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MINIMISING FUNGICIDES BY ALTERNATING FORMULATIONS AND INTERVALS TO IMPROVE POTATO BLIGHT MANAGEMENT AND FARM RETURNS

J. KILONZI, M. NYONGESA, P. PWAIPWAI, J. OYOO and J. MAFURAH[1]

Kenya Agricultural and Livestock Research Organization, Tigoni, P. O. Box 338-0217, Limuru, Kenya
[1]Egerton University, P. O. Box 536-20115, Njoro, Kenya
Corresponding author: kilonzijack@gmail.com

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ABSTRACT

Late blight (*Phytophthora infestans*) is one of the most devastating and economic disease impacting both ware and the seed potato industry. The disease causes huge crop losses, and its management attracts heavy expenses. Efficacy successes in chemical management, as opposed to biofungicides, has resulted in the development of a number of fungicide formulations to counter emergence of chemically insensitive *P. infestans* strains. The objective of this study was to evaluate the efficacy and cost-benefits of applying different fungicides in alternate; while varying the spray intervals to manage potato blight and improve on net farm returns. A survey was conducted in Nyandarua County in Kenya, using a structured questionnaire, administered to potato farmers. Milraz® (Propineb 700 g kg^{-1} + Cymoxanil 60 g kg^{-1}), Ridomil® (Metalaxyl 4% + mancozeb 64%) and Mistress 72® (Cynamoxil 4% + Mancozeb 64%) were used *in vitro* and field experiments. Plated pea agar and detached leaflets were amended with the fungicides at concentrations of 0, 35, 70 and 100% of the manufacturers recommended rate before inoculating with *P. infestans*. In the field experiment, the fungicides were applied at intervals of 7, 14 and 21 days spray as single, two or three fungicides in alternations. Results showed that, mycelial and blight lesion growth was curtailed by concentrations of 70 and 100%; while 35% concentration of the fungicides reduced mycelial growth and lesion size by 53 and 2%, respectively. In the field experiment, there were no yield and AUDPC differences ($P<0.05$) among the fungicides and their combinations. However, spraying the fungicides at weekly and bi-weekly intervals gave the highest yields of 17.65 and 16.4 t ha^{-1}, compared to tri-weekly and unprotected plots that recorded 7.93 and 0.43 t ha^{-1}, respectively. In addition, application of triple fungicides in alternation reduced late blight severity by 51%; while application of two fungicides in alternation reduced severity by 39% compared to single fungicide application (21%) on average. Maximum net benefit ratio was observed on plots protected using three fungicides (31.58); followed by two fungicides applications (26.81) applied biweekly in alternate relative to single fungicide applied weekly.

Key Words: Cost benefit, late blight, *Phytophthora infestans*

RÉSUMÉ

Le mildiou (*Phytophthora infestans*) est l'une des maladies les plus dévastatrices et économiques affectant à la fois l'industrie des pommes de terre de consommation et de semence. La maladie cause non seulement d'énormes pertes de récoltes, mais aussi, sa gestion attire de lourdes dépenses. Les succès d'efficacité dans la gestion chimique, par opposition aux biofongicides, ont abouti au développement d'un certain nombre de formulations de fongicides pour contrer l'émergence de souches de P. *infestans* chimiquement insensibles. L'objectif de cette étude était d'évaluer l'efficacité et les coûts-avantages de l'application de différents fongicides en alternance, tout en faisant varier l'intervalle de pulvérisation pour gérer la brûlure de la pomme de terre et améliorer les revenus nets de la ferme. Une enquête a été menée dans le comté de Nyandarua au Kenya, à l'aide d'un questionnaire structuré, administré aux producteurs de pommes de terre. Milraz® (Propineb 700 g kg^{-1} + Cymoxanil 60 g kg^{-1}), Ridomil® (Metalaxyl 4% + mancozeb 64%) et Mistress 72® (Cynamoxil 4% + Mancozeb 64%) ont été utilisés dans des expériences in vitro et sur le terrain. La gélose aux pois sur plaque et la notice détachée ont été modifiées avec les fongicides à des concentrations de 0, 35, 70 et 100 % du taux recommandé par les fabricants avant l'inoculation avec P. *infestans*. Dans l'expérience sur le terrain, les fongicides ont été appliqués à un intervalle de 7, 14 et 21 jours de pulvérisation en tant que simple, deux ou trois fongicides en alternance. Les résultats ont montré que la croissance des lésions mycéliennes et de brûlure était réduite par des concentrations de 70 et 100 % ; tandis qu'une concentration de 35 % des fongicides a réduit la croissance mycélienne et la taille des lésions de 53 et 2 %, respectivement. Dans l'expérience sur le terrain, il n'y avait pas de différences de rendement et d'AUDPC (P<0,05) entre les fongicides et leurs combinaisons. Cependant, la pulvérisation des fongicides à intervalles hebdomadaires et bihebdomadaires a donné les rendements les plus élevés de 17,65 et 16,4 t ha^{-1}, par rapport aux parcelles tri-hebdomadaires et non protégées qui ont enregistré 7,93 et 0,43 t ha^{-1} , respectivement. De plus, l'application de triples fongicides en alternance a réduit la gravité du mildiou de 51 %; tandis que l'application de deux fongicides en alternance a réduit la sévérité de 39 % par rapport à l'application d'un seul fongicide (21 %) en moyenne. Le rapport bénéfice net maximum a été observé sur les parcelles protégées par trois fongicides (31,58) ; suivi de deux applications de fongicides (26.81) appliquées toutes les deux semaines en alternance par rapport au fongicide unique appliqué chaque semaine.

Mots Clés : coût-avantage, mildiou, *Phytophthora infestans*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important food and cash crop in sub-Saharan Africa (SSA), mainly grown by smallholder farmers. The tuber crop has potential to abate hunger due to its high yield per unit area of land (Devaux *et al.*, 2021). Potato is grown under a wide capricious climatic conditions, ranging from tropical to temperate regions which experience a wide range of biotic stresses (Devaux and Ortiz, 2014). Kenya is among the leading producers of potato in the SSA region (FAOSTAT, 2018).

One of the major biotic stresses impacting on potato production is late blight (*Phytophthora infestans*), whose history dates back to 1840s, but still cause yield losses of up to 75% and total loss may be incurred if not sufficiently managed (Kilonzi *et al.,* 2020a; Poudel *et al.,* 2020). The world annual economic losses caused by the disease are about 170 US billion dollars, and the decrease is thus regarded as a threat to global food security (Wu *et al.*, 2012). In Kenya, the crop is predominantly grown in highlands, which experience favourable weather conditions that support multiple life cycles of *P. infestans* within a short period. This results in accelerated epidemics of the decrease (Maziero *et al.*, 2009).

Management of this devastating disease depends on cultivars resistance and fungicides application(Ritchie *et al.,* 2018). However, farmers' choice of variety to grow is often based on market dynamics(Muhinyuza *et al.*, 2012). Furthermore, some of the resistant varieties have succumbed to new races of *P. infestans* (Andrivon *et al.,* 2011). The new races of *P. infestans* distress adversely the durability of the host resistance, consequently resulting in use of fungicide as the sole alternative in managing late blight (Sharma *et al.*, 2013). Chemical control has, therefore, become the key effective approach in managing late blight. Unfortunately, fungicide applications has been associated with increased costs of production, emergence of fungicide resistant strains and trade-offs for the human and environmental concerns (Carvalho, 2017).Moreover, application of single fungicide formulation has contributed to emergence of chemically insensitive strains, which have been described as more virulent, aggressive and adapted to new ecology and newly released resistant varieties globally due to evolution (Matson *et al.*, 2015; Amarasekare *et al.*, 2016). In addition, accumulation of chemical fungicide increases residue in crop products and soil, consequently distressing beneficial microbial communities involved in nutrients recycling, bioremediation and crop pathogen managements (Hussain *et al.,* 2009). Molecules of fungicides have also been reported in water bodies, influencing water qualities for human and aquatic life (Wightwick e*t al.*,2012; Ullah and Dijkstra, 2019). Studies on the use of biocontrols alone to manage the disease have been slow and infective during high epidemics (Yao *et al.*, 2016; Kilonzi *et al.*, 2020b). Therefore, there is need to develop an integrated disease management programme, including combining cultural and minimal chemicals application to offer both preventive and curative measures. The objective of the study was to determine the effects of alternating fungicides formulation on late blight severity, yield, and net farm returns to inform effective and reliable recommendations for managing late blight.

METHODOLOGY

Fungicides formulations *in vitro*. Freshly blighted leaves were collected from Nyandarua County in Kenya and transported to KALRO Tigoni laboratory in a cooler box within 12 hours. Pure culture of *P. infestans* were prepared, following Forbes (1997) procedures; and identified using the procedure described by Wang *et al.* (1993). Fungicide formulations that included; Milraz® (propineb 70% + cymoxanil 6%), Ridomil® (metalaxyl 4% + mancozeb 64%) and Mistress 72® (cymoxanil 4% + mancozeb 64%) were applied at rates of 2.5, 2.0 and 2.0 g L^{-1}, respectively, following recommendations of the manufacturer.

Pea agar plated in a 90 mm petri dish, was amended with 40 μL of each of the fungicide suspension at concentrations of 0, 35, 70 and 100% the manufacturer's recommended rate (MRR) as described above. A mycelial plug was cut using a core borer (0.2 cm diameter); and placed at the centre of petri dish (90 mm diameter) containing amended pea agar. Treatments included Fungicides (Milraz®, Ridomil®, and Mistress 72®) and their concentrations of 0, 35, 70 and 100%, laid out in completely randomised design, in split plot arrangement, with four replications. The plates were incubated at 18 ± 2 °C for up to 11days. Positive controls were plates amended with manufacturers' recommended concentrations of each fungicide; while the negative control consisted of untreated plates (having *P. infestans* alone).

Data on mycelial radial growth was measured at two day standing intervals, starting from the 3rd up to the 11th day after inoculation. Inhibition percentage over the control treatment was calculated as shown in Equation 1, from the 3rd up to the 11th day and analysed, using Statistical Analysis System (SAS) software version 8.2.; *viz*;

Growth inhibition (%) =

$$\frac{\text{Growth in control plate} - \text{Growth in treatment}}{\text{Growth in control plates}} \times 100$$

...................…………………........... Equation 1

Blight lesions in detached leaflet assay. Healthy leaflets of approximately 6 cm long × 4.5 cm wide were excised from the middle canopy of six weeks old rooted apical cuttings, from screen house, using sterilised incision scissors. The leaflets were washed using sterilised distilled water and their bases covered with moist cotton wool. A suspension of *P. infestans* was adjusted to 1×10^4 zoospores mL^{-1}, using hemocytometer and 40 µL droplet placed on the abaxial side of the leaflets. The leaflets were air dried on laboratory bench and incubated at 18 °C for 24 hours.

Fungicides (named in previous section) were applied at concentrations of 35, 70 and 100%suspensions by dipping the leaflets for 5 seconds on the abaxial side. The leaflets were air dried for two minutes and then placed upside down (abaxial surface up) in 20 cm (length) × 18 cm (width) × 6 cm (depth) plastic dishes lined with a wet serviette paper (6 leaflets per dish) and incubated at 18 ºC.

Experimental design was as previously described in the *in vitro* experiment. Positive controls were leaflets treated with MRR concentrations of each fungicide; while negative control consisted of fungicide untreated detached leaflets

Observations were made starting on the 3rdto 11th day after inoculation and lesion size was measured every two days for 11 days to calculate lesion area using Equation 2 (Fontem *et al*., 2005):

$$S = \frac{\pi(L+W)^2}{4}$$ …………...……..... Equation 2

Where:

S, L and W represents area, length and width of the lesion for each detached leaflet respectively. π = 3.14

Fungicides formulations and regime in the field. A field experiment was conducted at the Kenya Agricultural and Livestock Research Organisation (KALRO) Tigoni farm, located in Limuru, Kiambu County, from October 2020 to July 2021. The field is located at 2300 m above sea level, and at latitude 10° 9' 22" south and longitude 36° 4' 72" east. The average mean annual rainfall is 1800 mm per *annum*, and temperature ranging ranges from 10 to 25 °C and at altitude of 2300 m above sea level (Jaetzold *et al*., 2006).

At the onset of rains, seed tubers of about 60 mm of *Shangi* variety were sourced from KALRO Tigoni and planted in furrows at a spacing of 75 cm by 30 cm. Diammonium Phosphate (DAP 18:46:0) was applied at a rate of 500 kg ha^{-1} and mixed well with soil before planting. Natural infection of late blight was relied on.

Fungicides namely; Mistress 72®, Milraz® and Ridomil® were applied as single, two or three fungicides in alternate, separately at an interval of three weeks, bi-weekly and weekly spray regime. Negative and positive controls consisted of unsprayed plots and plots sprayed weekly using single fungicide respectively. Spraying commenced when first blight symptoms were observed. The alternate application strategy was, the cheaper fungicide sprayed more times to save on costs. Therefore, fungicide application was as follows: single fungicides application (Mistress®, Ridomil® and Milraz®),two fungicides application in alternate (Ridomil® followed by Milraz®, Mistress® followed Ridomil® and Mistress 72® followed Milraz®) and three fungicides application in alternate (Mistress® followed by Ridomil® then Milraz® in cyclic manner).

The treatments namely; fungicide spray intervals (weekly, biweekly and triweekly) and alternate applications (single, two and three fungicides) were laid out in randomised complete block design, in a split plot arrangement, with three replications. Spray interval was the main plot and the fungicide application alternates was sub plot treatment. The sub plots measured 3 m x 3 m with a path, of 2 m width between main plots to minimise fungicide drifts effects. Further drift management relied on collecting data on interior rows only.

Data on potato seedling emergence were taken 2 weeks after planting. Late blight severity and incidence data collection began 30 days after emergence, before fungicide applications. 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 about 10% of the leaf), 2 = up to 25% lesion size on a leaflet 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 (Yuen and Forbes, 2009). The results were summarised using Equation 3 to calculate Area Under the Disease Progress Curve (AUDPC) (Equation 4).

$$PDS = \frac{\Sigma \text{ individual numerical rating}}{\text{Total number of plants assessed x maximum score in the scale}} \text{ x } 100$$

....Equation 3

AUDPC was calculated using

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) \times (t_{i+1} - t_i)$$

.... Equation 4

Where:

y_i, t_i, and i^{th} represents assessment of disease (percentage) at i^{th} observation, time (days) at i^{th} observation and i^{th} represent total number of observation, respectively (Simko and Piepho, 2012).

Disease incidence data (number of plants showing late blight symptom in each plot) were collected and converted to percentage disease incidence (PDI) using the Equation 5:

$$PDI = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

.... Equation 5

At maturity, potato tubers were harvested from the inner two rows of each plot and graded as ware (>60 mm), seed (30 to 60 mm) and chatt (< 30 mm) grades according to Kenya Plant Health Inspectorate Service (KEPHIS) potato grading system.

Marketable tubers (ware and seed grade) were weighed and the yield converted to tonnes per hectare.

Relative yield loss due to late blight was determined as the percentage yield reduction of the unsprayed plots, compared with protected plots using Equation 6 as described by Kassaw *et al.* (2021):

$$\text{Relative yield loss} = \frac{Yp - Yc}{Yp} \times 100$$

....Equation 6

Where:

Yp referred to yield from protected plots;andYc represented yield from control plots.

Partial budgeting was used to determine costs and revenues among the fungicides regimes. Costs that applied uniformly to all treatments were not considered (Halloran *et al.*, 2013). Related costs that varied among treatments included fungicide price, fungicide application wage and knapsack hire. Potato prices were based on current local pricing of potatoes when packed in 100 kg bags at farm gate price. Fungicides were purchased from local agro stockist in Limuru town. Labour costs were based on Kiambu County labour wages scheme. The yield observed was adjusted by 10% to approximately match field yield experienced by farmers (Muchiri *et al.,* 2017).

Economic analyses were conducted using partial budgeting in Kenya Shillings (Kes). To compare fungicide formulation, the ratio of the net benefits (Gross margin) to total variable costs was calculated using Equation 7. The treatment that showed the highest ratio was regarded as more economical.

$$\text{Cost Benefit ratio} = \frac{\text{Net benefit (NB)}}{\text{Total variable costs (TVC)}}$$

…………………....……………. Equation 7

RESULTS

Evaluation of fungicides *in vitro*. There was no observable change in mycelial growth of *P. infestans* in plates amended with the fungicides at concentrations of 70 and 100%. On the other hand, concentration of 35% of the fungicides allowed substantive growth of the pathogen, three days after incubation up to about 38 mm. However, the negative control plates progressively developed to 80 mm radial growth after 11 days of incubation. In the first and last 3 days of the inoculation, mycelial growth was slow compared to other periods of growth. Maximum growth rate was observed between 5 and 7 days after inoculation (Fig. 1).

Blight lesion size observed on detached leaflets treated with concentration of 35% of the fungicides was not significantly different (P<0.05) from the untreated leaflets. Maximum lesion size was observed in the untreated treatments, followed by 35% then concentrations of 70 and 100%of the manufacturer's rate. The largest lesion size increase was observed from day 3 to 9 after inoculation. Concentration 100% of all the fungicides curtailed lesion development. Even though fungicides were not significantly different, Milraz® tended to decelerate late blight development slightly more than the other fungicide formulations (Fig. 2).

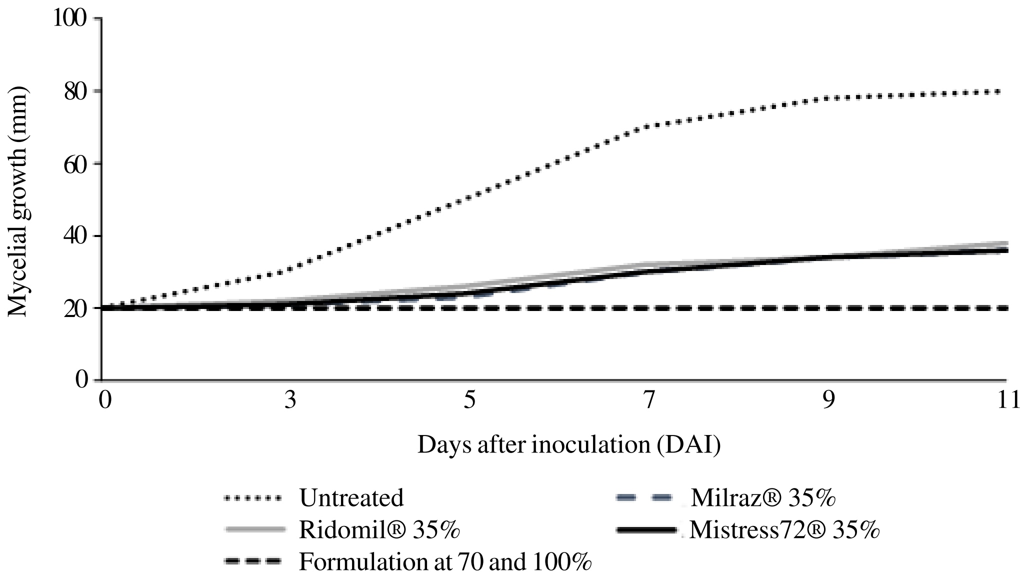

Figure 1. Effect of fungicides and their alternating combinations on *P. infestans* mycelial growth *in vitro.*

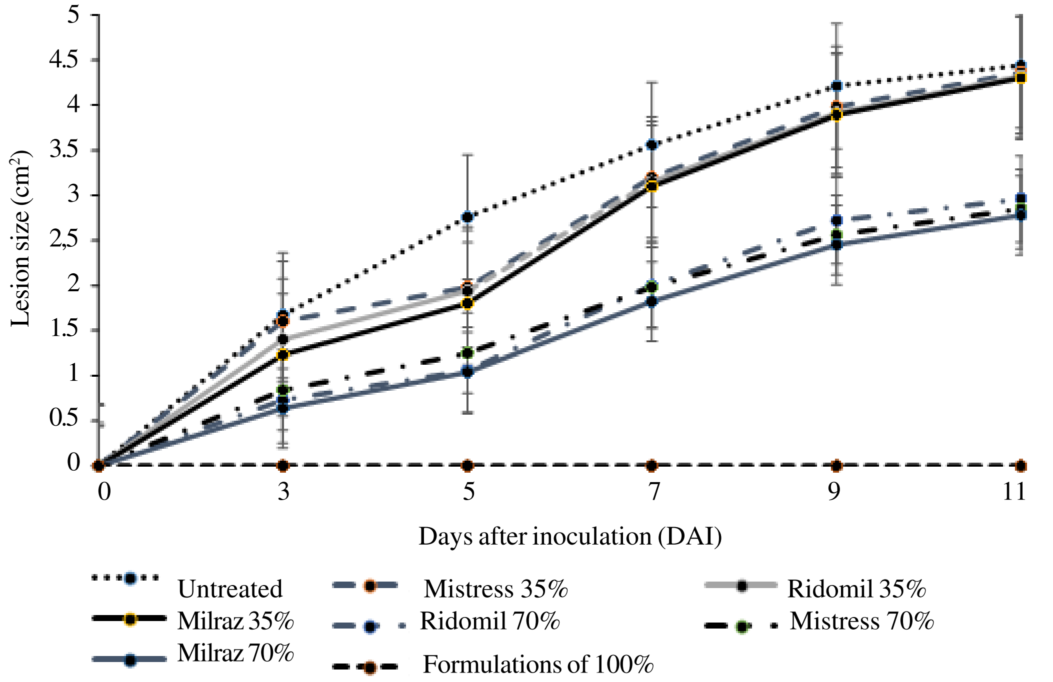

Figure 2. Effect of fungicide formulations and concentrations on lesion size (cm^2) of late blight on detached potato leaf assay *in vitro*.

Late blight disease progress curve. In the open field experiment, it was observed that disease severity increased exponentially in all the treatments across the growing period (Fig. 3). The unsprayed plots recorded the highest disease severity (80%); followed by plots protected using single fungicide. Disease progress was decelerated by all the fungicides in all days after emergence, relative to unprotected plots. Late blight severity was not significantly different among the fungicides ($P<0.05$) during the cropping seasons. However, Milraz® tended to impede disease severity slightly more than the other fungicides. Fungicide formulations applied singly had higher disease severity than when two or three fungicides were applied in alternations. The highest disease suppression (78%) was observed in plots protected using three fungicides, applied in alternation during the growth period. None of the treatment stopped the growth and development of late blight symptoms across the growing period, once the crop was infected (Fig. 3).

Area under disease progress curve. There was a significant effect of fungicides on the area under disease progress curve (AUDPC) and yield, in which the unprotected had the highest disease severity and consequently, the lowest yield. Yield and AUDPC results were not significantly different among the treatments. Conversely, AUDPC and yield observed in plots in which fungicides were applied in alternation as single, two and triple fungicide were significantly different. Furthermore, AUDPC and yield observed in plots protected using any two fungicide combinations did not differ significantly when applied in alternate. On the other hand, plots in which three fungicides were applied in alternates were significantly different from other treatments and showed the lowest disease severity (1089) and highest yield (20.23 t ha^{-1}) (Table 1).

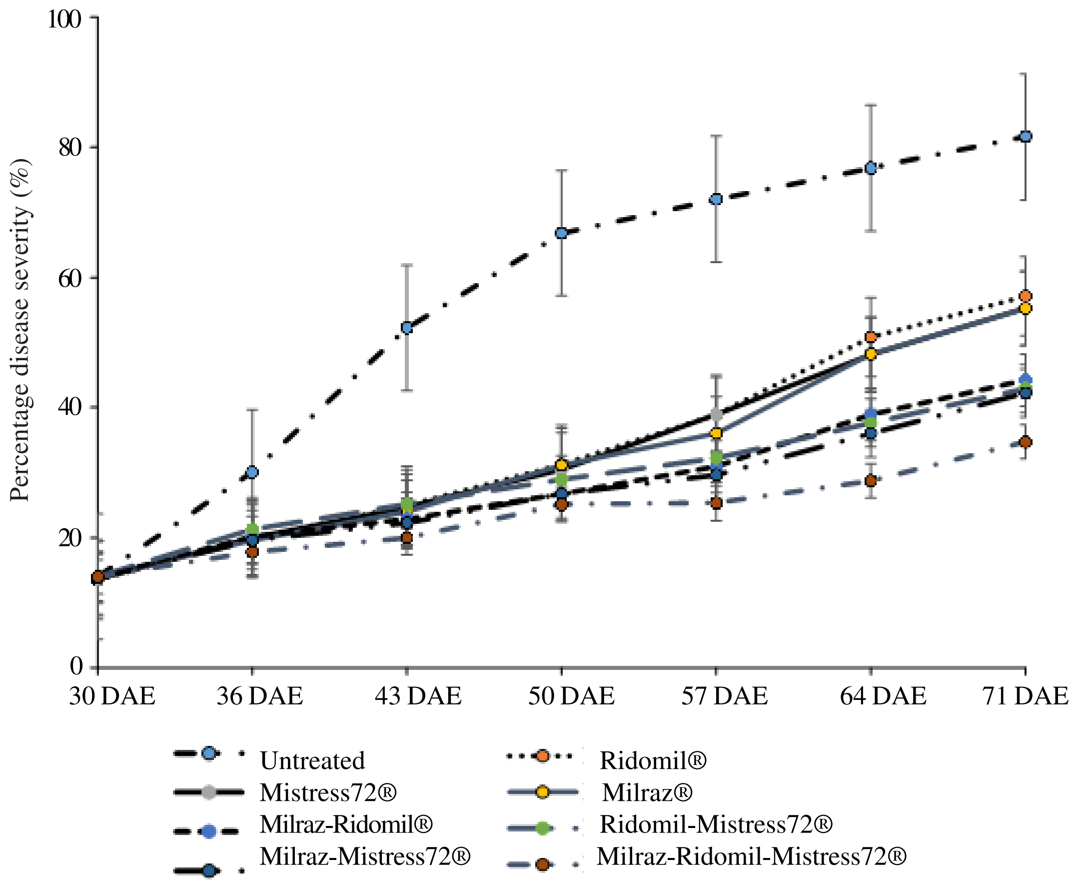

Figure 3. Effect of fungicides formulations on late blight disease progression.

TABLE 1. Effect of fungicides on late blight severity and yield

Fungicide	AUDPC	Yield (t ha^{-1})
Untreated	2100a	0.43a
Ridomil®	1612b	13.36b
Mistress 72®	1551b	13.70b
Milraz®	1510b	13.97b
Milraz®- Ridomil®	1335c	16.62c
Ridomil®- Mistress 72®	1365c	16.64c
Milraz®- Mistress 72®	1289c	17.00c
Milraz®- Ridomil®- Mistress 72®	1089d	20.23d
$HSD_{(<0.05)}$	69.30	1.114
CV (%)	3.13	5.32

Fungicide application interval. Fungicide application interval had significant consequence on late blight pressure and contribution to final yield outcome (Table 2). Weekly spray intervals recorded the lowest disease severity across the growing period, followed by bi-weekly; while tri-weekly regime was intermediate between untreated and bi-weekly spray intervals, for all fungicides. The trend was analogous with the observed AUDPC that subsequently contributed to significant yield difference among the treatments. Consequently, weekly spray intervals had higher yield (17.65 t ha^{-1}) compared to the unsprayed plots that recorded the lowest yield of 0.43 t ha^{-1} (Table 3).

Interaction between spray interval and fungicides. The interaction of fungicide formulations and application interval had significant effects on late blight management (Figs. 4 and 5). The highest AUDPC that coincided with the lowest yield was observed on unsprayed plots; followed by treatments protected using single fungicide applied tri-weekly. Similarly, within each spray regime, applying single fungicide recorded higher disease severity and lower yield. A significantly higher yield was observed in plots sprayed with two and three fungicides in alternate applied weekly and bi-weekly.

Expectedly, yield observed from the experiment was negatively correlated (r = -0.98) with the late blight severity (AUDPC) (Fig. 6). The highest yield loss was observed in plots tri-weekly, with single fungicide. Fungicide application using either two or three fungicide formulations in alternations reduced yield loss; with weekly spray plummeting yield loss more by 98%.

Costs and revenues. The farm gate price for a 100 kg^{-1} bag of potato tubers was Kenya shillings (Kes) 1,800 on average. The prices per kilogramme of Mistress 72®, Ridomil® and Milraz® were Kes 2,000, 2,800 and 3,250, respectively. The costs guided the application frequency, with the least costly been applied multiple times in cases of alternations. The application rate for Mistress 72®, Ridomil® and Milraz® was 2.5, 2.0 and 2.0 kg ha^{-1} according to MRR, respectively. Fungicide application at weekly, bi-weekly and tri-weekly resulted to 7, 4 and 3 sprays per cropping season, respectively. Fungicide application was Kes 400 per man-day corresponding to 4 man-days ha^{-1}; while knapsack hire was Kes 100 per hectare per day according to Ministry of Agriculture Limuru, Kiambu. Ridomil® which is one of the most widely used fungicide applied weekly was referred as conventionally applied fungicide.

TABLE 2. Effect of late blight development on late blight severity and yield of potato across days after emergence

Regime	Days after emergence							AUDPC	Yield
	30	36	43	50	57	64	71		
Untreated	13.9a	23.9a	29.8a	36.7a	56.2a	68.7a	86.6a	2100a	0.43a
Tri-weekly	13.8a	18.3b	28.9b	34.8b	41.2b	54.83b	66.8b	1796b	7.93b
Bi-weekly	13.7a	17.9b	27.8c	28.3c	34.6c	41.2c	47.3c	1375c	16.40c
Weekly	13.8a	18.0b	21.1d	26.5c	32.3c	37.1d	40.9d	1262d	17.65d
$HSD_{(<0.05)}$	0.602	1.345	0.919	1.578	1.493	1.176	1.163	1.163	0.520
CV (%)	5.55	9.48	5.42	7.55	5.93	5.64	3.22	3.22	5.32

TABLE 3. Cost/benefit analysis for application of different fungicides regimes for control of late blight in potato in Kenya

Variable	Weekly					Bi-weekly				Tri-weekly			
	RD®	Ms-RD®	Ms-Mz®	RD-MZ®	Ms-RD -Mz®	Ms-RD®	Ms-Mz®	RD-MZ®	Ms-RD -Mz®	Ms-RD®	Ms-Mz®	RD-MZ®	Ms-RD -Mz®
Yield ($t\ ha^{-1}$)	17.60	20.12	21.14	20.13	25.30	19.92	19.90	20.02	24.23	10.04	10.03	9.84	11.14
Adjusted yield ($t\ ha^{-1}$)	15.84	18.10	19.01	18.12	22.77	17.92	17.91	18.02	21.81	9.04	9.03	8.86	10.03
Gross benefits (Kes)	285,120	325,800	342,180	326,160	409,860	322,560	322,380	324,360	392,580	162,720	162,400	159,480	180,540
Costs (Kes)													
Knapsack hire	700	700	700	700	700	400	400	400	400	300	300	300	300
Ridomil®	19,000	8,400	0	11,200	6,000	5,600	0	5,600	2,800	2,800	0	5,600	2,800
Mistress 72®	0	8,000	8,000	0	5,600	4,000	4,000	0	4,000	4,000	4,000	0	2,000
Milraz®	0	0	9,750	9,750	6,500	0	6,500	6,500	3,250	0	3,250	3,250	3,250
Labour(Kes)													
Spraying	2,800	2,800	2,800	2,800	2,800	1,600	1,600	1,600	1,600	1,200	1,200	1,200	1,200
TVC (Kes)	22,500	19,900	21,250	24,450	21,600	11,600	12,500	14,100	12,050	8,300	8,750	10,350	9,550
NB	262,600	305,900	320,930	301,710	388,260	310,960	309,880	310,260	380,530	154,420	153,650	149,130	170,990
(NB ÷TVC)	11.67	15.37	15.10	12.33	17.98	26.81	24.79	22.00	31.58	18.60	17.56	14.41	17.90

TVC- Total variable costs, NB- Net Benefit, RD®- Ridomil®, RD- Ms®- Ridomil® followed by Mistress 72®, Mz- Ms®-Milraz® followed by Mistress 72®, RD-MZ®- Ridomil® followed by Milraz® andMz-Rd-Ms®-Ridomil®followed by Mistress 72® followed by Milraz®, Kes-Kenya shillings

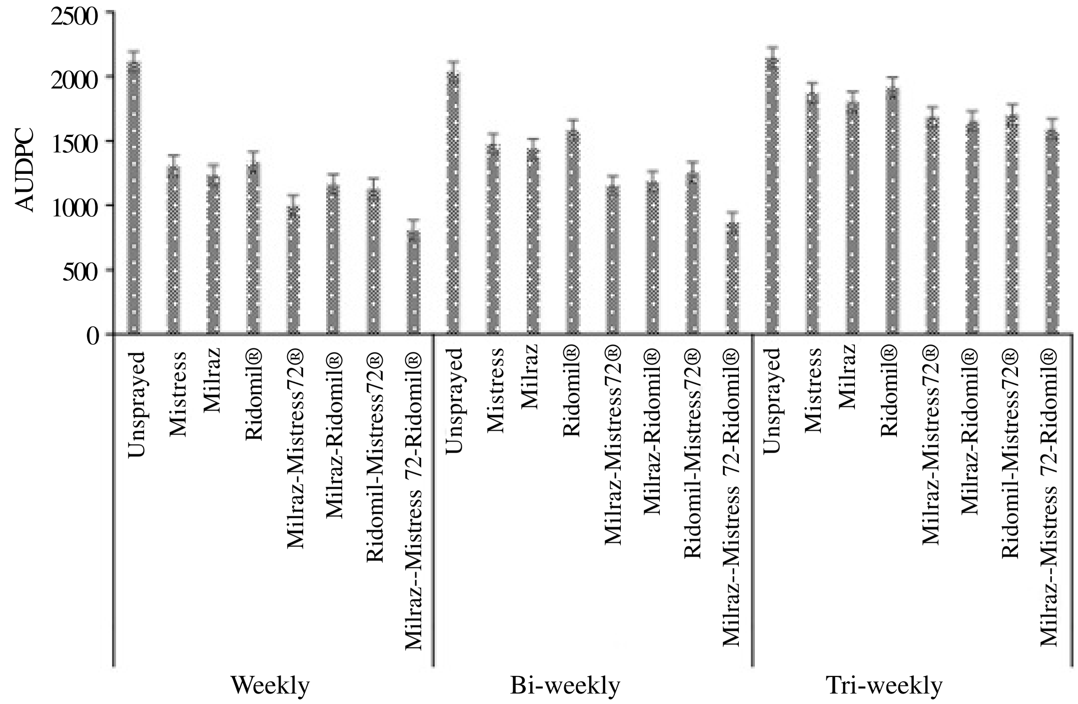

Figure 4. Interaction effect of fungicide regime and fungicide formulations on late blightAUDPC

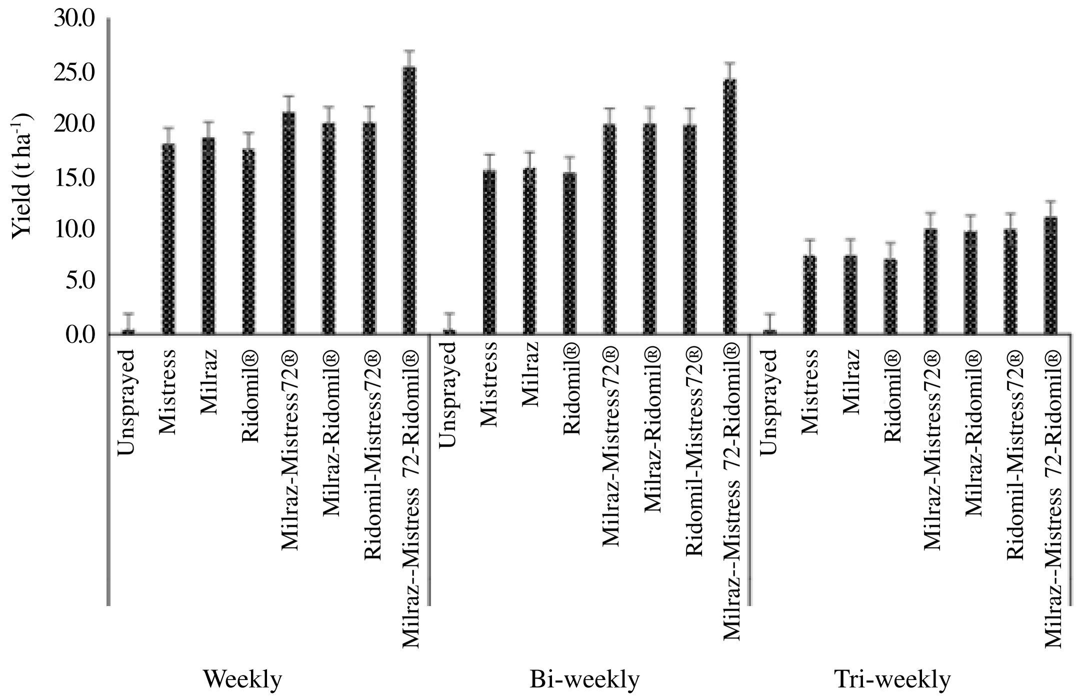

Figure 5. Interaction effect of fungicide regime and fungicide formulations tuber yield.

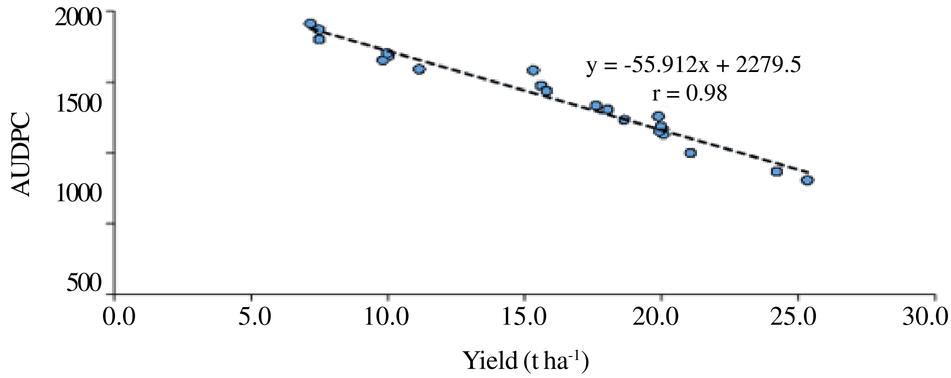

Figure 6. Correlation between Area Under Disease Progress Curve and yield of potato (t ha^{-1}).

Cost benefit analysis. On average, biweekly spray resulted in higher net benefit than weekly, despite the yield differences. Application of three fungicides in alternations biweekly recorded the highest cost benefit ratio, owing to their low costs and higher returns. Similarly, application of multiple fungicides had higher yields than plots sprayed with single fungicide. However, multiple fungicides application at tri-weekly spray interval was less beneficial though increased net farm income more than when single fungicide was applied weekly. Although, spraying of three fungicides in alternate provided greater economic returns when applied weekly, cost benefit ratio indicate that the latter was more profitable when applied biweekly (Table 3).

DISCUSSION

Results of the *in vitro* results elucidate (Fig. 1) that, even though the fungicide formulations were not significantly different, metalaxyl containing fungicide (Ridomil®) showed the lowest effect on *P. infestans* mycelial growth and late blight lesion development, relative to other fungicides. Similar results were observed by Khadka *et al.* (2020). Adaptation to metalaxyl adverse effects often occur spontaneously following overuse of the fungicide, consequently resulting in strains that are less sensitive to metalaxyl (Pule *et al.*, 2013).

In addition, field experiment findings suggested that application of multiple fungicides in alternate was more efficacious in managing late blight than using single fungicide (Table 1). Previously, Muchiri *et al.* (2017) reported that use of co-formulated fungicide was more effective in managing late blight, as well as increasing net farm income. Despite this advantage, farmers continue to face substantive yield losses following high disease epidemics. Therefore, applying more than two fungicides having different active ingredients, as observed in this study, could be a solution in managing yield losses associated with late blight.

In previous reports, frequency of applying fungicides has been found to play a key role in suppressing late blight development (Lal *et al.*, 2017; Majeed *et al.*, 2017; Kilonzi *et al.*, 2020b). Our results from the field experiment are in agreement with these findings in which a shorter spray interval was more efficacious than biweekly interval in all fungicide formulations. However, we found that, application of more than one fungicide in alternate showed better results in managing the disease than when applied singly (Table 2). On the contrary, triweekly application of fungicides in alternate was not sufficient in averting late blight development. This could be attributed to the long duration of the spray interval that to allow mycelial and sporangia regrowth and ultimately spore production causing more and new infections.

Application of more than one fungicide formulation in alternate reduced AUDPC by 48 and 35%, respectively, compared to single fungicide that contributed to 26% disease decline (Fig. 1). Alternating multiple fungicides offer different modes of actions, including curative and preventive mechanisms reduce chances of *P. infestans* growth (Kassaw *et al*., 2021). For instance, metalaxyl targets RNA synthesis inhibition and mancozeb is a multisite broad spectrum protectant. Cymoxanil stops post-infection slowing down the fungal growth by targeting thymidine synthesis (Ziogas and Davidse, 1987); while propineb interferes with respiration and metabolism of carbohydrates and proteins. The chemicals provide an improved protection due to their systemic and protectant actions that target multisite of *P. infestans,* thereby increasing their efficacy (Tiwari *et al.*, 2021).

Applying fungicides at weekly spray intervals resulted in lower yield losses as compared to triweekly spray interval (Table 2). Yield loss was further reduced when more than one fungicides were applied in alternate at weekly and bi-weekly interval, resulting to improved economic returns based on cost benefit ratio over control. Similar results were observed by Kefelegn *et al.* (2012) and Kassaw *et al.* (2021).

Further cost effectiveness was observed where more than one fungicide was used, where a less costly is alternated with a high pricy fungicide (Table 3). To optimise late blight management, farmers apply fungicides at a higher frequency, thus raising cost of production that rarely match observed yield. Even though, multiple fungicide spray managed and reduced the spray frequency per cropping season, there is need to incorporate the practice in an integrated disease management programme including use of resistant variety to further reduce the use fungicide.

CONCLUSION

Concentrations of 70 and 100% of the fungicide reduce late blight (*P. infestans)* by about 50% compared to unprotected plots. Multiple fungicide application in alternates is more efficacious and ultimately improves farm economic returns. It is apparent that the absolute effects of using three fungicides in alternates contributes to the highest reduction in yield loss. Moreover, applying three fungicides in alternate at bi-weekly interval contributes to increased net farm income.

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REFERENCES

Amarasekare, K.G., Shearer, P.W. and Mills, N.J. 2016. Testing the selectivity of pesticide effects on natural enemies in laboratory bioassays. *Biological Control* 102:7-16. doi: 10.1016/ j.biocontrol.2015. 10.015.

Andrivon, D., Avendaño-Córcoles, J., Cameron, A.M., Carnegie, S.F., Cooke, L.R., Corbière, R. and Zimnoch-Guzowska, E. 2011. Stability and variability of virulence of *Phytophthora infestans* assessed in a ring test across European laboratories. *Plant Pathology* 60(3):556-565. https://doi.org/10.1111/j.1365-3059.2010.02392.x

Carvalho, F.P. 2017. Pesticides, environment, and food safety. *Food and Energy Security* 6(2):48-60. doi:10.1002/fes3.108.

Devaux, A., Goffart, J. P., Kromann, P., Andrade-Piedra, J., Polar, V. and Hareau, G. 2021. The potato of the future: opportunities and challenges in sustainable agri-food systems. *Potato Research* 64(4): 681-720. https://doi.org/10.1007/s11540-021-09501-4

Devaux, A., Kromann, P. and Ortiz, O. 2014. Potatoes for sustainable global food security. *Potato Research* 57(3):185-199. https://doi.org/10.1007/s11540-014-9265-1

FAOSTAT. 2018. Statistics for global food production. Food Agricultural statistics, United Nations, Peru. https://www.potatopro.com/world/potato-statistics. Accessed 14th November 2021.

Fontem, D.A., Olanya, O.M., Tsopmbeng, G.R. and Owona, M.A.P. 2005. Pathogenicity and metalaxyl sensitivity of *Phytophthora infestans* isolates obtained from garden huckleberry, potato and tomato in Cameroon. *Crop Protection* 24(5):449-456. https://doi.org/10.1016/j.cropro.2004.09.012

Forbes, G.A. 1997. Manual for laboratory work on *Phytophthora infestans* CIP Training Manual. International Potato Centre, Lima, Peru.

Jaetzold, R., Schmidt, H., Hornetz, B. and Shisanya, C. 2006. Farm management handbook of Kenya-Natural conditions and farm management information, 2nd Edition. Volume II/B. Ministry of Agriculture, Nairobi, Kenya.

Halloran, J.M., Larkin, R.P., Defauw, S.L., Olanya, O.M. and He, Z. 2013. Economic potential of compost amendment as an alternative to irrigation in Maine potato production. *American Journal of Plant Science* 4:238-245. https://doi.org/10.4236/ajps.2013.42031

Hussain, S., Siddique, T., Saleem, M., Arshad, M. and Khalid, A. 2009. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. *Advances in Agronomy* 102(09):159-200. https://doi.org/10.1016/S0065-2113(09)01005-0

Kassaw, A., Abera, M. and Belete, E. 2021. The response of potato late blight to potato varieties and fungicide spraying frequencies at Meket, Ethiopia. *Cogent Food & Agriculture* 7(1):1-17. doi: 10.1080/23311932.2020.1870309.

Kefelegn, H., Chala, A., Kassa, B. and Tiwari, G.B. 2012. Evaluation of different potato variety and fungicide combinations for the management of potato late blight (*Phytophthora infestans)* in Southern Ethiopia. *International Journal of Life Sciences* 1(1):8-15.

Khadka, R.B., Chaulagain, B., Subedi, S., Marasini, M., Rawal, R., Pathak, N. and Sharma-Poudyal, D. 2020. Evaluation of fungicides to control potato late blight (*Phytophthora infestans*) in the plains of Nepal. *Journal of Phytopathology* 168(5): 245-253. https://doi.org/10.1111/jph.12886.

Kilonzi, J.M., Mafurah, J.J., Nyongesa M.W. and Kibe, A.M. 2020a. Efficacy of *Trichoderma asperellum* seed treatment and Ridomil® application in managing late blight on potato, *World Journal of Agricultural Research* 9(2): 42-52. doi: 10.5539/jas.v12n7p32.

Kilonzi, J.M., Mafurah, J.J. and Nyongesa, M.W. 2020b. Cost benefit analyses in managing late blight through *Trichoderma asperellum* seed treatment and Ridomil® application on potato. *Journal of Agricultural Science* 12(7):32-52. doi: 10.5539/jas.v12n7p32.

Lal, M., Yadav, S., Sharma, S., Singh, B.P. and Kaushik, S.K. 2017. Integrated management of late blight of potato. *Journal of Applied and Natural Science* 9(3):1821-1824.

Majeed, A., Muhammad, Z., Ullah, Z., Ullah, R. and Ahmad, H. 2017. Late blight of potato (*Phytophthora infestans*) I: Fungicides application and associated challenges. *Turkish Journal of Agriculture - Food Science and Technology* 5(3):261-266. https://doi.org/10.24925/turjaf.v5i3.261-266.1038

Matson, M.E.H., Small, I.M., Fry, W.E. and Judelson, H.S. 2015. Metalaxyl resistance in *Phytophthora infestans*: Assessing role of RPA190 gene and diversity within clonal lineages. *Phytopathology* 105(12):1594-1600. https://doi.org/10.1094/PHYTO-05-15-0129-R

Maziero, J. M. N., Maffia, L. A. and Mizubuti, E.S.G. 2009. Effects of temperature on events in the infection cycle of two clonal lineages of *Phytophthora infestans* causing late blight on tomato and potato in Brazil. *Plant Disease* 93(5):459-466. doi: 10.1094/PDIS-93-5-0459.

Muchiri, F.N., Narla, R.D., Olanya, O.M., Nyankanga, R.O. and Ariga, E.S. 2017. Efficacy of fungicide mixtures for the management of *Phytophthora infestans* (US-1) on potato. *Phytoprotection* 90:19-29. https://doi.org/10.7202/038983ar

Muhinyuza, J.B., Shimelis, H., Melis, R., Sibiya, J. and Nzaramba, M.N. 2012. Participatory assessment of potato production constraints and trait preferences in potato cultivar development in Rwanda. *International Journal of Development and Sustainability* 1(2):358-380. http://isdsnet.com/ijds-v1n2-23.pdf

Poudel, A., Pandey, M., Shah, K., Acharya, B. and Shrestha, J. 2020. Evaluation of fungicides for management of late blight (*Phytophthora infestans*) of potato. *Agrica* 9(1): 10 -19. https://doi.org/10.5958/2394-448x.2020.00004.8

Pule, B.B., Meitz, J.C., Thompson, A.H., Linde, C.C., Fry, W.E., Langenhoven, S. D. and Rij, N.C. Van. 2013. *Phytophthora infestans* populations in central, eastern and southern African countries consist of two major clonal lineages. *Plant Pathology* 62: 154-165. https://doi.org/10.1111/j.1365-3059.2012.02608.x.

Ritchie, F., Bain, R.A., Lees, A.K., Boor, T.R. and Paveley, N.D. 2018. Integrated control of potato late blight: Predicting the combined efficacy of host resistance and fungicides. *Plant Pathology* 67(8):1784-1791. https://doi.org/10.1111/ppa.12887

Sharma, B.P., Forbes, G.A., Manandhar, H.K., Shrestha, S.M. and Thapa, R.B. 2013. Determination of resistance to *Phytophthora infestans* on potato plants in field, laboratory and greenhouse conditions. *Journal of Agricultural Science* 5(5):148-157. https://doi.org/10.5539/jas.v5n5p148.

Simko, I. and Piepho, H. 2012. Analytical and theoretical plant pathology. The area under the disease progress stairs: Calculation, advantage and application. *Extra* 102(4): 381-389.

Tiwari, I., Shah, K. K., Tripathi, S., Modi, B., Subedi, S. and Shrestha, J. 2021. Late blight of potato and its management through the application of different fungicides and organic amendments: A review. *Journal of Agriculture and Natural Resources* 4(1): 301-320. https://doi.org/10.3126/janr.v4i1.33374.

Ullah, M. and Dijkstra, F. 2019. Fungicide and bactericide effects on carbon and nitrogen cycling in soils: A meta-analysis. *Soil Systems* 3(2):23-29. doi: 10.3390/soil systems3020023.

Wang, H., Qi, M. and Cutler, A.J. 1993. A simple method of preparing. *Nucleic Acid Research* 21(17):4153-4154.

Wightwick, A.M., Bui, A.D., Zhang, P., Rose, G., Allinson, M., Myers, J.H. and Allinson, G. 2012. Environmental fate of fungicides in surface waters of a horticultural-production catchment in South eastern Australia. *Archives of Environmental Contamination and Toxicology* 62(3):380-390. https://doi.org/10.1007/s00244-011-9710-y

Wu, Y., Jiang, J. and Gui, C. 2012. Low genetic diversity of *Phytophthora infestans* population in potato in north China. *African Journal of Biotechnology* 11(90):15636-15642. doi: 10.5897/AJB12.484

Yao, Y., Li, Y., Chen, Z., Zheng, B., Zhang, L., Niu, B. and Wang, Q. 2016. Biological control of potato late blight using isolates of *Trichoderma*. *American Journal of Potato Research* 93(1):33-42. https://doi.org/10.1007/s12230-015-9475-3

Yuen, J.E. and Forbes, G.A. 2009. Estimating the level of susceptibility to *Phytophthora infestans* in potato genotypes. *Phytopathology* 99:782-786.

Ziogas, B.N. and Davidse, L.C. 1987. Studies on the mechanism of action of cymoxanil in *Phytophthora infestans*. *Pesticide Biochemistry and Physiology* 29(2):89-96. doi: 10.1016/0048-3575(87)90066-6