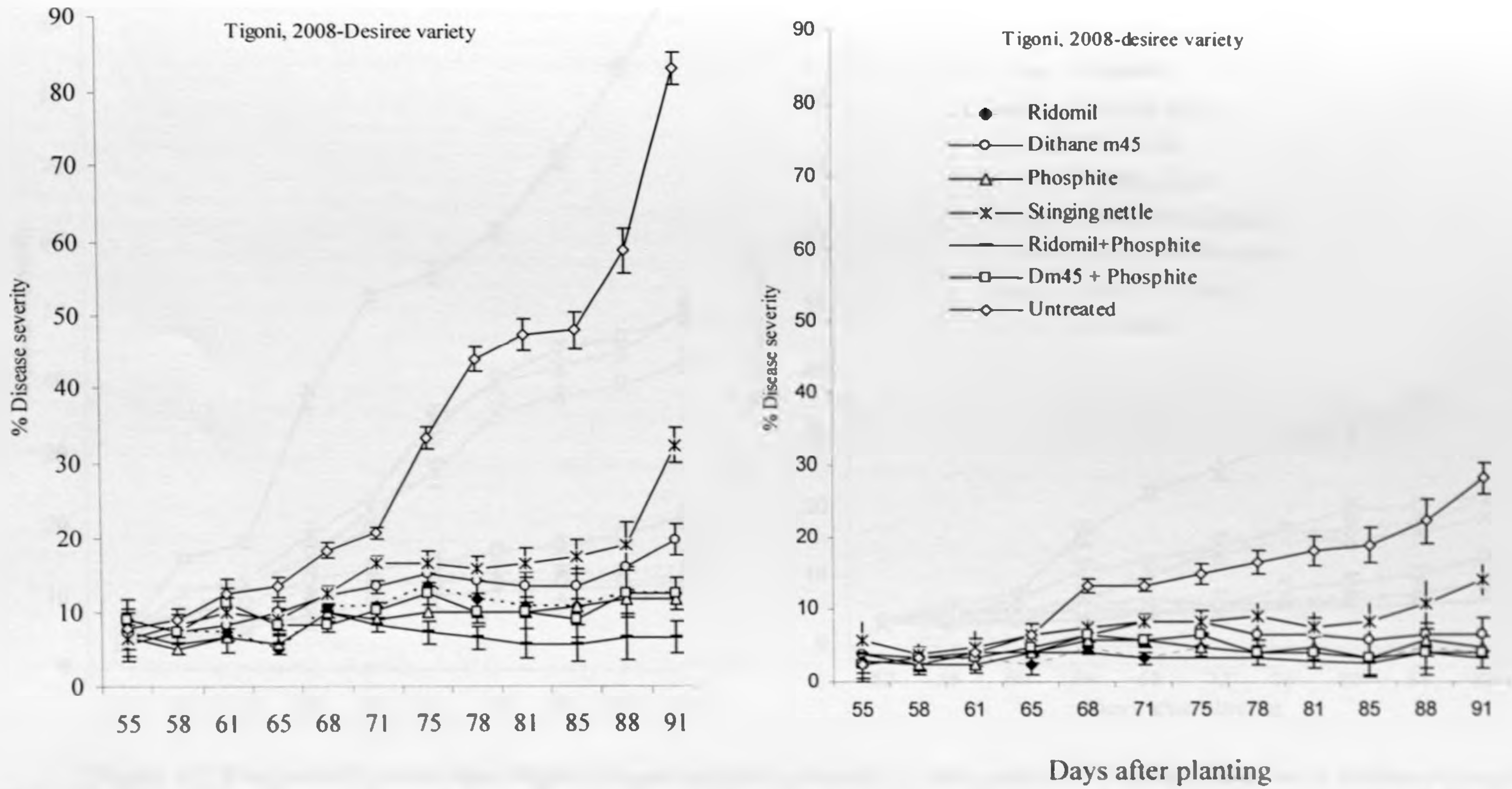

Figure 4.1 Progress of potato late blight (*Phytophthora infestans*) in two potato cultivars treated with different fungicides at Tigoni Research Station during 2008 cropping season (Verticar Bars indicate SE of the mean)

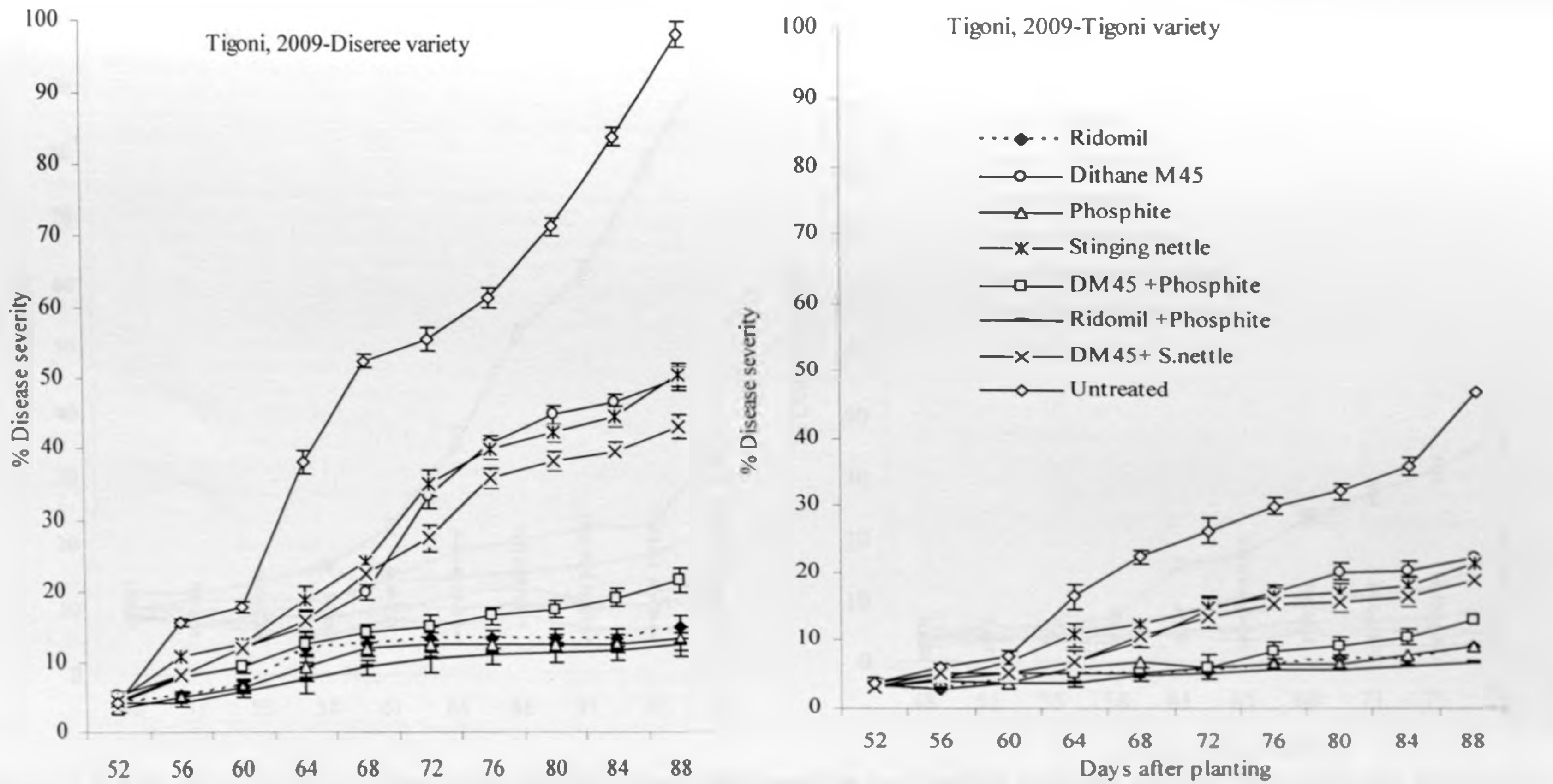

Figure 4.2 Progress of potato late blight (*Phytophthora infestans*) in two potato cultivars treated with different fungicides at Tigoni Research Station during 2009 cropping Season (Verticar Bars indicate SE of the mean)

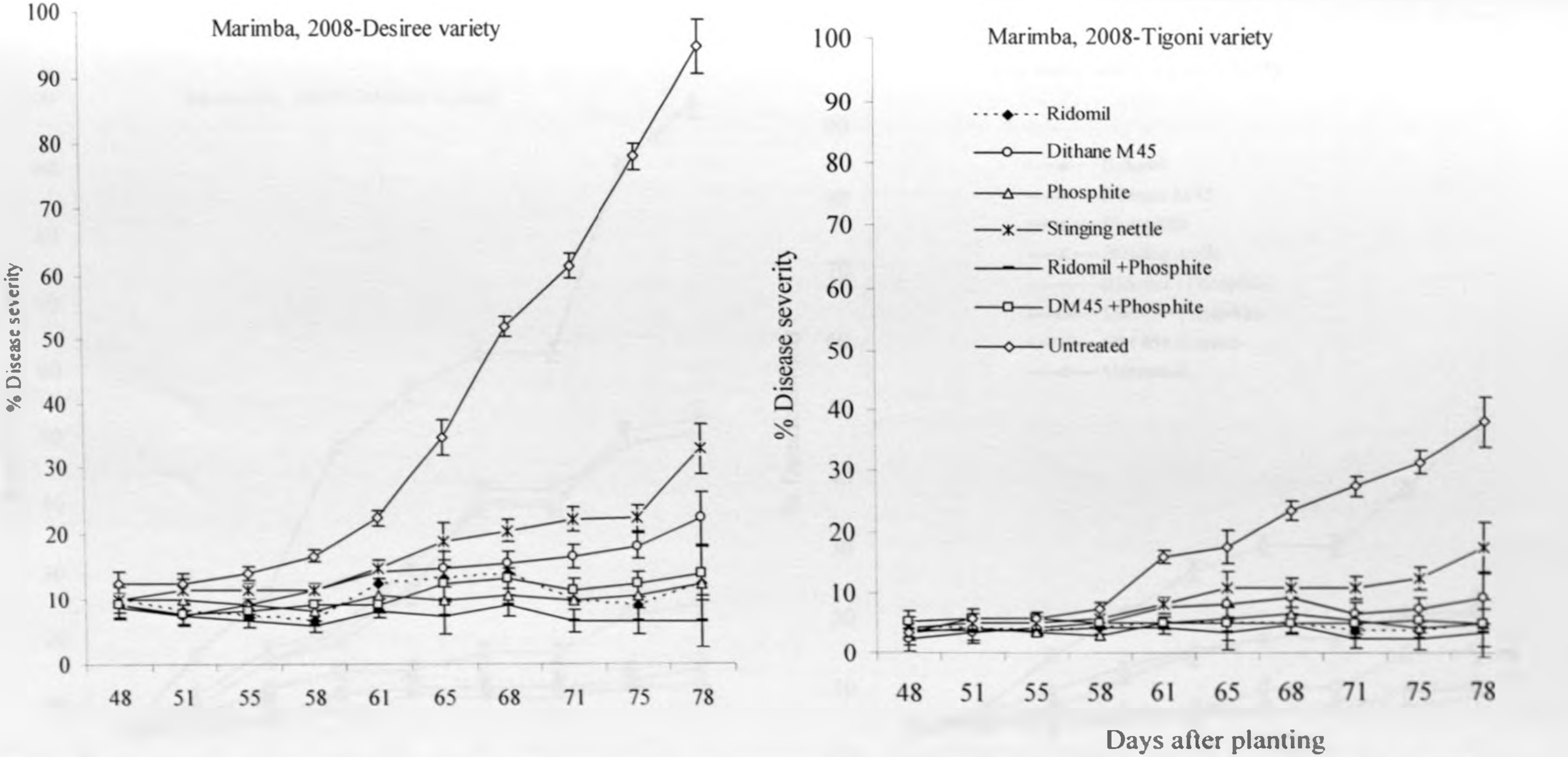

Figure 4.3 Progress of potato late blight (*Phytophthora infestans*) in two potato cultivars treated with different fungicides at Marimba Research Station during 2008 cropping season (Verticar Bars indicate SE of the mean)

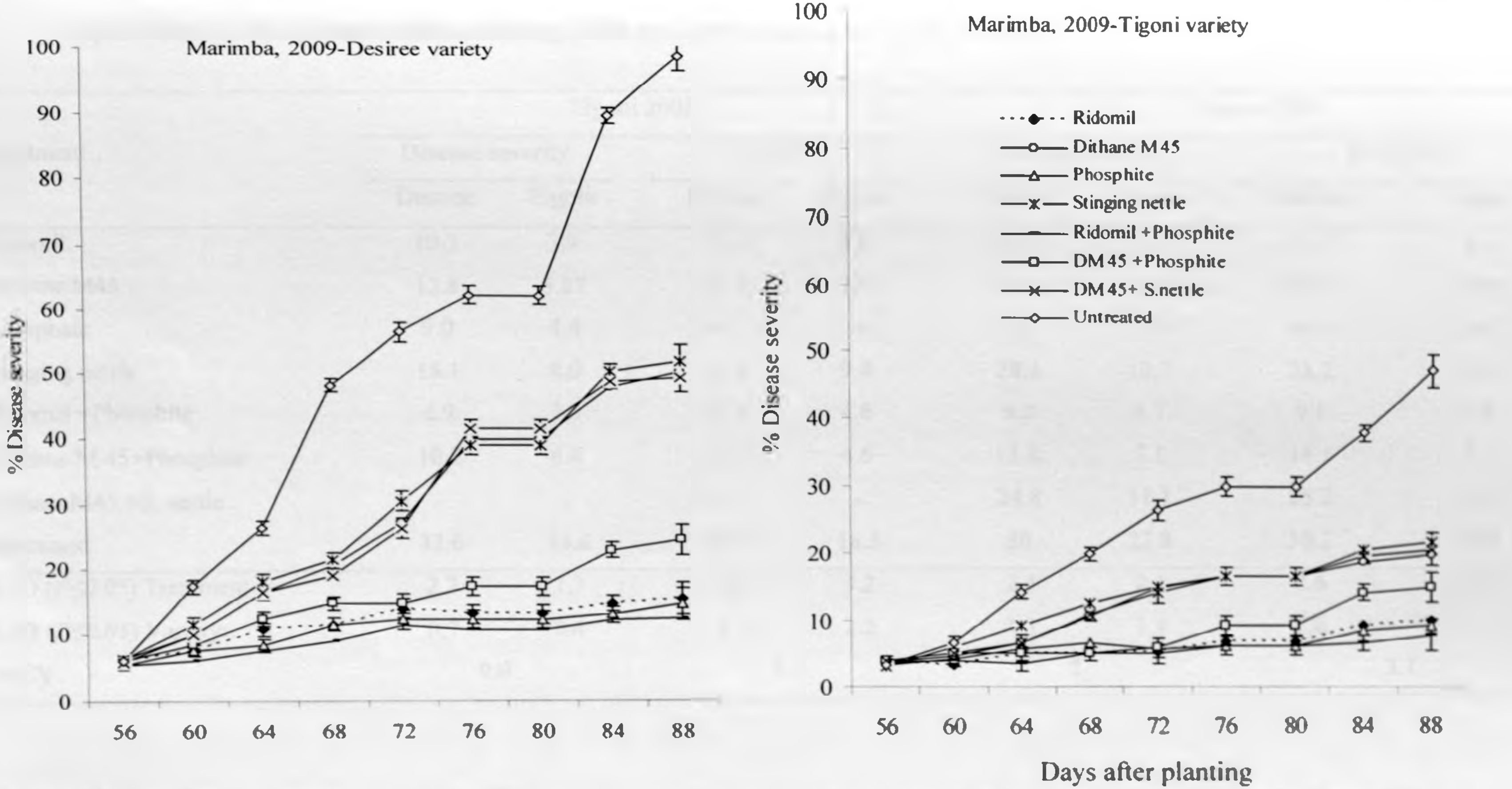

Figure 4.4: Progress of potato late blight (*Phytophthora infestans*) in two potato cultivars treated with different fungicides at Marimba Research Station during 2009 cropping season (Verticar Bars indicate SE of the mean)

Table 4.2. Mean % disease severity and Relative area under disease progress curve (R.AUDPC) of different fungicides applications in two potato cultivar during 2008 and 2009 seasons in Tigoni location

Treatment	Tigoni 2008				Tigoni 2009			
	Disease severity		RAUDPC		Disease severity		RAUDPC	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	10.2	3.9	13.0	4.9	10.9	5.5	11.2	5.6
Dithane M45	12.8	5.87	15.5	7.5	27.6	12.5	27.1	12.6
Phosphate	9.0	4.4	11.3	5.6	10	5.9	10.3	5.8
Stinging nettle	15.1	8.0	18.4	9.8	28.4	12.7	28.2	13.1
Ridomil +Phosphite	6.9	3.5	8.9	4.6	8.8	4.7	9.1	4.8
Dithane M 45+Phosphite	10.2	4.4	12.8	4.6	13.8.	7.1	14.1	7.1
DithaneM45 +S. nettle	-	-	-	-	24.8	11.1	25.2	11.4
Untreated	33.6	13.6	39.4	16.5	50	22.8	50.2	22.8
LSD ($P \leq 0.05$) Treatment	2.7	1.7	6.6	3.2	2.4	2.1	2.6	2.2
LSD ($P \leq 0.05$) Variety	0.7	0.4	2.7	2.2	1.3	1.1	1.6	1.3
% CV	9.0		7.1		2		3.1	

Table 4.3 Mean % disease severity and Relative area under disease progress curve (R.AUDPC) as a result of different fungicides applications in two potato cultivar during 2008 and 2009 seasons in Marimba location.

Treatment	Marimba 2008				Marimba 2009			
	Disease severity		RAUDPC		Disease severity		RAUDPC	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	10.4	4.4	16.4	5.5	12.1	6.2	12.4	6.3
Dithane M45	14.0	6.3	17.1	11.1	29.1	13.3	24.7	12.9
Phosphate	10.2	4.6	16.2	5.8	11	6.2	11.4	6.4
Stinging nettle	17.8	9.1	21.5	14.3	30.5	13.7	30.4	13.9
Ridomil +Phosphite	7.4	3.7	12.7	4.7	9.6	5.3	10	5.5
Dithane M 45+Phosphite	10.8	5.4	16.4	6.9	15.8	8.1	16.4	8.1
DithaneM45 +S nettle	-	-	-	-	29	12.8	28.9	12.1
Untreated	40.1	18.0	49.1	24.3	53.2	24.5	52.5	24.1
LSD ($P \leq 0.05$) Treatment	2.5	2.3	8.4	5.2	2.8	2.2	3.1	2.7
LSD ($P \leq 0.05$) Variety	0.94	0.8	5.1	4.7	1.4	1.1	1.1	1.2
% CV	8.5		8.1		0.4		1.3	

4.4.2. Effect of different fungicides on size and number of late blight lesions

Mean lesion length increased steadily in both sites and also in both cultivars in all the plots where the different fungicides were applied while the lesion expanded rapidly from untreated plots from 61day in Tigoni and 58 day in Marimba location during 2008 season (Figure 4.5). In Tigoni site the lesion length expanded up to 27.0 mm and 15.0 mm in Desiree and Tigoni variety respectively within 46 days after disease was noticed in unsprayed plots (Table 4.4). While in Marimba site the lesion expanded up to 37mm and 24 mm in Desiree and Tigoni in unsprayed plots respectively within 30 days in the unsprayed plots (Table 4.4).

There was a significant difference in lesion length expansion in all sprayed plots using different fungicides compared to unsprayed plot in both sites. Potato crop treated with Ridomil 2.5 kg/ha followed by Phosphite 2.5l/ha, had a significantly less lesion length expansion compared with other treatment but did not differ significantly with plots treated with Phosphite 2.5l/ha alone in Desiree cultivar at Tigoni site, Similarly Ridomil 2kg/ha followed by Phosphite 2.5l/ha had significantly less effect on expansion rate in Tigoni variety and differed significantly with all other treatments. In both sites Ridomil alone did not differ significantly with Phosphite, however incorporating Phosphite in plot treated with Ridomil increased the efficacy significantly in reduction of lesion expansion rate .Similarly Phosphite resulted to significance less lesion expansion rate compared to Dithane M45 alone when sprayed to Desiree cultivar in both site but the efficacy of Dithane M45 was increased when Dithane M45 was alternated with phosphoric acid and did not differ significantly with the phosphoric acid alone and this was observed in both sites for Desiree cultivar (Table 4.4). However in both sites incorporating Phosphite to plot treated with either Ridomil or Dithane M45 alone did not lead to any significant reduction in lesion size expansion in Tigoni cultivar (Table 4.4). Stinging nettle extract also reduced the lesion size significantly (*$P<0.05$*) compared to untreated plots but it also had significantly low effect compared with other treatment in Marimba site. Stinging nettle extract did not differ significantly in lesion expansion rate when compared with Dithane M45 in Tigoni site on both potato cultivars (Table 4.4).

There was a significance difference between the cultivars in lesion expansion rate with Desiree cultivar having the significantly higher rate of lesion expansion than Tigoni cultivar in both sites (Figure 4.5, 4.6, 4.7 and 4.8). Similarly Marimba site resulted into a higher lesion expansion compared to Tigoni site and this was observed during the two seasons.

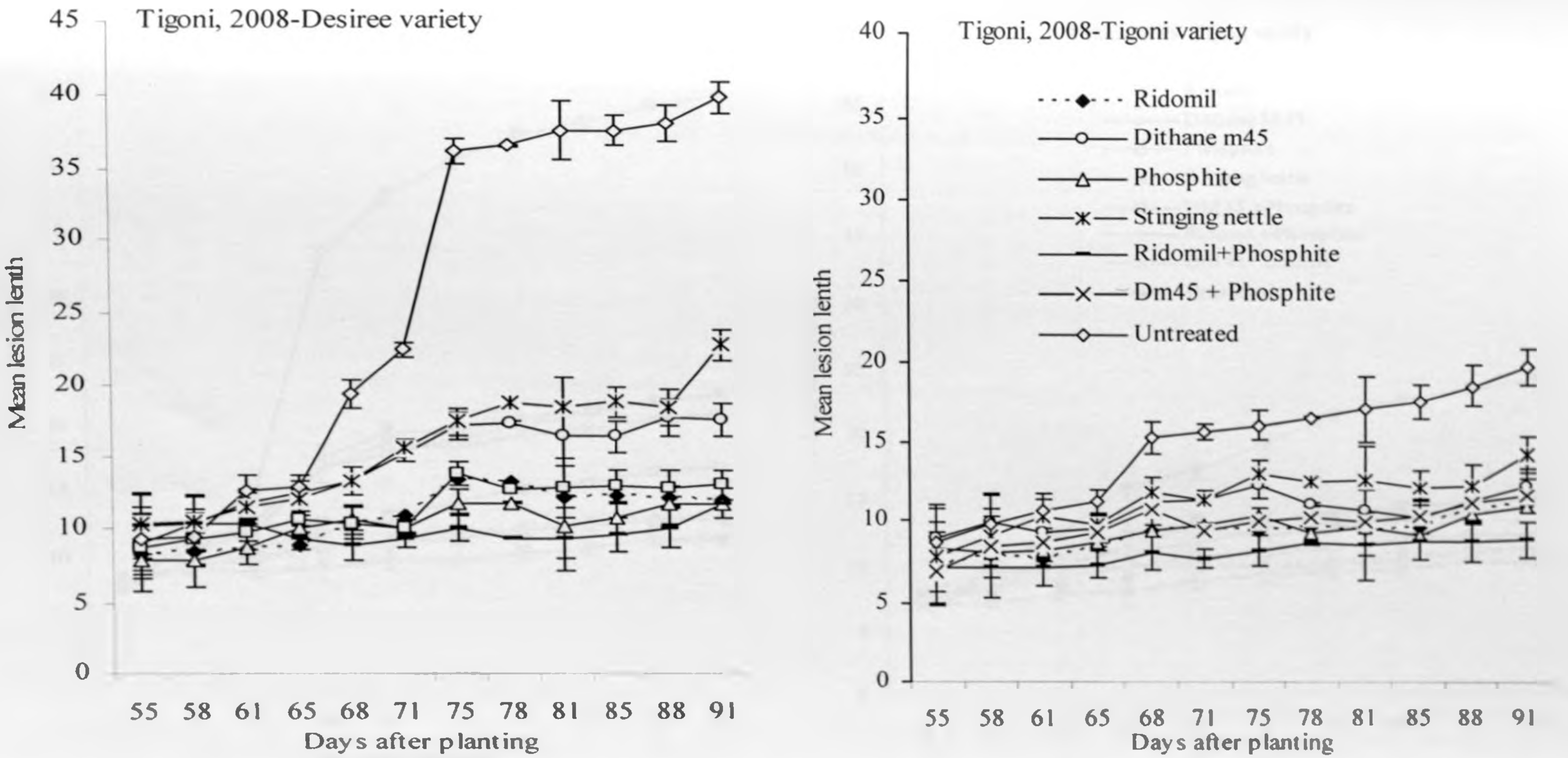

Figure 4.5. Mean lesion length on two potato cultivars planted at NPRC, Tigoni as a result of application of different fungicides during 2008 season (Verticar Bars indicate SE of the mean)

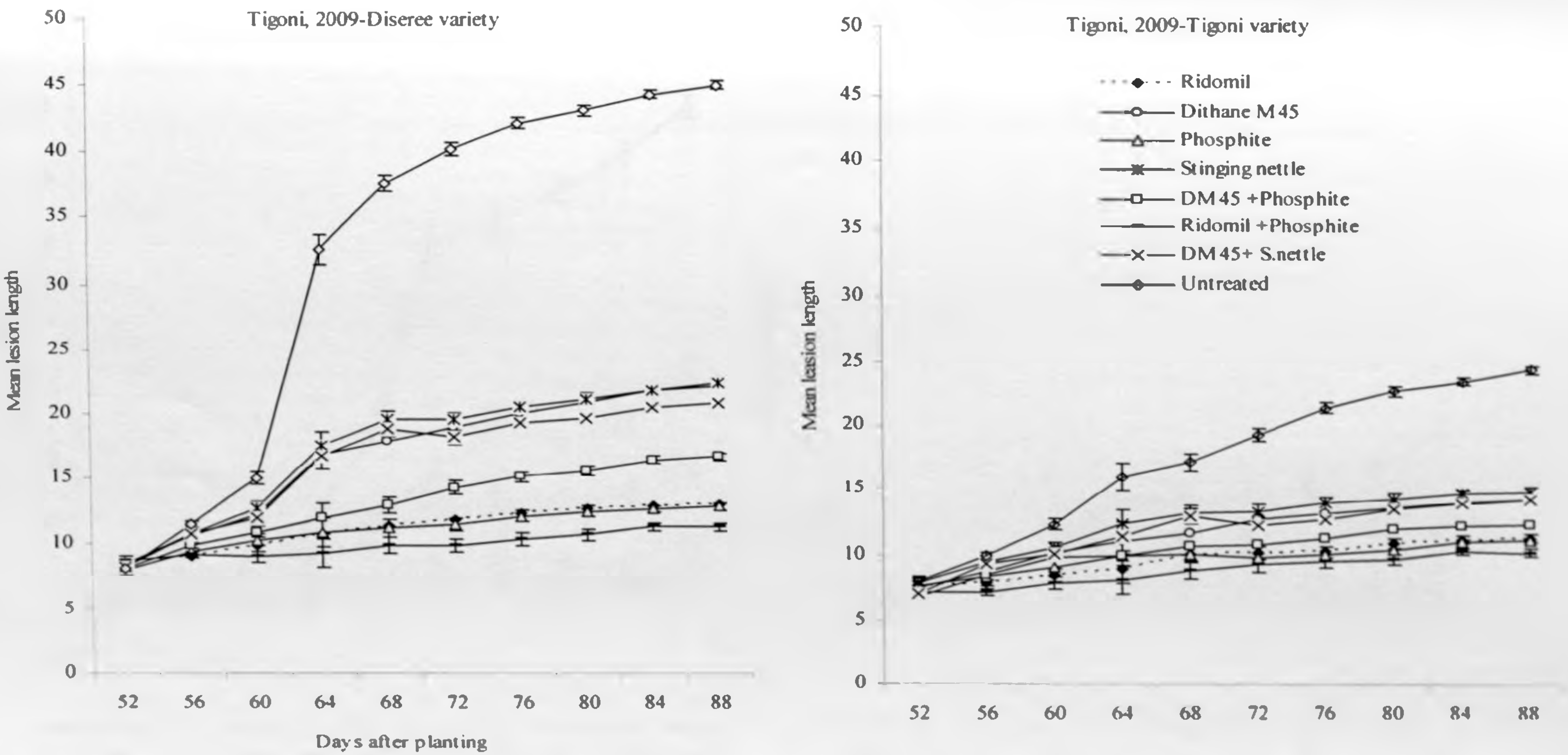

Figure 4.6. Mean numbers of lesions on two potato cultivars at NPRC, Tigoni as a result of application of different fungicides during 2009 season. (Verticar Bars indicate SE of the mean)

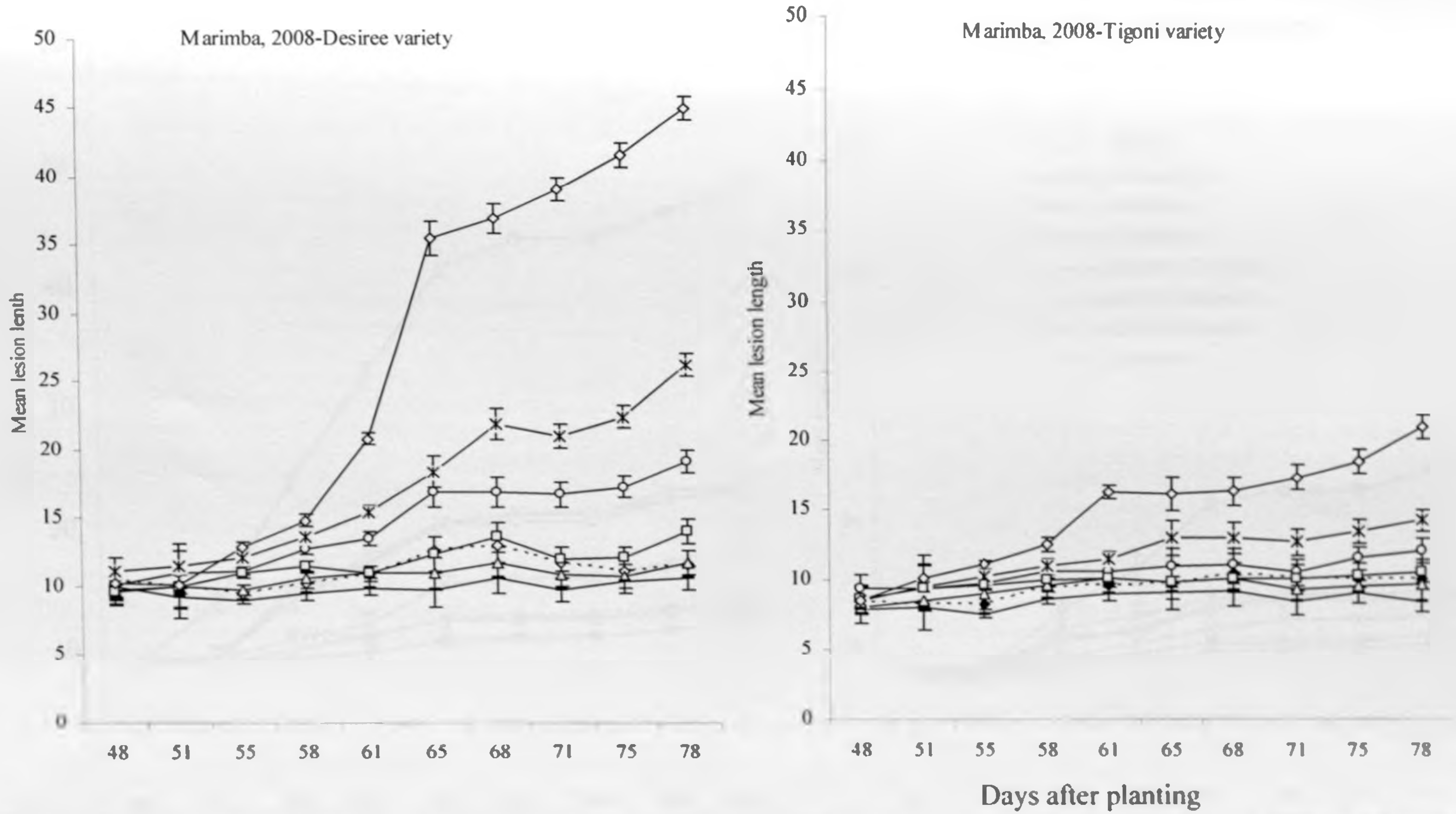

Figure 4.7. Mean lesion length on two potato cultivars planted at Marimba as a result of application of different fungicides during 2008 season (Verticar Bars indicate SE of the mean)

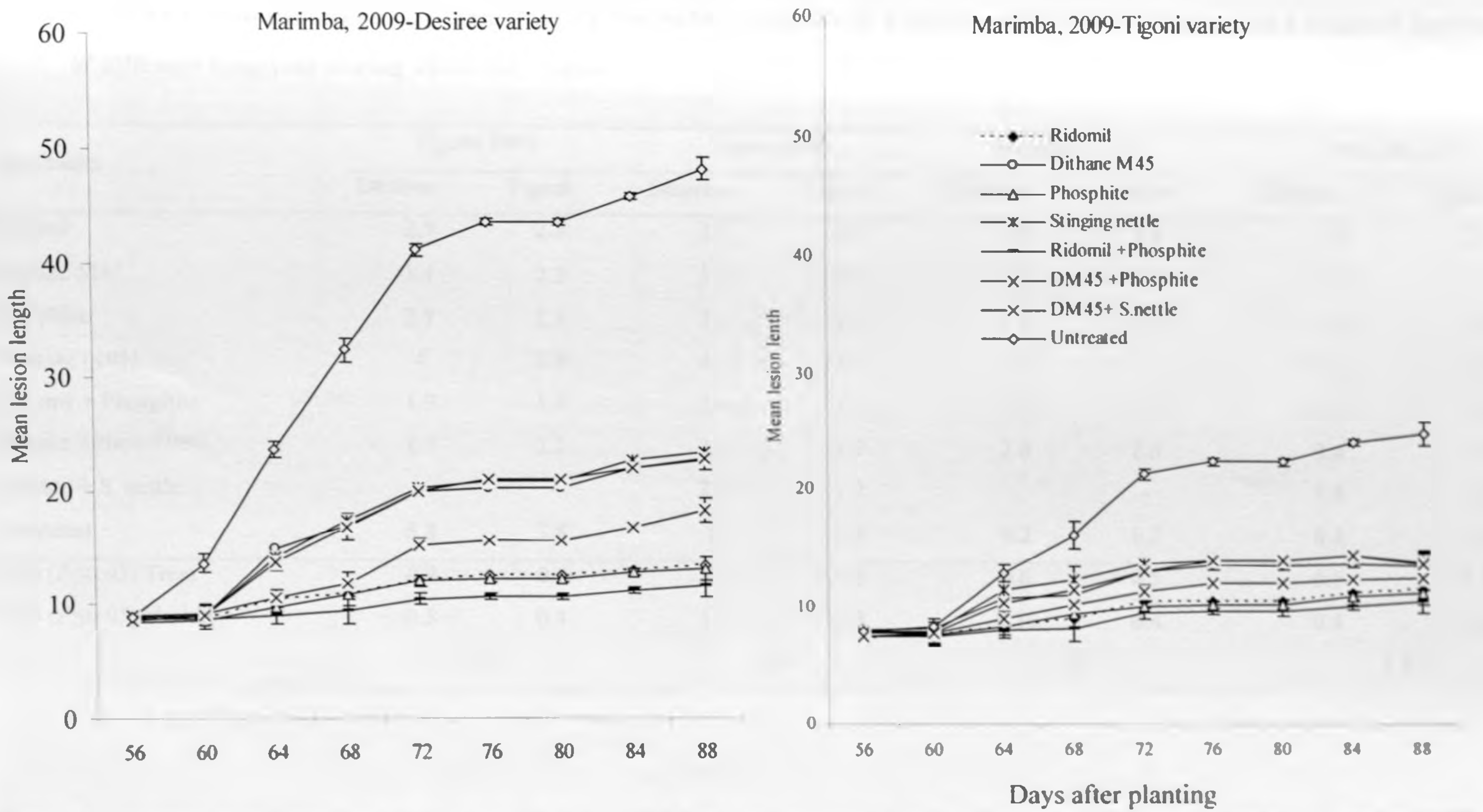

Figure 4.8. Mean lesion length on two potato cultivars at Marimba as a result of application of different fungicides during 2009 and 2009 season (Verticar Bars indicate SE of the mean)

Table 4.4 Mean Lesion expansion rate (mm/day) on two potato cultivars at Tigoni and Marimba locations as a result of application of different fungicide during 2008/2009 seasons

Treatments	Tigoni 2008		Tigoni 2009		Marimba 2008		Marimba 2009	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	2.5	2.3	2.8	2.4	2.8	2.3	2.9	2.4
Dithane M45	3.4	2.3	5.7	2.9	3.7	2.7	4.3	3
Phosphite	2.7	2.1	2.8	2.5	2.8	2.4	2.8	2.4
Stinging nettle	4	2.9	4.3	3.2	4.3	3	4.4	3.1
Ridomil + Phosphite	1.9	1.4	2.4	2.1	2.5	1.2	2.2	2.1
Dithane +Phosphite	2.7	2.2	4.2	2.2	2.8	2.5	3.4	2.7
Dithane + S. nettle	-	-	2.5	2.2	-	-	4.8	3..0
Untreated	6.8	3.8	8	4.4	9.2	6.2	8.4	4.5
LSD ($P \leq 0.05$) Treat	0.9	0.7	3	0.5	0.6	0.5	0.5	0.4
LSD ($P \leq 0.05$) Variety	0.5	0.4	1.5	0.3	0.33	0.4	0.4	0.3
% CV	2.9		2.9		1.9		0.8	

The lesion numbers at Tigoni site remained low between 58 and 61day after planting for all treatments but increased rapidly in plot treated with stinging nettle extract and in unsprayed treatment (Figure 4.9, 4.10).The same trend was observed in marimba although the increase of lesion number was faster than in Tigoni site and it started after 55 days after planting during 2008 (Figure 4.11, 4.12).

There were significant (*P<0.05*) differences observed in all treatments in mean lesion density compared to plots where no treatment was applied in both potato cultivars and sites (Table 4.5). Lesion density at Tigoni site increased in all plots without fungicide spray application attaining a peak at 46 days after the disease was noticed (figure 4.8.), while in Marimba the peak was attained within 30 days after the disease onset. Mean lesion density between the two potato cultivar differed significantly in both sites with cultivar Tigoni having less number of lesion than Desiree cultivar (Table 4.5). At Tigoni site, plots where Ridomil followed by Phosphite was applied resulted to the least number of lesions when sprayed on both cultivar and differed significantly with Dithane M45 (2.5kg/ha), followed by Phosphite (MDP), Dithane M45 alone and stinging nettle extract on Desiree cultivar, however on Tigoni cultivar Ridomil followed by Phosphite (MDP) did not differ significantly with all the fungicides except where the stinging nettle extract was applied. Dithane M45 alternated with Phosphite and Dithane M45 applied alone differed significantly when compared with Phosphite (Table 4.5).

Ridomil alone did not differ significantly with plot treated with Phosphite alone and incorporating Phosphite in plots treated with Ridomil did not increase the efficacy of Ridomil in reducing the lesion number in both potato cultivars (Table 4.5). However Dithane M45 applied alone differed significantly with Phosphite in Desiree cultivars and incorporating phosphite in plots treated with Dithane M45 alone did not lead to an increased efficacy in reducing the lesions number. Also stinging nettle extract differed significantly with all the fungicides except Dithane M45 on Desiree cultivar (Table 4.5). At Marimba, a similar trend was observed where Ridomil followed by Phosphite resulted in the lowest number of lesions when sprayed on both cultivars; however it did not differ significantly with plots treated with Ridomil and Phosphite alone on both cultivars (Table 4.5, Figure 4.11, 4.12).

Phosphate, when applied alone did not differ significantly with Ridomil alone in both cultivars but differed significantly with Dithane M45 in both cultivars (Table 4.5). Incorporating phosphoric acid to plots treated with Dithane M45 significantly led to increased efficacy in reducing the lesion numbers.

Stinging nettle extract led to significantly reduced number of lesions compared with untreated plot, however plots treated with stinging nettle extract had significantly higher number of lesions compared to plots treated with other fungicide on both cultivars except on plots where DM45 was applied on Desire cultivar. The mean number of lesion on both sites were not significant different during 2008 season.

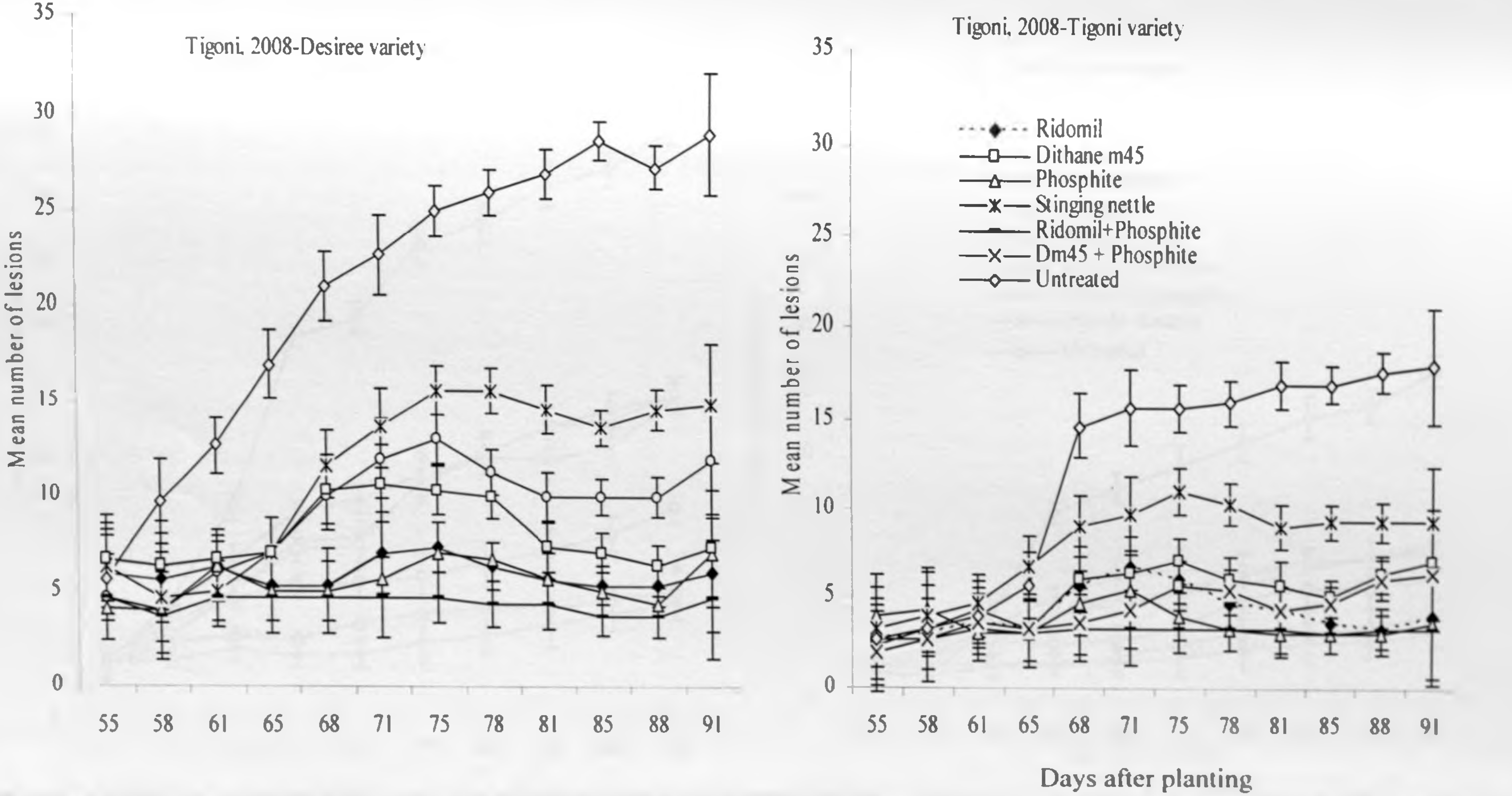

Figure 4.9. Mean numbers of lesions on two potato cultivars at NPRC, Tigoni as a result of application of different fungicides during 2008 season. (Verticar Bars indicate SE of the mean)

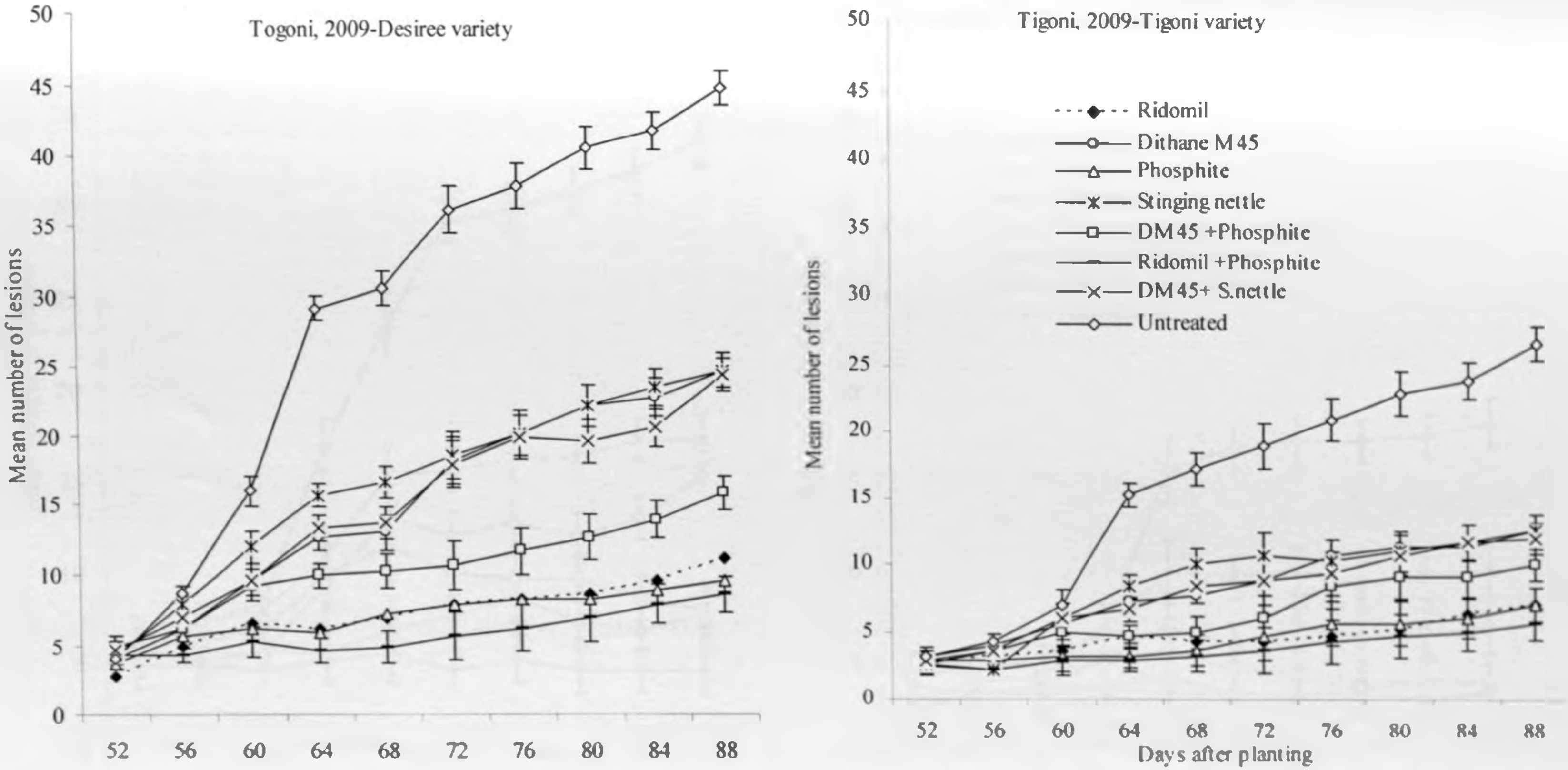

Figure 4.10 Mean number lesions on two potato cultivars planted at NPRC, Tigoni as a result of application of different fungicides during 2009 and 2009 seasons. (Verticar Bars indicate SE of the mean)

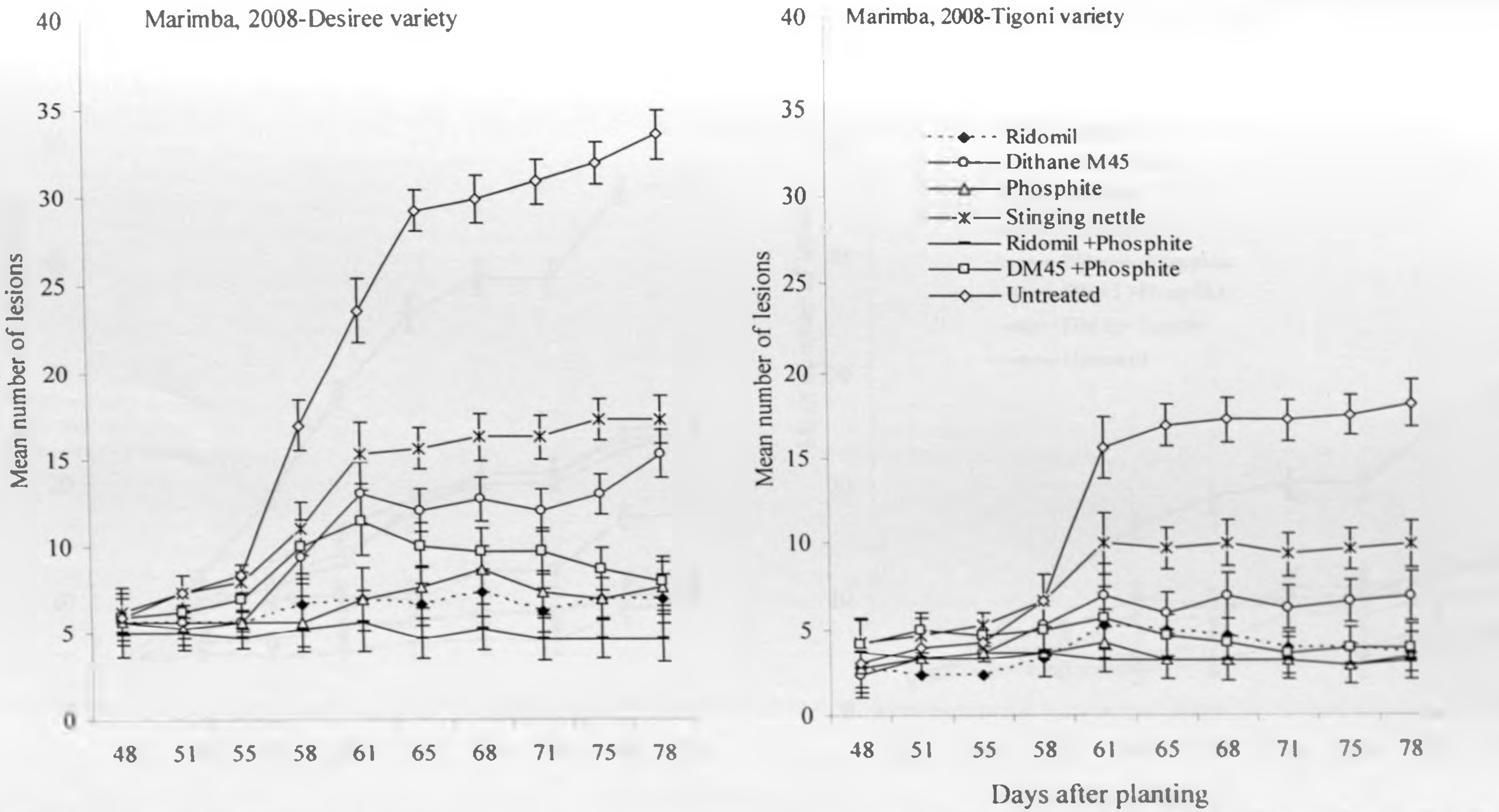

Figure 4.11. Mean numbers of lesions on two potato cultivars at Marimba as a result of application of different fungicides during 2008 season. (Verticar Bars indicate SE of the mean)

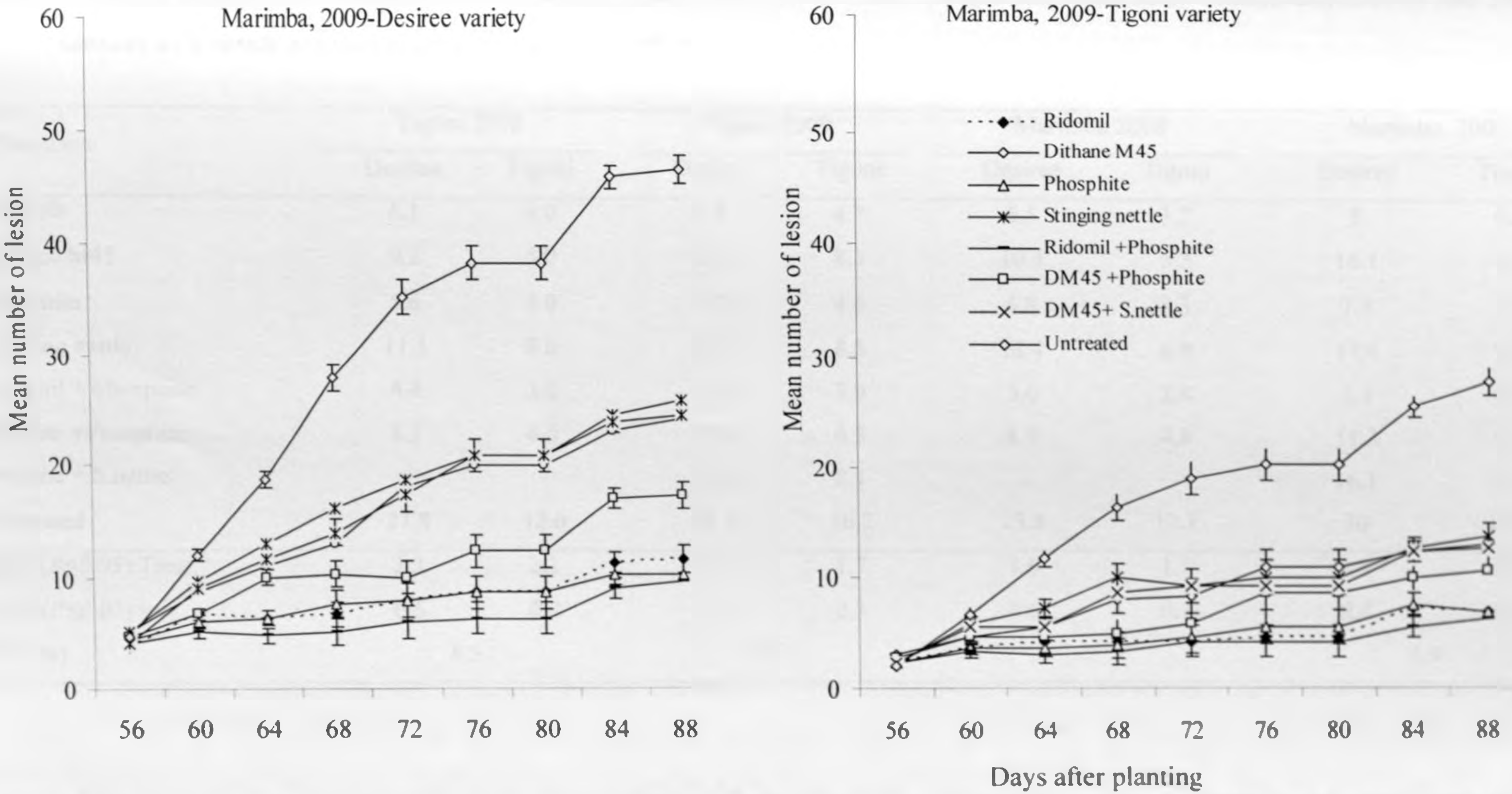

Figure 4.12. Mean numbers of lesions on two potato cultivars at Marimba as a result of application of different fungicides during 2009 season. (Verticar Bars indicate SE of the mean)

Table 4.5. Mean Number of Lesions per Plant on two potato cultivars at NPRC, Tigoni and Marimba Station durin 2008 and 2009 seasons as a result of different fungicides applications

Treatments	Tigoni 2008		Tigoni 2009		Marimba 2008		Marimba 2009	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	6.1	4.0	7.5	4.7	6.5	3.7	8	4.8
Dithane M45	9.2	5.0	15.5	8.3	10.4	5.3	16.1	8.6
Phosphite	5.6	4.0	7.2	4.6	6.8	3.3	7.8	4.9
Stinging nettle	11.1	8.0	16.7	8.8	13.4	8.0	17.4	9.1
Ridomil + Phosphite	4.4	3.0	5.9	3.9	5.0	3.4	6.3	4.1
Dithane +Phosphite	8.3	4.0	10.6	6.3	8.9	4.8	11.3	6.8
Dithane + S.nettle	-	-	15.1	8.1	-	-	16.1	8.6
Untreated	21.8	12.0	29.1	16.2	23.8	12.1	30	16.9
LSD ($P \leq 0.05$) Treat.	2.3	2.1	2.1	1.7	1.8	1.4	1.8	1.6
LSD ($P \leq 0.05$) var	0.6	0.4	0.4	0.3	0.6	0.7	0.4	0.4
CV (%)	8.5		10.1		7		6.9	

4.4.3 Effects of fungicides on tuber blight

Tuber blight incidence was observed during harvesting in all the treatments and in both locations (Table 4.6 & 4.8). There were significant ($p \leq 0.05$) differences among different treatments, locations and between the two potato cultivar in tuber disease incidence at harvest (Table 4.6 & 4.8). Tuber blight incidence on control plots, plots treated with Ridomil followed by Phosphite, Dithane M45 followed by phosphite, phosphite (MDP) and Ridomil did not show any significantce different on tuber blight incidence among themselves on Desiree cultivar, although they differed significantly with the tubers within the plots where Dithane M45 and stinging nettle extract were applied within the same cultivar (Table 4.6).Tuber blight incidence on Tigoni cultivar was significantly lower in potato crop when foliage was treated with phosphite and Ridomil either alone or in combination with conventional fungicide used in the management of potato late blight than when foliage was not treated. Stinging nettle extract did not differ significantly ($p \leq 0.05$) in tuber blight incidence at harvest with untreated control in Tigoni cultivar.

Plot treated with Dithane M45 had significantly higher tuber disease incidence in both potato cultivar compared to the plot where Dithane M45 was applied first followed by phosphoric acid. Potato crop treated with Ridomil alone did not show any significant different in both potato cultivar both at harvest and after storage. Incorporating phosphite in the late blight management had shown to improve the management of potato tuber blight. Tuber blight incidence differed significantly between the two potato cultivar with the cultivar Tigoni having significantly higher tuber blight incidence than Desiree cultivar (Table 4.6 and 4.7). Ridomil at 2.5 kg/ha followed by phosphite resulted in the lowest tuber disease incidence of 0.53% on Desiree cultivar while potato tuber from plots treated with Dithane M45 followed by Phosphite resulted in the lowest incidence on Tigoni cultivar (Table 4.6). Also potato crop treated with Ridomil alone had higher tuber disease incidence on Desiree cultivar while those potato tubers harvested from plot treated with Dithane M45, stinging nettle extract and unsprayed plots had the highest tuber incidence on Tigoni cultivar (Table 4.6).

The result also showed that tuber blight incidence after storage was not significantly different after application of various treatment on Desiree cultivar (Table 4.6). However on Tigoni cultivar tubers harvested from plot treated with Ridomil followed by phosphite and Phosphite alone had zero tuber disease incidences and differed significantly with those harvested from plot where stinging nettle extract was applied (Table 4.6). Stinging nettle extract resulted in the highest mean tuber blight incidence of 1.83% while the plot treated with Ridomil had the lowest mean tuber disease incidence of 0.17 although all the treatments were not significantly different

Tigoni Table 4.6. Tuber blight Incidence at harvest and after storage of two potato cultivars treated with different fungicide during 2008/ 20009 seasons at NPRC Tigoni

	Tigoni 2008				Tigoni 2009			
Treatments	Blight at harvest		Blight after storage		Blight at harvest		Blight after storage	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	1.4	5.2	0.3	1.3	0.8	2.6	0.5	0.9
Dithane M45	3.2	7.4	0.7	1.0	1.4	3.8	0.7	1.2
Phosphite	1.4	4.3	0.3	0.3	0.7	1.4	0.4	0.5
Stinging nettle	4.0	7.3	1.0	0.0	1.2	2.4	0.6	1.3
Ridomil + Phosphite	0.5	3.3	0.3	0.7	0.5	1.3	0.2	0.1
Dithane +Phosphite	0.7	2.8	0.7	0.7	0.9	2.6	0.7	1.1
Dithane M45 +S.nettle	-	-	-	-	0.4	1.2	0.2	0.4
Untreated	0.9	7.4	1.0	2.7	1.3	3.5	0.5	1.6
LSD($P \leq 0.05$) Treatment	2.5	2.1	1.9	1.6	1.4	1.2	1.1	0.8
LSD ($P \leq 0.05$) variety	1.0	0.7	0.5	0.3	0.2	0.32	0.3	0.2
CV (%)	24		57.1		11.6		24.8	

Table 4.7 Tuber blight incidence at harvest of two potato cultivars treated with different fungicide during 2008/ 20009 seasons at Marimba

Treatments	Marimba 2008		Marimba 2009	
	Tuber incidence at harvest		Tuber incidence after storage	
	Desiree	Tigoni	Desiree	Tigoni
Ridomil	1.4	1.3	0.7	1.5
Dithane M45	1.8	3.1	1.7	2.9
Phosphite	1.1	1.1	0.7	1.1
Stinging nettle	2.1	4.8	1.3	2.9
Ridomil + Phosphite	0.8	0.8	0.3	1.0
Dithane +Phosphite	1.5	2.5	1.1	1.9
Dithane M45 +S.nettle	-	-	0.8	2.0
Untreated	1.9	3.4	0.8	1.7
LSD ($P \leq 0.05$) Treatment	2.1	0.3	0.9	0.7
LSD ($P \leq 0.05$) variety	0.5	0.3	0.3	0.16
CV (%)		36.4		8.7

4.4.4 Effect of fungicides on tuber yield

In 2008 tuber yield was higher in Tigoni than Marimba in the unsprayed plots. The plants treated with Ridomil followed by Phosphite and Ridomil alone had the highest potato total yields (21.27 t/ha) and (19.2 t/ha) on Tigoni and Desiree cultivar respectively during 2008 short rain season (Table 4.9). The total tuber yield also differed significantly between all fungicide treatments and the control apart from potato plant where Dithane M45 was applied and this was the same in both cultivars. Similarly potato cultivar Tigoni in plot treated with stinging nettle extract did not result to significantly higher yield compared to unsprayed plot (Table 4.8).

Plants treated with Dithane M45 and Ridomil alone did not differ significantly in total yield compared to those plots where Phosphite (MDP) was incorporated with either of the two fungicides (Table 4.8, 4.9, 4.10 & 4.11). The application of Phosphite resulted in significantly higher total tuber yield compared to those treated with Dithane M45 on Desiree cultivar but it did not differ significantly on Tigoni cultivar (Table 4.8). Stinging nettle extract did not result in any significant increases in tuber yield when compared with untreated spray on Tigoni cultivar but resulted into significantly higher yield on Desiree cultivar (Table 4.8).

All the treatments resulted to a significantly ($P<0.05$) higher marketable yields compared to the plot where no fungicide was applied, except in plot where Dithane M45 was applied on Desiree cultivar which did not show significant difference compared to untreated plots. Potato crops where Ridomil and Ridomil followed by Phosphite (MDP) were applied resulted in the highest marketable yields of 16.93 and 18.53 in Desiree and Tigoni cultivars respectively (Table 4.10). Alternating Phosphite with Dithane M45 resulted to significantly higher marketable yield compared to plots where Dithane M45 alone was applied on Desiree cultivar but in Tigoni cultivar there was no significant effect (Table 4.8). Similarly, incorporating Phosphite on Ridomil treated plots resulted to significantly higher yield compared to potato plant where only Ridomil alone was applied on Tigoni cultivar but not on Desiree cultivar (Table 4.8 & 4.9.). On all the treatments, potato cultivar Tigoni resulted to significantly higher yield compared to Desiree cultivar, expect on plots where Ridomil alone was applied on Desiree cultivar resulting to significantly higher yield compared to Tigoni cultivar (Table 4.8). The marketable tuber yield ranged from 3.87 t/ha, 4.78 t/ha in untreated check on Desiree and Tigoni cultivar respectively to 16.93 t/ha on Desiree cultivar on plots treated with Ridomil alone and 18.53 t/ha on plots treated with Ridomil 2kg/ha followed by Phosphite (Table 4.8).

Application of Dithane M45 followed by Phosphite, Ridomil and Ridomil followed by Phosphite and Phosphite alone resulted in significantly higher number of tuber per hill (P<0.05) than the untreated plants in Tigoni (Table 4.8 & 4.9). Dithane M45, stinging nettle exract did not result significantly higher number of tubers per hill compared to the untreated plot in the Desiree cultivar. However all the applied treatment resulted to significantly (P<0.05) higher number of tuber per hill when applied to Tigoni cultivar. The incorporation of Phosphite only increased the number of tubers per hill significantly when alternated with Dithane M45 as compared to Dithane M45 alone in both potato cultivars. However when Phosphite was alternated with Ridomil it did not result to significantly higher number of tuber per hill as compared to the plot where Ridomil alone was applied.

Table 4.8: Effect of fungicides on number of potato tuber per hill, marketable yields and total tuber yields during 2008 season, at Tigoni

	Tigoni 2008					
Treatments	No of tuber/hill		Marketable yield(t/ha)		Total yield (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	7.5	11.0	16.9	13.4	19.2	16.0
Dithane M45	4.9	8.0	5.1	11.8	9.1	13.5
Phosphite	7.7	10.0	13.8	16.8	15.6	17.0
Stinging nettle	5.8b	8.0	9.4	10.8	7.7	12.0
Ridomil + Phosphite	7.1	12.0	14.2	18.5	18.3	21.0
Dithane +Phosphite	6.9	8.0	10.2	14.4	11.8	16.0
Untreated control	4.6	6.0	3.9	4.8	4.8	6.6
LSD ($P \leq 0.05$) Treatment.	1.6	1.4	5.2	4.8	6.8	5.8
LSD ($P \leq 0.05$) variety	0.6	0.4	1.6	1.6	0.7	0.6
CV (%)	6.6		26.6		17.1	

Table 4.9 Effect of fungicides on number of potato tuber per hill, marketable yields and total tuber yields during 2009 season, at Tigoni

Treatments	Tigoni 2009					
	No of tuber/hill		Marketable yield(t/ha)		Total yield (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	9.3	13	13.5	20.8	14.4	22.5
Dithane M45	6.7	8.3	7.9	14	9	15.3
Phosphite	9.7	14	14.4	23.3	15.6	24
Stinging nettle	6.3	8	10.1	13.3	11	15.3
Ridomil + Phosphite	10.7	14.3	17.5	25.7	18.6	26.47
Dithane +Phosphite	7.7	10.7	14.7	20.4	15.7	21.5
Dithane + S.nettle	6.3	7.7	9.5	12.2	10.2	13.4
Untreated control	5.3	6.3	6.9	8	7.5	9.9
LSD ($P \leq 0.05$) Treatment.	1.4	1.2	6.8	4.9	6.7	5.2
LSD($P \leq 0.05$) variety	0.7	0.7	2.6	2.2	2.5	1.9
CV (%)	10.6		18.5		16.5	

Table 4.10 Effect of fungicides on number of potato tuber per hill, marketable yields and total tuber yields during 2008 season, at Marimba

Treatments	Marimba 2008					
	No of tuber/hill		Marketable yield(t/ha)		Total yield (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	6.3	9.3a	18.4	15.5	20.1	16.4
Dithane M45	4.7	6.8	6.6	7.9	8.8	9.7
Phosphite	8.33	8.7	13.4	17	14.2	18
Stinging nettle	6.4	6.2	7.5	8.9	7.6	8.9
Ridomil + Phosphite	8.4	12.3	18.6	16.8	20.1	17.8
Dithane +Phosphite	5.9	6.6	8.4	10.0	8.8	11.1
Untreated control	3.3	4.0	3.8	4.3	5.3	5.8
LSD ($P \leq 0.05$) Treatment.	1.7	1.6	6.0	5.7	5.9	4.8
LSD ($P \leq 0.05$) variety	0.8	0.6	1.2	0.9	1.17	1.3
CV (%)	5.8		26.1		26.3	

Table 4.11 Effect of fungicides on number of potato Number of tubers per hill, marketable yields and total tuber yields during 2009 season, at Marimba

Treatments	Marimba 2009					
	No of tuber/hill		Marketable yield(t/ha)		Total yield (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	7.7	11.3	16.1	18.3	17.1	19.6
Dithane M45	6.0	8.3	7.5	8.9	8.6	9.9
Phosphite	8.3	10.7	13.4	15.3	13.7	16.2
Stinging nettle	5.9	6.5	6.9	8.6	8.1	9.7
Ridomil + Phosphite	10.3	13.7	21.4	20.9	22.7	21.9
Dithane +Phosphite	6.3	7.7	8.5	9.7	8.9	10.7
Dithane + S.nettle	5.7	7.7	7.7	8.4	8.8	10.1
Untreated control	4.0	4.3	3.9	4.4	5.6	6.0
Lsd ($P \leq 0.05$) Treat.	1.7	1.4	4.4	3.7	4.5	3.6
Lsd ($P \leq 0.05$) variety	0.6	0.7	0.9	.6	1.1	0.8
CV (%)	13.4		10		10.7	

More potato ware than either seeds or chatts were produced in Marimba than in Tigoni sites (Table 4.12 & 4.14). Ridomil 2.5 kg/ha followed by Phosphite treated potato plant resulted to more ware of 10.2t/ha and 10.9t/ha in Desiree and Tigoni cultivars respectively which differed significantly with unsprayed plot and stinging nettle exract on both cultivars (Table 4.15). Dithane M45 and stinging nettle extract were not significantly different in term of ware yield on both cultivars when compared with the unsprayed plots. Also Dithane M45 when applied alone differed significantly compared to plots treated with Ridomil alone, Phosphite alone and Dithane M45 followed by Phosphite. (Table, 4.14). Incorporating Phosphite to plot treated with Ridomil did not lead to significant increase of ware in both cultivars, while it led to significantly higher yield when incorporated to Dithane M45 treated plot in Desiree cultivar. Different potato cultivar did not differ significantly ($p \leq 0.05$) in ware production.

In terms of seed production there was no significant ($p \leq 0.05$) difference among all treatments in Tigoni cultivar, and the unsprayed treatment except in plots where Phosphite was applied which resulted to significantly higher seed than unsprayed plots, (Table 4.12 & 4.13), however plots treated with Ridomil differed significantly ($p \leq 0.05$) with those treated with Dithane M45 on Desiree cultivar (Table 4.12 & 4.13). Similarly chatt yields from unsprayed plots and all the other treated plots with different fungicide was not significantly different ($p \leq 0.05$). All treatments produced more ware than seed or chatts, except stinging nettle and unsprayed plots where more seeds were produced than ware (Table 4.11, 4.12, 4.13, 4.14, 4.15). Mean yield of the two cultivars and in two locations were not significantly different.

Table 4.12. Yields in (t/ha) of different grades of potato tubers harvested as a result of application of fungicides on two potato cultivars during short rain 2008 croping season at Tigoni

Treatments	Tigoni 2008					
	Ware weight (t/ha		Seed weight (t/ha)		Chatt weight/ha	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	7.9	9.0	8.5	5.8	1.9	1.6
Dithane M45	3.4	5.0	2.6	5.9	0.9	1.2
Phosphite	8.1	10.0	4.9	8.1	1.4	1.0
Stinging nettle	3.3	4.0	6.0	7.4	1.2	1.2
Ridomil + Phosphite	10.0	11.0	4.6	7.6	1.4	0.9
Dithane +Phosphite	7.2	9.0	4.9	6.5	0.73	1.2
Untreated control	2.1	2.0	3.7	3.6	0.7	1.4
LSD ($P \leq 0.05$) Treatment.	1.0	0.8	1.0	0.7	0.5	0.4
LSD($P \leq 0.05$) variety	27.8	25.4	19.8	21.4	16.0	15.4
CV (%)	27.8		19.8		16.0	

Table 4.13. Yields in (t/ha) of different grades of tubers harvested as a result of application of fungicide on two potato cultivars during long rain 2009 croping season at Tigoni

Treatments	Tigoni 2009					
	Ware weight (t/ha)		seed weight (t/ha)		Chatt weight (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	6.7	13.7	6.8	7.2	0.9	1.7
Dithane M45	3.6	7.7	4.3	6.3	1.1	1.3
Phosphite	9.1	14.3	5.3	8.2	1.2	0.7
Stinging nettle	5.2	8.2	4.9	6.2	0.8	0.9
Ridomil + Phosphite	11.3	17	6.2	8.7	1.1	1
Dithane +Phosphite	10.8	12.5	4	8	1	1
Dithane + S.nettle	5.5	5.4	4	6.8	0.7	1.2
Untreated control	2.9	2.8	4	5.2	0.7	1.9
LSD ($P \leq 0.05$) Treatment.	4.4	4.1	4.1	3.9	0.5	0.3
LSD ($P \leq 0.05$) variety	1.7	1.6	1.6	1.4	0.27	0.2
CV (%)	21.2		19.4		17.2	

Table 4.14. Yields in (t/ha) of different grades of tubers harvested as a result of application of fungicide on two potato cultivars during short rain 2008 croping season at Marimba

Treatments	Marimba 2008					
	Ware weight (t/ha)		Seed weight (t/ha)		Chatt weight (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	13.7	11.5	5.0	4.0	1.8	0.9
Dithane M45	4.4	3.7	2.1	4.2	1.2	0.8
Phosphite	8.9	11.4	4.5	5.6	0.8	0.9
Stinging nettle	2.8	4.0	4.7	5.0	1.0	0.9
Ridomil + Phosphite	12.5	11.8	6.0	5.1	1.3	1.0
Dithane +Phosphite	5.4	5.5	3.0	4.5	0.4	1.1
Untreated control	1.7	1.3	2.1	3.0	0.7	1.1
LSD ($P \leq 0.05$) Treatment.	5.5	4.9	2.5	2.6	0.7	0.5
LSD ($P \leq 0.05$) variety	0.6	07	1.1	0.8	0.4	0.4
CV (%)	25.4		29.8		33.4	

Table 4.15. Yields in (t/ha) of different grades of tubers harvested as a result of application of fungicide on two potato cultivars during long rain 2009 croping season at Marimba

Treatments	Marimba 2009					
	Ware weight (t/ha)		Seed weight (t/ha)		Chatt weight (t/ha)	
	Desiree	Tigoni	Desiree	Tigoni	Desiree	Tigoni
Ridomil	11.7	12.7	4.4	5.5	0.9	1.3
Dithane M45	3.6	3.7	3.9	5.2	1.1	1.0
Phosphite	9.6	10.2	3.8	5.0	0.6	0.9
Stinging nettle	3.2	4.3	3.7	4.3	1.2	1.1
Ridomil + Phosphite	17.1	15.3	4.4	5.5	1.2	1.o
Dithane +Phosphite	5.6	6.0	3.7	3.7	0.4	1.0
Dithane + S.nettle	3.8	4.3	3.9	4.1	1.1	1.7
Untreated	1	1.3	2.9	3.1	1.7	1.6
LSD ($P \leq 0.05$) Treatment.	4.0	3.6	2.0	1.8	0.7	0.5
LSD ($P \leq 0.05$) variety	0.89	0.7	0.7	0.5	0.3	0.2
CV (%)	5.8		17.2		22	

4.5 Discussion.

4.5.1 Effect of Phosphite and stinging nettle on foliar disease incidence

From the study all the tested fungicide reduced the disease severity; AUDPC, lesion size and lesion number and led to an increased yields of two the potato cultivars. This results support the second hypothesis that Phosphoric acid and stinging nettle extract can reduce disease development in established epidemics. However, it was also clearly revealed that the two bio-product do not have equal effects. It is also clear that the dynamics of an epidemic and the environmental conditions in which the epidemic occurs can influence the efficacy of a particular fungicide in suppressing an epidemic.

Phosphite and Ridomil treated plants had less late blight damage compared to the plants treated with Dithane M45 and stinging nettle extract. In general, better absorption of phosphite under various environmental conditions has been shown to be effective for control of diseases incited by oomycetes (Easton & Nagle, 2004, 2007). The relatively higher late blight disease recorded in Dithane M45 treated plants suggests that protectant fungicides by themselves may not be sufficient in late blight control under some tropical environments. Therefore this study did not agree with reports by (Namanda *et al.*, 2004) which showed that Dithane M45 can be effective in reducing the impacts of late blight. It is possible that timing and interval of application for mancozeb may not have been sufficient to optimize its effectiveness in this experiment. Difference in late blight severity was observed between the 2008 and 2009 seasons.

The differences in disease levels between different locations may be attributed to the difference in weather conditions that were experienced. Variation in late blight incidence and severity has been reported previously among the various locations of Kenya (Nyankanga *et al.*, 2004; Olanya *et al.*, 2006).

First, in each of the location of investigation, Ridomil followed by phosphoric acid had the highest suppressive effect on established epidemics. The effect was most easily detected in both sites and in Desiree cultivar, which is susceptible to late blight. Phosphite was more effective on late blight management than Dithane M45 and Ridomil when applied alone and this was in agreement with results published by Mayton *et al.*, (2008). Incorporating Phosphite on either Ridomil or Dithane M45 fungicide improves the suppressive effect of Ridomil and Dithane M45 on established epidemics and more so on the protectant fungicide (Dithane M45) on the susceptible cultivar.

Ridomil followed by Phosphite consistently suppressed lesion size in the field plots on both locations and potato cultivars. All the fungicides tested had a significant effect on lesion numbers. Although Ridomil followed by Phosphite reduced the lesion expansion rate, it did not differ significantly compared to Phosphite and Ridomil when applied alone. Johnson *et al.*, (2004) reported that Phosphoric acid was effective in controlling lesion development and sporulation and these results were consistent with this study in that phosphoric acid was found to be effective in reducing the lesion sizes as well as spore productions.

Phosphite applied alone and when incorporated with conventional fungicides was more effective in suppressing an established epidemic in both locations. Nonetheless, the results of our experiments indicate that Dithane M45 and stinging nettle might have some useful efficacy in the field environmental conditions. Phosphite 2.5 l/ha would probably have greater efficacy in temperatures cooler as it was observed in Marimba and this may be attributed to the factors probably contributed to this difference. Most pathogen races has developed resistance to metalaxyl (Stevenson, 1993) and this may explain why incorporating Phosphite increases the effictiveness against late blight strains in infected tissue than is Ridomil or Dithane M45 alone as it was reported by Stevenson (1993) that alternating fungicides may lead to an improved efficacy.

4.5.2 Effect of Phosphite and stinging nettle on tuber disease incidence

Phosphite (MDP) has shown to have useful potential to suppress tuber and foliar blight in an integrated late blight management strategy. A review by Erwin and Ribeiro (1996) stated that phosphites had been used to control 19 species of *Phytophthora* (Erwin and

Ribeiro 1996). The result of this study revealed that there was substantial variance in the amount of tuber blight among different fungicide in both locations and seasons and this was in consistent with previous reports by (Dorrance and Inglis, 1998; Platt and Tai, 1998). The variation in tuber blight control amongst the different treatments and the control could be attributed by the fact that tuber blight incidence has been reported to be associated with epidemic severity in the foliage (Fry *et al.*, 1983). The Fry research group (Fry *et al.*, 1979) and (Stewart *et al.*, 1994; Platt and Tai, 1998) had previously reported that tubers from plots that are not protected by fungicide sometimes have a lower incidence of tuber blight than do tubers from plots that have been protected and this was in agreement with finding of this study observed in Desiree variety, although in Tigoni variety the untreated plots had higher tuber disease incidence though it did not differ significantly with those plots which were treated with stinging nettle extract and Dithane M45. The epidemic on unprotected plants proceeds much more rapidly than the epidemic protected plants which may suggest that the supply of sporangia from lesions in the foliage over the tubers may be over shorter time period for unprotected plant, also the rapid epidemic on unprotected plants limits tuber size, and it has been reported that smaller tubers are smaller targets for sporangia.

Epidemics in plots with different treatments may peak in terms of sporangia production at different times due to differences in environmental conditions, with one time being more favorable to tuber blight than another. The soil environment under plants with foliage is more humid than the soil environment under plants without foliage which tends to be more dry and this difference in soil environment may influence the initiation of tuber blight. Most previous studies have indicated that Phosphoric acid applied in the field and to harvested tubers can protect those tubers from *P. infestans* upon subsequent inoculation (Cohen and Coffey, 1986; Cooke and Little, 2001; Johnson et al., 2004) from their experiment, Cooke and Little (2001) provided evidence that foliar applications can have some effect on infections occurring during the growing season and this is confirmed by this study where significant differences were detected indicating that phosphonates either applied alone or in combination with ridomil ordithane M45 can protect tubers from infection prior to harvest, however stinging nettle extract did not show any significant effect on reducing tuber blight incidence both at harvest and after storage in both cultivars. In addition to investigating the incidence of tuber blight at harvest, the incidence of tuber blight in the tubers that were subsequently stored was also assessed. Foliar applications of phosphite in the field was demonstrated to be very effective in suppressing the development of subsequent blight as it resulted in tuber incidence of 1.1 % compared to control which had 1.7 on stored tubers and this result were consistence with earlier finding by Cooke, & Little (1996). When total tuber blight at harvest and after storage for one month was evaluated, tubers from phosphite 2.5t/ha treated plots had the least disease. Additionally, when the phosphite 2.5t/ha (phosphonates) was incorporated with other fungicide were compared to all treatments where convectional fungicide were applied alone, there was significantly less mean propotion of tuber blight in tubers from the convectional fungicide when Phosphite (MDP) was incorporated. Incorporation of Phosphite with Dithane M45 and Ridomil was found to be an effective strategy in management of potato late blight. Application of Ridomil followed by phosphite 2.5t/ha potato plots was found to be very effective in reduction of total tuber disease incidence on potato.

4.5.3 Effect of Phosphite and stinging nettle on tuber yield and yield components

Potato tuber yields were significantly different among different fungicide treatments in the experiment and this was in agreement with findings of Hamm. and Clough, (1999) who reported that different fungicides have different effect on potato tuber yields. The indication that more ware were produced compared to seed and chatt grades in both seasons in the experiment shows that these methods of controlling late blight and tuber blight are effective in promotion of ware production which may be due to translocation of photosyntate materials for along time.

Incorporating Phosphite into convectional fungicide has been found to be effective in the management of potato late blight. Dithane M45 treated plots had no significant difference in Desiree cultivar while alternating phosphate with dithane M45 resulted in significantly higher yields and wares compared to plots where dithane M45 alone was applied. Stinging nettle extract had no significant different in affecting yield and ware production compared to unsprayed plots in Tigini cultivar while it application affected the yields significantly in Desiree cultivar.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General discussion

Phosphite (MDP) and stinging nettle extract had positive effect on the reduction of disease severity, lesion length and lesion numbers and also led to higher yield when compared to untreated plots. Foliar application of Phosphite (MDP), stinging nettle extract, Ridomil and Dithane M45 resulted in different protection levels against late blight pathogen. Protection also decreased with potato variety, however the final protective effect resulting from Phosphite (MDP) when incorporated with Dithane M45 and Ridomil was higher than that obtained from each fungicide when applied alone and this was in agreement with earlier findings of Johnson *et al*,. (2004) who found that the effect of phosphorous acid varied depending on cultivar and pathogen, and also showed that the application schedule influences disease incidence and severity in the field.

Lesion expansion was suppressed, and lesion numbers were reduced when established lesions on potato plant caused by *P. infestans* were treated with all the tested fungicides. Phosphite alternated with Ridomil, phosphite 2.5t/ha (MDP) alone, and Ridomil alone reduced sporulation more consistently than Dithane M45 and stinging nettle extract when applied alone. The fungicide restricts disease development after initial disease establishment on potato plants. Fungicides, such as Phosphite 2.5t/ha have been reported to reduce sporulation (Schwinn, and Margot, 991). Also the result are agreeing with earlier findings by Johnson., *et al*.,(2004) and Vakoch, (1996) who observed that phosphonates and mancozeb were shown to reduce sporulation of *P. infestans* on potato leaves. Lesion density was less affected by the stinging nettle extract and Dithane M45 fungicides in the study, although phosphonates were more effective than Dithane M45 whereas their greatest effect was on reducing the disease development and lesion size. In the study, phosphoric acid when alternated with convectional fungicide did restrict lesion expansion and inhibit disease development better than the conventional fungicides when applied alone. Stinging nettle extract resulted in reduced disease development and lesion size and it was found to be effective in managing late blight when applied after initial symptoms were noticed.

Results were generally consistent from the two experiments when fungicides were applied before the initial symptoms were observed on potato plant. Phosphonate plus Ridomil when applied reduced disease development, lesion number and size. Late blight is extremely dificult to manage in the Kenyan highland once the disease is present in a potato field, because after row closure the microclimate created by crop canopy usually favors infection which spread rapidly whenever the conditions are favorable. In addition, duration of epidemic development is potentially long because the canopy closes relatively early and the growing season is long. *P. infestans* lesions on potato stem are important epidemiologically because they enable the late blight pathogen to perpetuate more readily during hot weather than leaf lesions (Johnson, 2004). Fungicides that limit sporulation and lesion expansion on stems may help abate late blight out breaks in fields, especially when disease levels are still relatively low. Inhibiting sporulation on potato foliage between tuber bulking and harvest may reduce the incidence of tuber infections. Fungicide application schemes that included phosphonate provided slightly better control of late blight in a North American fungicide trial than schemes that did not include them (Inglis, .*et al* /1998). Restricting lesion expansion may also be beneficial in reducing tuber infection by limiting total sporulating plant tissue in the crop canopy. Fungicides need to be integrated into a total disease management program and used in response to identified needs for maximum efficiency and benefit (. Fry, 1977, Fry, Apple, and Bruhn, 1983). However, disease may appear in a field before management practices are applied. Effective management of late blight after the disease is reported in a field includes using effective fungicides and application methods which was also reported by (Bruhn, *et al*,.1982, Hamm, and Clough, 1999,) and shortening fungicide application intervals, decreasing the frequency of sprinkler irrigation applications, and irrigating when the crop canopy will be wet for the least amount of time. With regard to fungicides, however, the most economical and effective timing for application is before infection (Fry, 1977, Schwinn, 1991).

It was also found that late blight incidence was reduced in tubers after harvest during 2008 season when the two potato cultivars were treated with phosphite 2.5t/ha alone, Dithane M45 alternated with phosphite 2.5t/ha, Ridomil alternated with phosphite 2.5t/ha and Ridomil alone in the tuber susceptible cultivar. Posphite 2.5t/ha had been reported to be able to control crop diseases caused by oomycetes (Cooke and Little 1996, 2001), both through a direct effect inhibiting oxidative phosphorylation in oomycete metabolism (McGrath 2004), and by an indirect effect stimulating the plant's natural defense responses (Smillie *et al.*,1989). Incorporation of phosphite in integrated crop management programmes might reduce fungicide use and also production costs, while maintaining disease levels under the threshold of economic damage. Incorporating phosphite 2.5t/ha with the convectional fungicide used was consistently better than either the phosphite 2.5t/ha or conventional fungicide alone. Potato cultivar had an effect on the effectiveness of posphite 2.5t/ha. Incidence of blighted tubers at Tigoni from tubers from resistant cultivar, Desiree was equivalent or more with that of the untreated plots. In Tigoni site, incidence of late blight in tubers was most effectively reduced when Dithane

M45 and Ridomil were alternated with phosphite 2.5t/ha and less reduced in tubers harvested where either of the fungicide alone and stinging nettle extract were applied. In 2009 season incidence of blighted tubers of Desiree cultivar was less reduced when foliage was treated with Ridomil, Dithane M45, phosphite 2.5t/ha and stinging nettle extract than when it was not treated.

Potato tuber yields were significantly different in all treatments compared to unsprayed plots. This shows that all the treatments had different effects on yields and farmer choice should be based on efficiency to control late blight and improved yields.

5.2. Conclusions and recommendations

This study revealed that Phosphite and stinging nettle were effective and may be used as practical method of reducing foliar late blight and tuber blight disease incidence. Incorporating phosphite with either Ridomil or Dithane M45 was the most effective in both foliar and tuber blight control than when either the phosphoric acid or the two fungicides were used alone. Although tuber blight disease incidence was recorded on plots where stinging nettle extract was applied, the study showed that stinging nettle extract resulted to increased yields. It was evident from the result that efficacy of Dithane M45 and Ridomil fungicides would be improved by incorporating Phosphite in the management strategies. Phosphoric acid and stinging nettle extract are affordable as late blight management strategies and they are also enviromental friendly and compatible with current production practice and require no investment inspecialized technology.

Application of Ridomil or Dithane M45 proved to be effective in the management of potato late blight which was in agreement with the previous work done, incorporating phosphite with either of the two fungicides spray played a great role on late blight reduction. The study also revealed that all the treatments resulted on increased yield . Although spraying Ridomil alone seemed to have the same effect on late blight management with spraying Ridomil followed by phosphite in short term, single solutions are ineffective in the long term. This is because of development of resistance by pathogen which has been already reported in some area. Therefore, farmers' selection of the management methods to be used should be based on the long term effect.

Based on the above conclusions, the following recommendations can be made:

1) Phosphite (MDP) and stinging nettle extract can be recommended as efficient methods of foliar blight management in potato production, however stinging nettle extract is less efficient in tuber blight management.

2) Alternating Ridomil or Dithane M45 with phosphite in the management of potato late blight strategy should be recommended as a long term efficient methods of both foliar and tuber blight management. However optimal application interval of phosphoric acid should be evaluated and established to enable effective control as well as farmers to achieve maximum benefits.

3) Further trials should be carried out to evaluate the bioactive molecules present in the stinging nettle extract and the quantity of those chemicals which are responsible for suppressing the late blight pathogen.

4) More attention should be given to breeding and screening more late blight resistant and acceptable cultivars that would lead to decreased fungicide use and thus promote more integrated environmentaly friendly strategies for control.

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APPENDICES

Appendix 1. Average temperature, relative humidity, rainfall, and number of rainy days recorded during the cropping months in 2008 and 2009 at Marimba

Year	Month	Rainfall (mm)	Relative humidity (%)	Temparature (C)	Rainy days
2008	September	89.1	78.4	16.2	10
2008	October	61.0	85.7	17.9	3
2008	November	408.2	86.2	16.4	19
2008	December	231.4	83.3	16.0	17
2009	January	0	48	19.8	0
2009	February	99	50	19.2	0
2009	March	133	84.7	18.8	14
2009	April	199.0	81.8	16.7	16
2009	May	189.0	81.8	16.7	16
2009	June	112.5	87.5	18.1	15

Source: Kenya Agrometeological Department, Nkubu, Meru

Appendix 2. Average Minimum & Maximum temperature, relative humidity, rainfall, and number of rainy days recorded during the cropping months in 2008 and 2009 at Tigoni

Year	Month	Rainfall (mm)	No. of rain days	Maximum temperature	Minimum temperature
2008	September	96.8	11	15.7	13.5
2007	October	56.8	8	17.2	14.3
2007	November	89.6	11	16.7	13.3
2007	December	67.1	7	17.4	13.9
2009	January	62.1	2	17.8	14.4
2009	February	85.5	1	17.9	14.6
2009	March	304.1	15	19.2	14.4
2009	April	492.1	16	16.9	14.4
2009	May	276.4	13	16.9	14.3
2009	June	132.1	8	14.7	13.6

Source: Kenya Agrometeological Department, NPRC, Tigoni

Appendix 3. Fungicide, Manufacturer, rates, amount and number of applications evaluated for late blight (*Phytophthora infestans*) control on potato during 2008 and 20009 cropping seasons at Tigoni and Marimba.

Fungicide Compound	Manufacturer	Active ingredients	Application Amount of a.i	Application rates	Number of applications
Phosphite	J.H, Biotechnology Laboratories	Mono and dipotassium phosphorous acid		2.5l(L/ha	5
Ridomil	Syngenta	Metalaxyl + Mancozeb	64% mancozeb,4 metalaxyl	2.5 (Kg/ha)	2
Dithane M-45		Mancozeb	84% mancozeb	2.5kg/ha	5
Victory	Fluence LTD (Agios Athanasius)	Metalaxyl	70% metalaxyl	5,100ppm	-
Stinging nettle	-	-	-	2.5kg/ha	5