



Effect of ridomil application regime in late blight (*Phytophthora infestans*) management on yield and pesticide residue in potato tubers

J. Kilonzi^{*1}, J. Mafurah², M. Nyongesa³, J. Oyoo⁴

^{1,2}Department of Crops, Horticulture and Soil, Egerton University, Njoro, Kenya

^{1,2,4}Kenya Agricultural Livestock and Research Organization (KALRO), Tigoni, Limuru, Kenya

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Abstract

Ridomil® (Metalaxyl 4% + Mancozeb 64%) is one of the most extensively used fungicide to manage late blight globally. The overuse of the chemical has recently raised human health and environmental concerns. The objective of the study was to determine the effect of Ridomil® application regime on yield and Metalaxyl residue in potato tubers. A field experiment was conducted where Ridomil® was applied at 7, 14 and 21 day spray intervals using manufacturer's recommended dosage of 2.5g L⁻¹. Fresh tuber samples were also randomly collected from Limuru and Wakulima markets in Kiambu and Nairobi counties respectively. Data on disease severity (RAUDPC), incidence and yield were collected and analyzed using SAS software. Assay of metalaxyl residue was extracted using Soxhlet method and quantified by Gas Chromatography Mass Spectrometer. Higher tuber yield and lower disease score were obtained in plots sprayed at 7 day interval compared to unprotected plots. Samples collected from Limuru market and plots sprayed at 7 and 14 day spray interval had 0.09, 1.69 and 0.08 mgkg⁻¹ respectively. Metalaxyl residues observed in samples from Wakulima market and plots sprayed at 21 day spray interval were below the limit of detection. The results indicate that potato consumed in most parts of Kenya could be unsafe following the application of Ridomil® at weekly or biweekly spray intervals.

* Corresponding Author: J. Kilonzi ✉ kilonzijack@gmail.com

Introduction

Human population is expected to reach 9.7 billion people by 2050 requiring 70% food increase to keep pace with the growing demand for food (Béné *et al.*, 2015). The current food demand, in the face of 21st century challenges, has led to unsustainable practices to improve food production so as to feed the ever increasing world population (Savary *et al.*, 2017). Increase in food production faces a number of challenges including reduced land size exerting pressure on existing agricultural systems (Mora 2014) Efforts to increase food production has been directed to dependence on agrochemicals including fertilizers, insecticides and fungicides (Hirooka and Ishii, 2013). Ogawa *et al.* (2011) found that, the use of Isotonil® resulted in additional rice yield due to increased effectiveness in leaf blast control. Potato has been identified as a key crop in enhancing food security and farm income worldwide (Devaux *et al.*, 2014). The tuber crop is consumed by over one billion people globally (Geremewe, 2019). However, potato is one of the most pesticide demanding agricultural crop because it is also prone to diverse biotic stresses. These biotic stresses include late blight disease caused by *Phytophthora infestans*, one of the major causes for low yields globally (Majeed *et al.*, 2017) whose management is solely through agrochemicals (Carputo and Frusciante, 2005).

Use of chemical fungicides contribute to increased yield due to their high efficiency in managing plant diseases (Kuai *et al.*, 2017). However, extensive use of pesticides to manage biotic stresses has led to nearly 38 billion USD spent on the chemicals each year (Pan-Germany, 2012). Gianessi and Reigner (2005) reported that without fungicides to protect vegetable crops, yield losses of 50-95% could occur. Chemical fungicide overuse to manage late blight has become a major practice globally (Wesseling, 2001; Lamichhane, 2017) leading to accumulation of chemical residues into soil and food system implying a threat to nature and ecosystem (Palma *et al.*, 2014). In addition, the chemical residue pose human health problems especially if they enter the food chain (Rahman *et al.*, 2014 ; Hussain *et al.*, 2009). Furthermore, the emergence of new fungicide

resistant *Phytophthora infestans* strains has instigated an increase in fungicide use (Kromann *et al.*, 2009) and a concomitant short interval applications to manage late blight (Majeed *et al.*, 2017b). The effect has been an increase in dissipation in potato tubers that could pose health hazards to (Juraske *et al.*, 2009 ; Popp *et al.*, 2013). Moreover fungicide drifts cause unintentional poisoning to farm workers and consumers (Lee *et al.*, 2011). The level of toxicity depends on the length and frequency of fungicide exposure which in severe cases, long term exposure cause coma and death (Pan-Germany, 2012).

Bioaccumulation of pesticide in higher trophic food chain level has been associated with several acute and chronic illnesses due to prolonged exposure (Noyes *et al.*, 2009). Mostafalou and Abdollahi (2013) reported chronic human diseases affecting nervous, reproductive, cardiovascular and respiratory system as a result of long term exposure to fungicides. Pesticide residues have also been associated with cancer including brain cancer, renal cell cancer and prostate cancer (Hou *et al.*, 2012 ; Vinson *et al.*, 2011 ; Lee *et al.*, 2011; Heck *et al.*, 2011; Cocco *et al.*, 2013). Prolonged exposure to fungicides threaten the health of the unborn babies (Shim *et al.*, 2009). Therefore, it is necessary to understand chemical residues in the management of pests in relation to their efficacy (Duan *et al.*, 2016).

Ridomil® (Metalaxyl 4% and Mancozeb 64%) is one of the most widely used fungicide to manage late blight globally applied up to 10 -15 times per cropping season depending on weather conditions (Taylor *et al.*, 2013). Metalaxyl and Mancozeb overuse on potato to manage late blight pose health risks arising from residues in food and drinking water (Lopes *et al.*, 2009; Atmaca *et al.*, 2018). Mancozeb is non-systemic preventive fungicide (Gullino *et al.*, 2010) usually broken down by plant to Carbon disulphide which accumulate in potato leaves and in pericardial layer of tubers if extensively applied (Alam *et al.*, 2012). The molecule is carcinogenic and antithyroid compound (ethylenethiourea) when exposed to light (Panganiban *et al.*, 2004). Prolonged exposure to mancozeb is associated with dermatitis and eye

problems (Cole *et al.*, 1997) and therefore it is hazardous to occupational workers (Wesseling *et al.*, 2005). However studies have shown Mancozeb being non-systemic do not persist into the tuber tissues. Dissipation into the pericardial layer could be removed by peeling which is a usual preparation procedure for potato before cooking (Rani *et al.*, 2013; Alam *et al.*, 2012). Recently Kim (2017) reported that prolonged exposure to carbamates resulted in asthma and chronic problems including cancer. Metalaxyl is an acylanilide systemic fungicide with curative action against downy mildew and late blight. Multiple application especially during long rain season has been found to dissipate into crop products including potato tubers (Laurie *et al.*, 2015). Abass *et al.* (2007) observed that metalaxyl affects liver functionalities. Recently Zhang *et al.* (2017) found that metalaxyl influence breast cancer progression. Kinetics of Metalaxyl have been studied in soil, grapes, cabbages and potatoes using different rates of fungicides and insecticides (Wang *et al.*, 2014; Yan *et al.*, 2015) but the scientists did not explore effects of fungicide regimes over seasons. Standards for metalaxyl have been set by European Food Safety Authority of European Commission that has set acceptable limits of 0.08 mgkg⁻¹ body weight and acute reference dose of 0.05 mgkg body weight in daily consumption in directive (EC) 91/414/EEC.

The objective of this study was therefore, to determine the levels of metalaxyl dissipated into the tuber following Ridomil® application at different spray interval.

Materials and methods

Study area

The study was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) Tigon, in Limuru, Kiambu County in two seasons from November 2018 to July 2019. The Centre is located at latitude 10°9' 22" south and longitude 36°4' 72" east. The area is situated at altitude of 2100 m above sea level and receives rainfall of 1800mm per annum. Temperatures ranges from 10°C to 25°C (Jaetzold *et al.*, 2006). Weather experienced in this area provide conducive conditions for late blight development during potato growth.

The experiment was conducted in a fallow field which had not been cropped with potato for the previous three years.

Planting and crop management

Tubers of seed grade II (60mm) of *Shangi* variety were obtained from KALRO Tigon. Primary and secondary cultivation were conducted during dry period in September 2018 and March 2019 before planting to remove weeds and break soil clods. Planting was done at the onset of rains. Diammonium Phosphate (DAP) at rate of 500kg ha⁻¹ was applied and mixed with soil in planting furrows. Weeds were managed manually while insect pest were controlled using Duduthrin (lambda cyhalothrin) (Twiga chemical company, Kenya) at a rate of 3.5m L⁻¹ which is abroad spectrum insecticide. Top dressing was done using Calcium Ammonium Nitrate (CAN) at rate of 440kg ha⁻¹ four weeks after transplanting. Harvesting was done on January and July in the short and long rain season respectively.

Fungicide application

Ridomil® at 2g L⁻¹ (manufacturer recommended dosage) was applied at 7 (manufacturer recommended interval), 14 and 21 day spray interval. The application began 22 days after emergence when late blight symptoms were noticed which depicts farmers practice. The negative control plots were left unprotected from late blight. Knapsack sprayer was calibrated prior to every spraying regime so as to deliver spray volume of uniform discharge. Spray drifts were managed using polythene paper and 2 metres path between the spray regimes.

Experimental design

Four fungicide spray regimes were compared namely: unsprayed, 7, 14 and 21 days interval. These treatments were laid in randomized complete block design with five replications. The experimental plots measured 3m × 3m with crop spacing of 0.75m × 0.3m separated by a path of 2 m width between the plots.

Data collection

Weather data were collected daily from KALRO Tigon weather station located about 100 m away from the trial site. Late blight severity and incidence were taken on weekly basis.

Severity was evaluated on the basis of the proportion of diseased foliage on a scale of 0 to 5 where 0 = healthy, 1 = one fresh lesion (small circular water soaked spot), 2 = up to 25% lesion plus foliar blight, 3 = up to 50% lesion, necrotic, foliar and stem blight, 4 = up to 75% lesion, necrotic, foliar and stem blight and slight defoliation and 5 = 100%; defoliation. The results were summarized to Relative Area Under the Disease Progress Curve (RAUDPC) as described by Yuen and Forbes (2009). At maturity, potato tubers were harvested from the inner rows of each plot and inspected for tuber blight symptoms. Tubers were graded into ware (>60mm), seed (30 to 60mm) and chatt (<30mm) grades based on Kenya Plant Health Inspectorate Service (KEPHIS) potato grading system and counted before weighing using Standard weighing scale. Yield data were converted to tonnes per hectare.

Sample Collection

Tuber samples were harvested 20 days after Ridomil® application at maturity. The negative control was samples from unprotected. Ten samples were randomly picked from the harvested tubers in each plot. Tubers were also sampled from 10 buyers during market day from Limuru market in Kiambu and Wakulima market in Nairobi County. Limuru market receives potato from Narok and Nyandarua counties while Wakulima market is served with potato from all counties and from Tanzania. The samples were washed with distilled water and air dried for 15 minutes. The tubers were packed in polyethylene and placed in ice boxes before transporting to Chemistry Department, Pesticides Research Laboratories at University of Nairobi within 24 hours. The samples were dried further in the laboratory using anhydrous sodium sulphate.

Standards, reagents and instrumentation

Metalaxyl certified pure analytical standard grade (purity, > 99%) was obtained from Pest Control and Products Board (PCPB) headquarters, Nairobi. Ethanol, sodium hydroxide, distilled water, magnesium sulphate, dcm, and other analytical grade chemicals were purchased from local stockists in Nairobi. Metalaxyl was determined by capillary gas chromatography mass

spectrometer (GC- MS) using Mass Spectrometer model HP5771A and NIST library 2008.

Sample processing

Potato tubers were washed with distilled water to remove soil particles. One tuber was randomly selected from the composite sample of each treatment and each tuber cut and homogenized using Hobart food processor. The homogenized sample was ground using pestle and mortar to homogenized powder.

Metalaxyl extraction

Metalaxyl was analyzed by GC-MS model HP5771A. A sample of 10g was picked using sterilized spatula and washed with 10mL of dichloromethane: n-hexane (1:1, v/v). The wet sample was chemically dried using a polar extraction solvent of 3g of anhydrous sodium sulphate for every gram of the sample before they were covered with aluminium foil and incubated overnight at 45°C. The dried sample was placed in a thimble in the soxhlet set up. Hexane and acetone were put in 250mL volumetric flask at a ratio of 1:1 and soxhlet extraction set for 16 hours in three replication (5g each).

Sample extract clean-up

Clean-up was done using silica. Briefly, chromatography clean-up column measuring 20cm long by 2cm in diameter was filled with 2g, 1.8g and 0.5g of anhydrous sodium sulphate (Na₂SO₄), silica oxide (SiO₂) and activated charcoal respectively in that order. Another Na₂SO₄ 2g was added followed by tapping the column to settle the particles uniformly. Conditioning of the column was done with 15mL of hexane-acetone mixture (1:1) and discarded.

Soxhlet extracts were transferred in to the column and the tube rinsed with 1mL of hexane-acetone mixture (1:1) five times and the filtrate immediately returned in to the column to avoid drying. Volumetric flask was used to collect the elute and then rotary-evaporated to 1.5mL before transferring in to GC sample auto vial. Nitrogen stream was added to concentrate the extract to 1mL. The extract was analyzed using GC-MS.

Recovery

Recovery was conducted to confirm efficiency of method used in analyzing the molecules residue. Triplicates of 10g of the tuber samples were injected with 50µL of pure Metalaxyl standard solution of 1ppm which was extracted. The samples were chemically dried using 3g of activated anhydrous sodium sulphate and blended into homogenized powder using pestle and mortar. The analysis was conducted as described above. Control samples (untreated tubers) and reagent blanks were also injected with 50µL of pure Metalaxyl standard solution of 1ppm. Extraction, clean up and analysis procedures were conducted same way as described above to find out interferences due to substrate and reagents.

GC- MS analysis and quantification of extract samples The DB-5 fused silica capillary column measuring 30m (long) by 0.25µm (diameter) was coated with 5% phenyldimethylpolysiloxane. Helium gas at 99.9% purity was passed at a flow rate of 1.0mL/min as a carrier gas. Operating temperatures were set as follows; oven temperature were initially set at 70°C for 1 minute, increased at a rate of 15°C per minute till it reaches 175°C, then at 2°C per minute to 215°C, followed by 10°C per minute to 265°C and finally 20°C per minute to 290 and held for 8 minutes. Injection volume (1µL) was injected at injection temperature of 250°C in split-less mode. Analysis of control samples from potted crop that included untreated potatoes were carried out alongside the treated samples. Field blanks consisted of distilled anhydrous sodium sulphate carried to track field contamination and then subjected to analytical procedures in three replicates.

Quality control involved analysis of control samples including untreated potatoes was carried out alongside the treated samples.

Data analyses

Yield and yield components and RAUDPC collected across the two seasons were analyzed using Statistical Analysis System (SAS) software version 8.2. Whenever Analysis of Variance (ANOVA) indicated significant difference ($P=0.05$) Tukey's Honest Significant Difference (HSD) was used to separate treatment means. Standard errors were calculated to separate the values of the treatments in chemical residues response.

Results

Effect of Ridomil® regime on late blight severity and yield

Disease severity progress was higher in the long rain season resulting in lower yield than in the short rain season (Table 1). The highest disease score (RAUDPC) and lowest yield were observed in unprotected plots followed by 21 days interval.

There was no significant difference ($P = 0.05$) between 7 and 14 day spray interval in terms of disease severity and yield in both short and long rain season. Spraying with Ridomil® at 7 and 14 days reduced late blight by 47% and 42% in the short rain season and by 37% and 34% in the long rain season respectively resulting in improved yield compared to unsprayed plots (Table 2). Protecting the crop using fungicide, reduced chatt size grade and improved on marketable size (Seed and ware grade) (Fig. 1). Tubers infection was only observed in the long rain season during harvesting in unprotected plots.

Table 1. Effect of season on late blight disease progress and yield.

Seasons	Days after emergence									
	28	35	42	49	56	63	70	77	AUDPC	Yield (t ha-1)
Long	2.96a	11.07a	39.55a	47.02a	42.84a	65.96a	77.53a	81.96a	2571.90a	12.54a
Short	7.92b	9.42b	13.39b	17.44b	21.69b	25.74b	28.85b	30.69b	1057.43b	15.81b
HSD ($P=0.05$)	0.408	0.550	0.846	0.758	1.01	1.481	1.439	1.351	36.07	0.490
CV%	30.89	21.08	12.55	9.23	12.29	12.68	10.63	9.42	7.80	13.57

Values followed by similar letters in the same column indicate the treatments do not differ significantly.

Table 2. Effect of spray regime on late blight severity and yield.

Spray regime	Short rain		Long rain	
	Raudpc	Yield	Raudpc	Yield
Unsprayed	0.26a	1.1a	0.62a	0.73a
21 day interval	0.19b	14.23b	0.51b	8.81b
14 day interval	0.15c	24.05c	0.41c	18.22c
7 day interval	0.14c	24.24c	0.34d	22.51d
HSD	0.015	1.067	0.036	0.529
CV%	19.07	9.51	9.20	6.23

Values followed by similar letters in the same column indicate the treatments do not differ significantly.

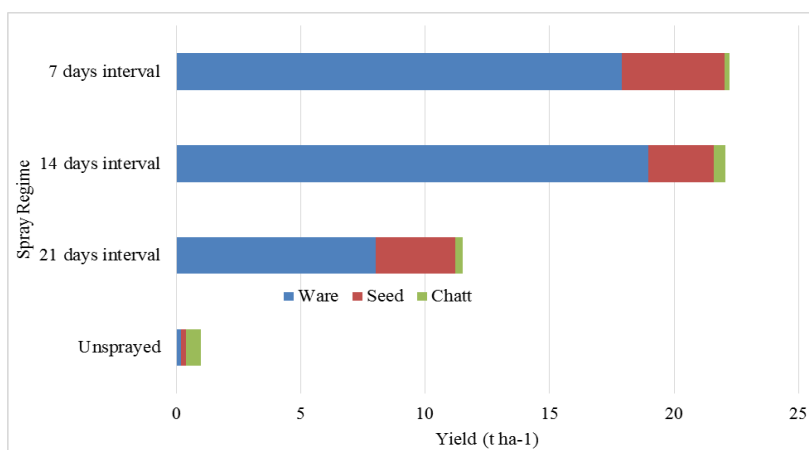


Fig. 1. Effect of spray regime on potato grades.

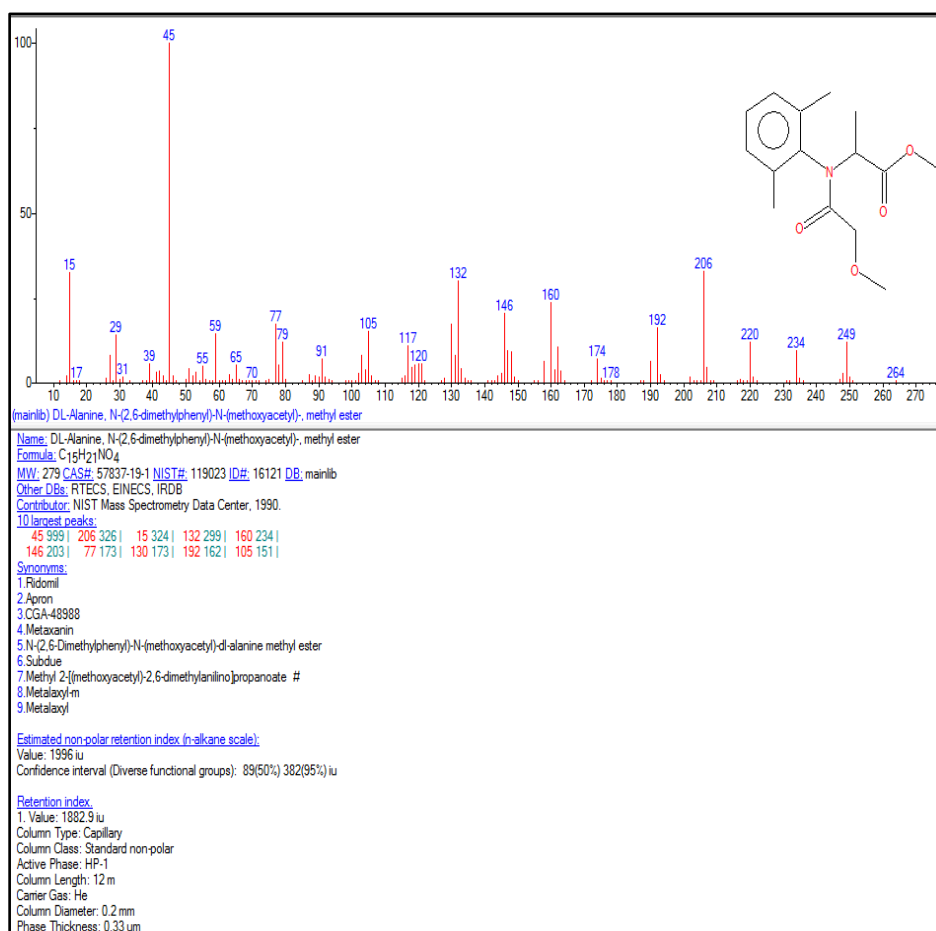


Fig. 2. Chemical structure and ionic mass spectra for Metalaxyl standard.

Assessment of Metalaxyl residues

Metalaxyl percentage recovery was 98.8%. Mass spectrum of Metalaxyl standard solution (Fig. 2) and ion chromatogram of Metalaxyl was after analysis using GC-MS. The ion chromatogram for the unsprayed, 21, 14 and 7 days spray intervals, Limuru market and Ukulima market was observed as shown in the Fig. 3. The highest metalaxyl residue was observed in 7 days spray interval followed by samples

collected from Limuru market which both had levels above acceptable limits of 0.08 mgkg⁻¹ following European Food Safety Authority directions if consumed daily. Spraying Ridomil® at 14 days spray interval gave a limit which was just on the limit line. Samples from plots sprayed at 21 days interval and Wakulima market indicated that they could be safer as they recorded undetectable limits below European Union limits (Table 3).

Table 3. Metalaxyl residues results.

Treatment	Metalaxyl (mgkg ⁻¹)
7 day spray interval	1.69±0.02
14 day spray interval	0.08±0.00
21 day spray interval	<LOD
Limuru market sample	0.09±0.00
Ukulima market sample	<LOD
Unprotected	<LOD
LOD	0.05 mg/Kg

Key; LOD and < represents Limit of Detection and less than respectively.

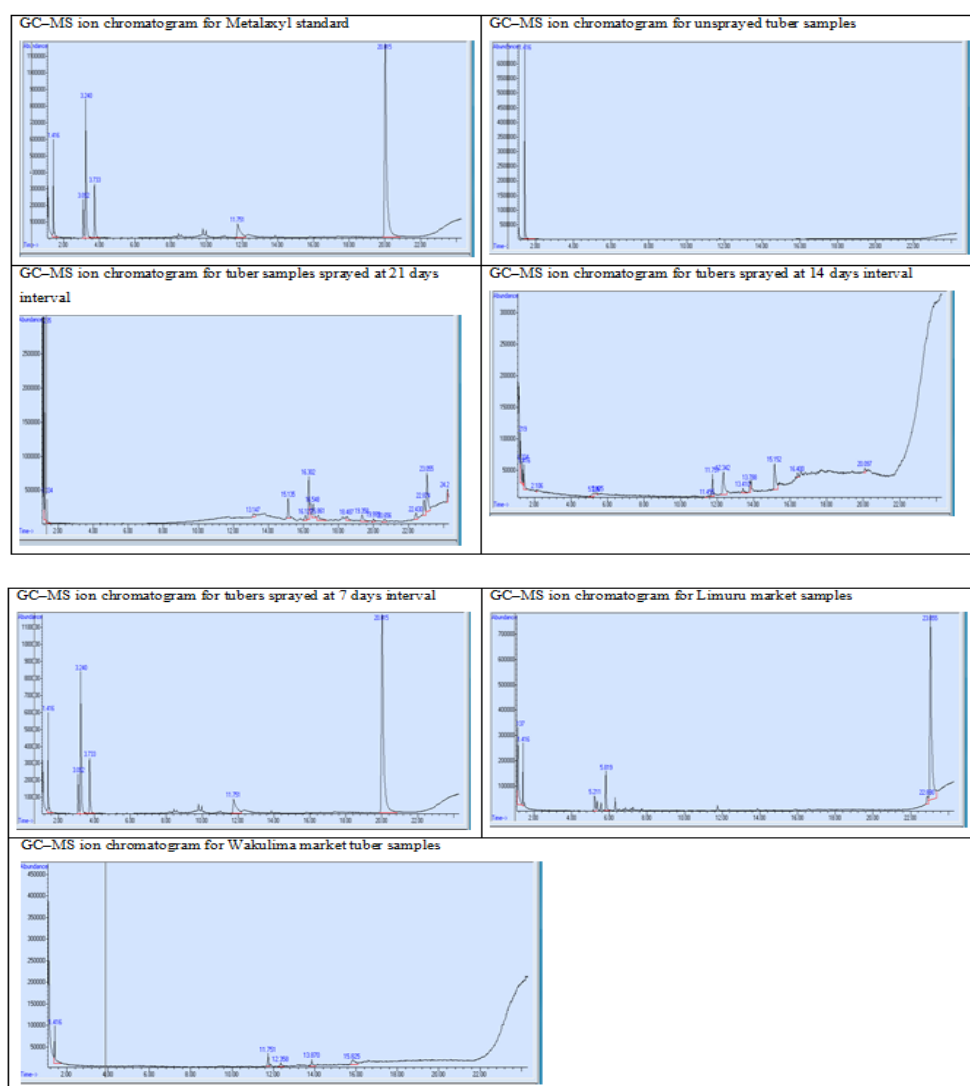


Fig. 3. GC-MS ion chromatograms for treatment

Fig. 3. GC-MS ion chromatograms for treatment.

Discussion

The discovery of metalaxyl (curative) co-formulation with mancozeb (preventive) improved the management of late blight but emergence of more aggressive *P. infestans* reduced the chemical's efficacy. Currently farmers are required to apply fungicides with increased frequency especially during long rain season in Kenya. Knowledge on the amount chemicals that flow into food products remains key in public health point of view. Potato is mostly harvested and sold at farm gate meaning the chemical formulation degradation is abridged. The present study aimed at determining the effects of fungicide application intervals on late blight severity, yield and chemicals residue on the freshly harvested tubers under Kenyan conditions.

The effect of seasonality on the Ridomil® spraying regime could be attributed to the prolonged wet weather conditions that support the growth and development of multiple cycles of *P. infestans* in the long rain season. Moreover, increased late blight severity resulted in defoliation of the infected crop causing tuber infection in the long rain season. The sporangia and zoospore from the defoliated leaves were washed down in to the tuber (Johnson, 2010).

Higher yield observed in 7 day spray interval was a result of the ability of the Ridomil® contact and systemic mode of action to kill *P. infestans* vegetative material either in plant tissues or plant surface. By this short spray interval, the pathogen had no time to reestablish to cause new infections (Majeed *et al.*, 2014). Increased chatt size in the unsprayed was owing to increased disease severity that reduce surface area for light interception and crop defoliation. Reduction in light interception affects crops' food assimilation and partitioning which is promoted by increased canopy (Ahmadi *et al.*, 2014).

The increased metalaxyl residue observed in plots sprayed at 7 days interval was a result of reduced time given to the crop to breakdown the molecule. Furthermore, higher levels in samples from Limuru market suggested freshly harvested tubers in Kenyan market may have high residue levels.

The process of metalaxyl uptake by potato, break down and dissipation is influenced by a number of factors. Metalaxyl dissipation is affected by rapid degradation of the chemical as a result of dilution of the toxicant associated with crop growth coupled with conducive environmental conditions. The major metabolic pathways include hydrolysis and oxidation of the methyl ester which are dissipated by plant uptake, microbial degradation, photodecomposition or leached. In soil, metalaxyl is predominantly formed by soil microorganism which migrate to lower soil horizon resulting in ground water contamination (Sukul and Spilteller, 2003 ; Sukul, 2006). Harvest period after the last fungicide application influence the amount of chemical residue dissipated into the tuber. Study conducted by Feng *et al.*, (2015) indicated that harvesting tubers within 5 days after the last fungicide application resulted in residue concentration exceeding the maximum residue levels. Use of higher application in effort to manage late blight especially when conducive conditions are prolonged which could endanger human health unknowingly. Increasing fungicide application rate resulted in increased chemical residue as observed by Alam *et al.* (2012). Environmental conditions including temperature, relative humidity and rainfall and plant physiology also influence chemical dissipation into crop products (Edward, 1975 ; Macharia *et al.*, 2009 ; Farha *et al.*, 2016).

Studies on possibility of Metalaxyl and Mancozeb residues in crop products and soil have been explored by scientists. Yan *et al.*, 2015 reported that Metalaxyl residue was observed but the levels were below 0.05 mgkg⁻¹ following 7 day interval application in China under controlled environments. Further studies suggested that metalaxyl residue levels were below detectable limits in potato tubers collected from either during short or long rain season (Kamau, 2017). Mancozeb deposition and dissipation in vegetables has also been explored but found to be below detectable limits due to its non-systemic nature (Sharma *et al.*, 2006). Lastly, results of this study show that farm workers face double exposure as a result of increased fungicide application frequency that cause dermal or aerosol exposure even at low

levels as reported by (Macfarlane *et al.*, 2013; Damalas and Eleftherohorinos, 2011) while mixing, applying the fungicides or working in treated field (EFSA, 2014 ; Christensen *et al.*, 2015). Studies have shown washing with tap water could decontaminate crop products by removing a percentage of pesticide residues especially from non-systemic fungicides including Mancozeb (Randhawa *et al.*, 2008; Cheverri *et al.*, 2004). Unfortunately, washing of the potato tubers after harvest to remove mud is rarely practiced. Some farmers leave the tubers in the field to harvest when market prices are conducive while a few harvest and store for a shorter period which affect chemical residue in tuber tissues. This has not been explored to determine how long the residue molecules breakdown. Therefore chemical dissipation and deposition into crop products is a dynamic process which require scientists to evaluate the products continuously to provide informative advice to value chain actors. Evidence from this study points out the need to embrace sustainable farming practices that focus on integrated disease management. This aligns with the need to enhance functioning natural ecosystem where natural enemies play their integral role in crop pests management.

Conflict of interest

The authors declares no conflict of interest in the publication of this manuscript

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Conclusion and recommendations

Ridomil® application at 7 day spray interval gave the highest late blight suppression and improved yield. However, samples from these plots protected with Ridomil® at 7 day spray interval as recommended by the manufacturer and Limuru market samples had a higher level of residue than acceptable limits.

Spraying Ridomil® at 14 days interval gave a level that was same as acceptable limit. We conclude that, dependence on fungicide to manage late blight predispose consumers to health risks.

Therefore there is need for embracing sustainable potato production practices that aim at reducing use of fungicides. In this study however, endorse for future studies on the effect of tuber storage, washing and delayed harvesting on the metalaxyl residue.

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