

Efficacy of *Trichoderma asperellum* Seed Treatment and Ridomil[®] Application in Managing Late Blight on Potato

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Received January 08, 2021; Revised February 09, 2021; Accepted February 23, 2021

Abstract Potato seed tubers latently infected with *Phytophthora infestans* initiate late blight that requires early fungicide application raising economic and human concerns. The objective of the study was to determine the efficacy of *Trichoderma asperellum* seed treatment and Ridomil[®] (Metalaxyl 4% and Mancozeb 64%) application to manage late blight. Ridomil[®] was applied at 21-, 14- and 7-day intervals on seed tuber and apical cuttings pre-treated with *T. asperellum* at 33 % (3×10^6), 66% (7×10^6) and 100% (1×10^7 CFU/mL) concentration by either dipping or injection. Results revealed that 7- and 14-day spray intervals were not significantly different ($P=0.05$) in terms of yield and late blight severity. Rooted apical cuttings had 7.4% higher disease severity resulting in 2.3% lower yield than crop from seed tubers. *T. asperellum* at 66% and 100% concentrations reduced disease severity by 26% and 27% respectively. Pericardial injection had 8.3% higher yield and conversely 7.8% higher disease severity than dipping. The combination of *T. asperellum* at 66% concentration with a 14-day spray interval provided better late blight management. The results suggest that seed treatment by dipping using 66% *T. asperellum* suspension could increase fungicide application interval by 7 days while improving on yield.

Keywords: *Trichoderma asperellum*, apical cuttings, Ridomil[®] (Metalaxyl 4% and Mancozeb 64%), *Phytophthora infestans*, dipping, injection

Cite This Article: Kilonzi Jackson Mutuku, Mafurah Juma Joseph, Nyongesa Moses Wabomba, and Kibe Antony Mwangi, "Efficacy of *Trichoderma asperellum* Seed Treatment and Ridomil[®] Application in Managing Late Blight on Potato." *World Journal of Agricultural Research*, vol. 9, no. 2 (2021): 42-52. doi: 10.12691/wjar-9-2-1.

1. Introduction

Potato is an important food crop globally for both consumption and industrial use and thereby contributing to income generation and livelihood improvement. The vegetable crop provides important nutrients including carbohydrates, vitamins and amino acids and a well-balanced protein to energy carbohydrate ratio [1]. In Kenya, the tuber crop is the second most important food crop after maize in terms of consumption and bulk harvested. It has a potential of alleviating poverty due to its ability to yield higher per unit area and mature (3 to 4 months) much earlier than maize (9 months) in potato growing regions. Potatoes contribute to both direct (800,000 growers) and indirect (2.5 million people) employment along the potato value chain [2]. However, potato yield is low up to 8 t ha^{-1} compared to a potential of 40 t ha^{-1} as a result of stresses including diseases, with late blight (*Phytophthora infestans*) being the major yield reducing biotic stress [3]. Late blight is a major problem in potato growing regions worldwide [4] and can be transmitted through the air, soil and seed [5]. Management

of late blight is mainly through fungicide applications at short intervals resulting in 8 to 15 applications per cropping season [6,7]. Potato seed tuber shortage in Kenya has resulted in a limited supply, consequently increasing the cost of seed up to 42% of the total cost of production. This has led to adoption of farm saved seed use by farmers resulting in accelerated disease epidemics owing to latent infection and transmission from one field to another [8]. Latently infected seed tubers initiate late blight epidemics early in the cropping season forcing farmers to adopt a short spray interval regime [9]. In addition, new strains of *P. infestans* that are resistant to fungicides and adaptive to newly released resistant cultivars have exacerbated the situation, especially when late blight conducive conditions are prolonged [10]. Increased late blight severity results in accelerated crop defoliation within short time, especially during wet periods, leading to production of zoospores and sporangia which infect the forming tubers [11]. Spores of *P. infestans* inhabiting the soil and potato culls cause early disease epidemics [12]. Use of tuber seed contaminated by the pathogen accelerates late blight severity and incidences [4]. Therefore, use of disease free planting material including rooted apical cuttings, which are produced through tissue culture, could reduce the

spread of *P. infestans* inoculum [13]. Some of the fungicides used to manage tuber blight are ineffective or have little to no activity against *P. infestans*, especially when applied after the disease has already established [11,14,15].

Seed health forms one of the key factors in determining the yield formation of potato [16,17]. Vegetative seed tuber multiplications run the risk of having increased late blight pressure since they are prone to latent infection [18]. This is the common practice in Kenyan potato industry. Reference [19] reported the need for improving the quality of the seed system in Eastern Africa to fill the seed deficit. Increase in seed supply will require use of modern seed potato production technologies including aeroponics, hydroponics, rooted apical and stem cuttings, that produce disease free and high generation materials [20]. However, little is known on apical cuttings' response to soil and air borne pathogens including *P. infestans* and on their yield potential in comparison to seed tuber.

Integrated disease management incorporating biological agents provides sustainable opportunities in managing late blight to enhance tuber yield to match the demand for both seed and ware potato [21,22]. *Trichoderma* spp. are the most widely used biological agent globally to manage a number of crop diseases. The fungus is known to grow faster than phytopathogens enforcing competitive action as well as secreting cell wall degrading enzymes that inhibit growth and survival of the pathogens [23]. *Trichoderma* species have been widely studied by scientists to manage potato diseases by inducing systemic plant disease resistance and also as a biofertilizer [24,25,26,27]. *Trichoderma harzianum*, *T. atroviride* and *T. viride* have been found to manage crop diseases including late blight on potato. Reference [28] found that *T. harzianum* reduced late blight epidemics on potato and tomato. Application of *T. viride* on potato foliage reduces late blight severity and improves yield [29]. Recently, [30] reported that when applied on plant foliage, *T. asperellum* induced plant defense mechanisms and promoted growth. However, the efficacy of *T. asperellum* through seed treatment prior to planting, to prevent late blight infection early in the cropping season that ultimately results in reduced fungicide application, has not been explored. Biocontrols alone are ineffective in managing late blight on potato because they are slow acting, and require certain conditions that favour their establishment [31], while chemical fungicides are known to have fast mode of action and target specific sites of the pathogen [32]. Therefore, combining biocontrols with the use of disease-free seed and reduced fungicide application could be a reliable and sustainable option in controlling late blight. This could result in reduced health, environmental and cost implications associated with pesticides overuse.

Therefore, the aim of the study was to establish effective *T. asperellum* seed treatment concentration against *P. infestans* in order to increase Ridomil® application interval on apical cuttings and tuber seed of potato to minimize fungicide overuse. This will substantially reduce the cost of production, minimize human hazards to farm workers as a result of reduced exposure and chemical handling and conservation of biodiversity. Furthermore, the use of biocontrol could

minimize emergence fungicide insensitive strains of *P. infestans*.

2. Materials and Methods

2.1. Description of Study Site

The study was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) Tigoni in Limuru (Kiambu County) during short (September 2018 to February 2019) and long (March to July 2019) rain seasons. The Centre is located at latitude 10°9'22"S and longitude 36°4'72"E at an altitude of 2300 m above sea level. The area experiences a bimodal rainfall pattern with average rainfall of 1800 mm per annum and temperature ranging from 10°C to 25°C. Two experiments were conducted where the first one aimed at determining the efficacy of *T. asperellum* seed treatment and Ridomil® application regime on seed tuber and rooted apical cuttings while the second was conducted to compare seed dipping with pericardial injection seed treatment methods.

2.2. Land Preparation and Planting

Rooted apical cuttings and tuber seed of cultivar *Shangi* (certified basic seed generation) were acquired from KALRO, Tigoni. Sprouted tubers were 45 mm grade while rooted apical cuttings were about 20 cm in height aged 4 weeks. *Shangi* yield range between 35 to 40 t ha⁻¹ and takes 3 to 4 months to mature. Although cultivar *Shangi* is susceptible to late blight, it is the most widely grown one in Kenya as it is suitable for making chips and for home consumption. Primary and secondary cultivation was conducted during dry periods (September 2018 and March 2019) before planting to remove weeds and soil clods. A fallow field which had not been cropped with potato for the previous three years was used. Planting of seed tuber and transplanting of rooted apical cuttings was done at onset of rains in November 2018 and April 2019 for short and long rain seasons, respectively. Diammonium Phosphate (DAP) at a rate of 500 kg ha⁻¹ was applied and mixed with soil in planting furrows. DAP was applied during planting of seed tubers and in split application for rooted apical cutting seedlings where the first half was applied at transplanting and the second half 2 weeks afterwards. Calcium Ammonium Nitrate (CAN) was applied at a rate of 440 kg ha⁻¹ six weeks after planting. During short rain season, supplemental irrigation was done to enhance pathogen development whereas during long rain season it was generally wet throughout the cropping season. Harvesting was done in February 2019 and July 2019 for short and long rain season experiment, respectively.

2.3. *Phytophthora infestans* Inoculum Preparation and Bulking

Freshly blighted leaves were sampled from a field in KALRO Tigoni and placed in a petri dishes lined with wet serviette. The petri dishes were incubated for 24 h at 18°C. Pure culture was prepared following procedure described by reference [33]. The pathogen mycelial and

sporangial growth in pure culture was observed using a stereomicroscope using Optika software version 3.2. The pathogen was sub-cultured on fresh pea agar media. The mycelial plug was scrubbed using a sterilized spatula and put in 3 Eppendorf tubes containing 10 mL of sterilized distilled water. The suspension was vortexed for 2 min using electric vortex (MRC laboratory equipment, Germany) and then incubated for 4 h at 4°C to enhance sporangia and zoospore formation. The pathogen inoculum was bulked by placing 20 µl droplets of *P. infestans* sporangia suspension on healthy leaves (abaxial side). The inoculated leaves in a plastic dish (15 x 12 x 5 cm) were incubated at room temperature $18 \pm 2^\circ\text{C}$ for 4 d on laboratory benches. The infected and healthy part was cut and placed on petri dishes. A 0.4 cm thick potato slices were placed on the infected leaf piece and then incubated for 10 d at room temperature ($18 \pm 2^\circ\text{C}$) on the laboratory benches [34]. Bulking of *P. infestans* was done by picking mycelia with a sterilized hypodermic needle from the infected tuber slices and suspending them in 20 mL Eppendorf tubes containing 10 mL of sterilized distilled water. The suspension was filtered through double folded cheese cloth into one litre bottle then incubated for 4 h at 4°C. Pathogenicity test was done on healthy 4 weeks old potato seedlings from glasshouse and tuber slices of cultivar *Shangi* (susceptible) using Koch's postulates. Quantification of *P. infestans* was conducted using Polymerase Chain Reaction (PCR) as described by [35]. Visualization of successful amplicons was done under UV light after agarose gel electrophoresis.

2.4. Experiment 1: Efficacy of *T. asperellum* Seed Tuber and Rooted Apical Cuttings Treatment and Ridomil® Foliage Application Regime

2.4.1. Seed Treatment with *T. asperellum* Suspension

Whole intact tubers were cleaned using running tap water to remove soil, rinsed with distilled water and surface sterilized by dipping in 5% sodium hypochlorite for 15 s. The tubers were rinsed with sterilized distilled water to remove excess alcohol and placed in shade to air dry for 15 min. *Trichoderma asperellum* suspensions at 33% (3.0×10^6 CFU/mL), 66% (7.0×10^6 CFU/mL) and 100% (1×10^7 CFU/mL) concentration of the Manufacturer Recommended Rate (MRR) were prepared using pure spore powder obtained from Real IPM, Kenya. *Trichoderma asperellum* spores of 0.1 g in weight were placed in an Eppendorf tube containing 10 mL (100% concentration). The suspension was mixed with 500 g of sterilized sorghum grains and then incubated at room temperature for 4 d to initiate sporulation. Afterwards, the grains were washed using 500 mL of sterilized distilled water and the concentration adjusted using a hemocytometer to 1×10^7 CFU/mL. *Trichoderma asperellum* at 66% and 33% concentrations were achieved by varying the 0.1 g samples by the percentages followed by standardization using hemocytometer. Mistress 72® (Cynamoxil 4% + Mancozeb 64%) suspension was prepared at a rate of 2 g L^{-1} (MRR) as positive control. From each standardized suspension, 3 litres were prepared and placed in a bucket. Cleaned intact tubers were placed in a netted bag and

dipped in the *T. asperellum* concentration for 15 s. The tubers were stored in a wooden store for 24 h and then inoculated with *P. infestans* zoospore suspension, adjusted to 4×10^4 zoospores/ml. The inoculated tubers were incubated at room temperature ($18 \pm 2^\circ\text{C}$) in the wooden store for 24 h before planting. Similarly, rooted apical cuttings were cleaned with distilled water and inoculated by dipping for 15 s before incubating as described above. Negative control was tubers inoculated with *P. infestans* only.

2.4.2. Experimental Design

Treatments were laid in randomized complete block design in split-split-plot arrangement with 3 replications. Fungicide application regime (7-, 14- and 21-day intervals) and planting material (rooted apical cuttings and tuber seed) were main-plot and sub-plot, respectively. Seed treatments (untreated, Mistress 72® and *T. asperellum* concentrations of 33%, 66% and 100%) were randomized within the sub-plots. Unsprayed plots and those protected using 7-day spray interval were negative and positive control respectively. In the sub-sub-plot, the untreated and seed treated with Mistress 72® formed the negative and positive control respectively. The sub-sub-plots measured $3 \text{ m} \times 3 \text{ m}$ with plant spacing of $0.75 \text{ m} \times 0.3 \text{ m}$. Paths between sub-plots measured 1.5 m in width and 2 m wide between the main plots to avoid fungicide drift.

2.4.3. Field Inoculation and Fungicide Application Regime

Artificial inoculation was performed using zoospores suspension adjusted to 4×10^4 zoospores/ml using hemocytometer. Inoculation was done using calibrated hand sprayer in the evening at a rate of 150 ml of sporangia solution/m². This was done twice during short rain season (18 and 40 Days After Emergence (DAE)) and once during long rain season (18 DAE) by application to the external rows to enhance uniform disease spread and infection. Overhead irrigation was done a day before inoculation and for the next two days after inoculation in morning and late evening to induce *P. infestans* infection during short rain season. Ridomil® application (2.5 g L^{-1}) was initiated upon appearance of first late blight symptoms (22 Days after emergence (DAE)), four days after pathogen inoculation, which is strategy used by the farmers.

2.5. Experiment 2: Comparing Dipping and Pericardial Inoculation Seed Treatment Methods

2.5.1. Seed Treatment and Fungicide Application Regime

To determine efficient method for seed treatment, a parallel experiment was conducted during short and long rain season to compare seed dipping with pericardial injection. A suspension of the *T. asperellum* at 100% concentration, Mistress 72® (2 g L^{-1}) as positive control and *P. infestans* as negative control suspensions, were prepared as described above for experiment one. From each treatment suspension, 45 µL was separately inoculated by pericardial injection on the surface sterilized

intact tubers at the apical end about 1 cm away from developing sprout using a 100 µl hypodermic syringe and needle. The negative check consisted of tubers inoculated with 45 µl of *P. infestans* suspension (adjusted to 4×10^4 zoospores/ml) [29]. Another set of intact tubers was placed separately in a netted bag and inoculated by dipping in the treatment suspension for 15 s and then stored in wooden store on a rack at 15-20°C for 24 h. Ridomil® spray regime was applied as described in experiment one above.

2.5.2. Experimental Design and Planting

The treatments were laid in a randomized complete block design in split-split-plot arrangement with 3 replications. Spray regime and treatment method were the main-plot and subplot, respectively, while seed treatment suspensions (*T. asperellum* at 100% concentration, Mistress 72® (2 g L⁻¹) and untreated) were randomized within the sub-plots. The experimental plots measurements and planting were as described in experiment one above.

2.6. Data Collection

Weather data were collected daily from November 2018 to July 2019 from KALRO Tigoni weather station located about 100 m away from the trial site. Emergence count was taken three weeks after planting while apical cutting survival rate was taken two weeks after transplanting. Sprout, stem and foliage infection symptoms were observed weekly. Height measurement, stems and branch count were taken at 21, 35 and 45 days after emergence on three pre-tagged plants per plot. Rooted apical cuttings produce several branches from a single stem while seed tubers give rise to several stems. Therefore, branch count was done on rooted apical cuttings and stem count on seed tubers, respectively. Score on late blight severity and incidence were taken on weekly basis starting at 26 DAE. 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 [36]. The results were used to calculate Percentage Disease Severity (PDS) which was then summarized using the formula below to compute Area Under the Disease Progress Curve (AUDPC)

$$PDS = \frac{\sum \text{individual numerical rating}}{\left(\frac{\text{Total number of plants assessed}}{\times \text{maximum score in the scale}} \right)} \times 100$$

AUDPC was calculated using the formula:

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

Where y_i represents assessment of disease (percentage) at i^{th} observation, t_i represents time (days) at i^{th} observation and n represents total number of observations.

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

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

Percent disease incidence was highly correlated with percent disease severity ($r^2 = 0.94$). AUDPC provided an adequate summary of the data for analysis.

At maturity, potato tubers were harvested from the inner rows of each 'plot' and inspected for tuber blight symptoms. Tubers were graded as ware (> 60 mm), seed (25 to 60 mm) and chat (< 25 mm) grades. The tubers in each grade class were counted and weighed. Yield data was converted to tonnes per hectare. Both symptomatic and asymptomatic tubers (10 samples) were cut transversely and incubated at 22-23°C for three weeks and inspected every third day for late blight symptoms to determine latent infection. Yield losses were estimated and the percentage of tubers infected:

$$\begin{aligned} & \text{Tuber infected \%} \\ & = \left(\frac{\text{Total no. of infected tubers harvested}}{\text{from the plot}} \right) \times 100. \\ & \quad \text{Total number of harvested tubers} \end{aligned}$$

2.7. Data Analysis

Emergence, height, branches and stem count, yield and yield components, percentage disease severity and AUDPC data were analysed using Statistical Analysis System (SAS) software version 8.2. Data on count were first transformed using square root ($\sqrt{x} + 0.5$) before analysing with SAS. Whenever Analysis of Variance (ANOVA) indicated a significant difference, ($P=0.05$) Tukey's Honest Significant Difference (HSD) was used to separate treatment means. Correlation between AUDPC and yield was determined using linear correlation and regression procedures.

3. Results

3.1. Effect of Weather on Late Blight Epidemics

Contrasting weather conditions were experienced during short and long rain seasons that influenced late blight development. The number of wet days in the short rain season were 21 and temperatures were generally warm (24.5°C), resulting in low Relative Humidity (RH) of 64%, while in the long rain season there were 62 wet days and generally cool temperatures (20 °C) and high RH of 77%. Late blight developed more rapidly in the long rain season than in the short rain season. The result revealed a high disease severity of 100% and 52% in the long and short rain season, respectively, in the unprotected plots (Figure 1) resulting in lower yield than in the short rain season.

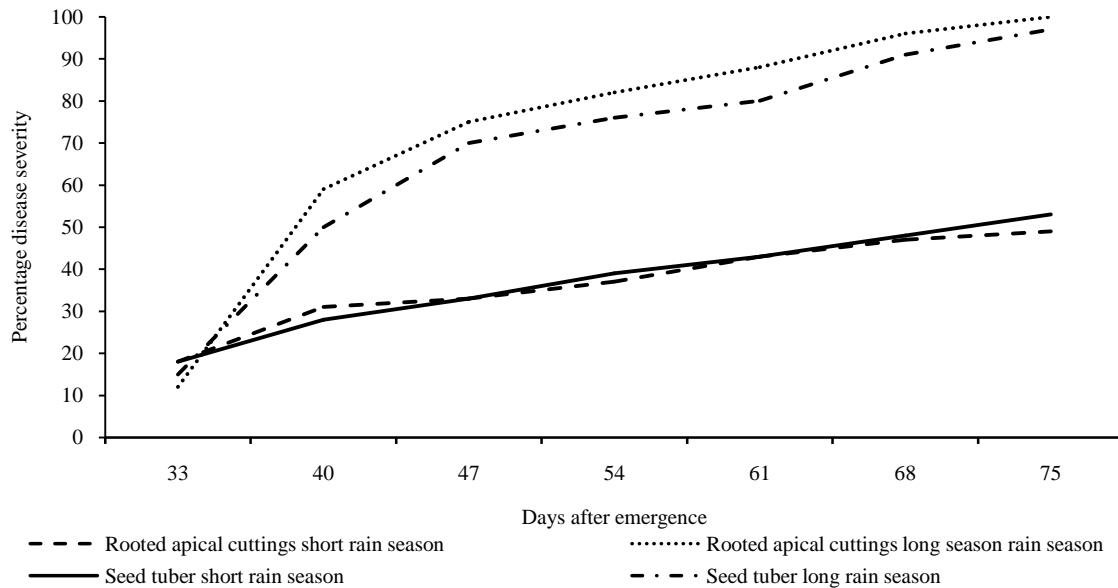


Figure 1. Effect of season on blight progress in unsprayed, untreated planting materials

3.2. Effects of Spray Regime, Planting Material and Seed Treatment and Their Interactions on Late Blight and Yield

The first late blight symptoms were observed 7 days after artificial field inoculation on untreated check and began from lower canopy and progressed upwards. The result was, an additional yield of 39% in the short rain season compared to long rain season. Percent disease severity across days after emergence and ultimate yield significantly differed among Ridomil® application intervals, *T. asperellum* concentrations and between seed type. There was no significant difference among 21-, 14- and 7- days spray intervals in the first 49 DAE. In the later crop growth stages, disease severity on plots sprayed at 7- and 14-day interval regimes were not significantly different. The highest disease severity score and lowest yield was observed in unsprayed plots followed by those sprayed at 21-day interval while 14-day spray interval was not significant different from 7-day spray interval in both seasons. Disease severity progress on rooted apical cuttings was higher than crops from seed tuber. The antifungal activities of *T. asperellum* against *P. infestans* dependent on the biocontrol's concentration. Suspension of *T. asperellum* at 33% concentration and untreated plots gave the highest disease score while *T. asperellum* at 66% and 100% concentration were not significantly different from Mistress 72®. Observed AUDPC on untreated plots was double the one observed on *T. asperellum* at 66% and 100% concentrations treated plots. Seed treated with *T. asperellum* at 66% and

100% reduced the rate of late blight severity upsurge resulting in disease severity reduction by 36% and 38% while contributing to augmented yield by 20% and 21%, respectively, compared to untreated plots.

In the short rain season, Ridomil® application provided better disease control and higher yield than in the long rain season (Table 1). Seasonal differences had significant effect on planting materials and ultimate yield. Rooted apical cuttings had lower severity and higher yield than crops from seed tuber in the short rain season while a higher disease score and lower yield was observed in the long rain season. This led to 8.94% higher yield in crops from rooted apical cuttings than one from tuber seed in the short rain season and conversely, 10.03% higher yield in crops from seed tuber than one from rooted apical cuttings in the long rain season (Table 2). Seed treatment gave a higher yield in the short rain season than in the long rain season (Table 3). Regime and seed type interaction resulted in higher yield in plots protected with 7- and 14-day spray intervals combined with seed tuber. Yield observed in rooted apical cuttings and seed tuber plots, sprayed with Ridomil® using similar spray interval was not significant different in the short rain but differed in the long rain (Table 4). Higher disease and yield lower score was observed in the untreated plots than one with pre-treated seed crop in all three fungicide application regimes including 7 day interval (recommended by manufacturer). Ridomil® application at 7- and 14-day spray interval in combination with *T. asperellum* at 66% and 100% concentrations provided the highest yield (Table 5).

Table 1. Effects of fungicide spray regime on late blight severity (AUDPC) and Yield (t ha⁻¹)

Regime	Short rain season		Long rain season	
	AUDPC	Yield (t ha ⁻¹)	AUDPC	Yield (t ha ⁻¹)
Unsprayed	1412.80a	5.88a	3242.10a	4.38a
21day interval	1063.70b	21.02b	2670b	8.81b
14day interval	854.10c	23.99c	2201.41c	20.36c
7day interval	806.70c	24.01c	2173.92c	20.40c
HSD (P=0.05)	50.25	0.85	71.45	0.59
CV (%)	7.23	6.73	4.12	6.57

Values followed by similar letters in the same column indicate that the treatments are not significantly different

Table 2. Effects of planting materials on late blight severity (AUDPC) and Yield (t ha⁻¹)

Planting materials	Short rain season		Long rain season	
	AUDPC	Yield (t ha ⁻¹)	AUDPC	Yield (t ha ⁻¹)
Apical cuttings	1036.3a	19.24a	2691.82a	12.94a
Seed tubers	1023.18b	18.21b	2451.98b	14.03b
HSD (P=0.05)	26.93	0.32	38.30	0.32
CV (%)	7.23	6.73	4.12	6.57

Values followed by similar letters in the same column indicate that the treatments are not significantly different.

Table 3. Effects of *Trichoderma asperellum* concentration on late blight severity (AUDPC) and Yield (t ha⁻¹)

<i>Trichoderma asperellum</i> rates	Short rain season		Long rain season	
	AUDPC	Yield (t ha ⁻¹)	AUDPC	Yield (t ha ⁻¹)
Untreated	1472.46a	14.84a	2862.58a	12.58a
33% <i>T. asperellum</i>	1486.b	15.00a	2791.75a	12.76a
66% <i>T. asperellum</i>	789.38c	21.06b	2410.79b	13.91b
100% <i>T. asperellum</i>	750.42c	21.34b	2399.92b	14.02b
Mistress 72 [®]	749.29c	21.38b	2394.46b	14.15b
HSD (P=0.05)	59.00	1.02	85.01	0.70
CV (%)	7.23	6.73	4.12	6.57

Values followed by similar letters in the same column indicate that the treatments are not significantly different.

Table 4. Effects of fungicide spray regime and planting material on yield (t ha⁻¹)

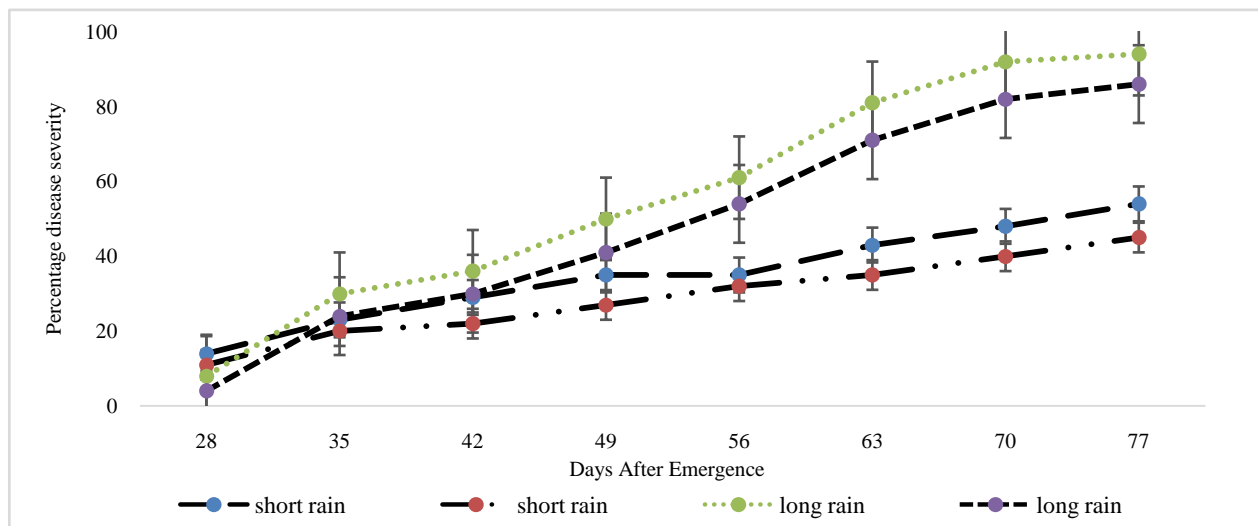
Spray regime	Short rain season		Long rain season	
	Seed tuber	Rooted apical cuttings	Seed tuber	Rooted apical cuttings
Unsprayed	1.17 ± 0.08a	0.96 ± 0.07b	0.83 ± 0.06a	0.64 ± 0.05b
21day spray interval	13.73 ± 0.99a	14.61 ± 0.92a	9.18 ± 0.25a	8.43 ± 0.25b
14day spray interval	23.26 ± 0.60a	24.65 ± 0.88a	21.02 ± 0.47a	19.57 ± 0.24b
7day spray interval	23.45 ± 0.62a	24.60 ± 0.86a	21.34 ± 0.37a	19.58 ± 0.23b
CV%	6.73		6.57	

Values followed by same letter within the same row indicate treatments do not differ significantly.

3.3. Effect of Seed Treatment Method on Late Blight Development and Yield

In both seasons, seed dipping had higher disease progress than the pericardial though they were not significantly different (Figure 2). Conversely pericardial injection had 7.73% higher yield compared to dipping. Results observed in plots protected with spray regime, seed treatment concentration and their interaction followed the same trend as described in experiment one above. Seed treatment with *T. asperellum* and Mistress

72[®] by pericardial injection gave the highest disease severity and yield compared the same treatments pretreated by dipping (Figure 3). Fungicide regime and treatment method interaction resulted in AUDPC observed in seed treatment method differing only in the long rain with pericardial injection recording the highest disease score and conversely the highest yield compared to dipping within the same spray regime. Higher yield was observed in pretreated crops by pericardial injection and protected with Ridomil[®] applied at 7- and 14-days intervals (Table 6).

**Figure 2.** Effect of season and seed treatment method on late blight development across days after emergence

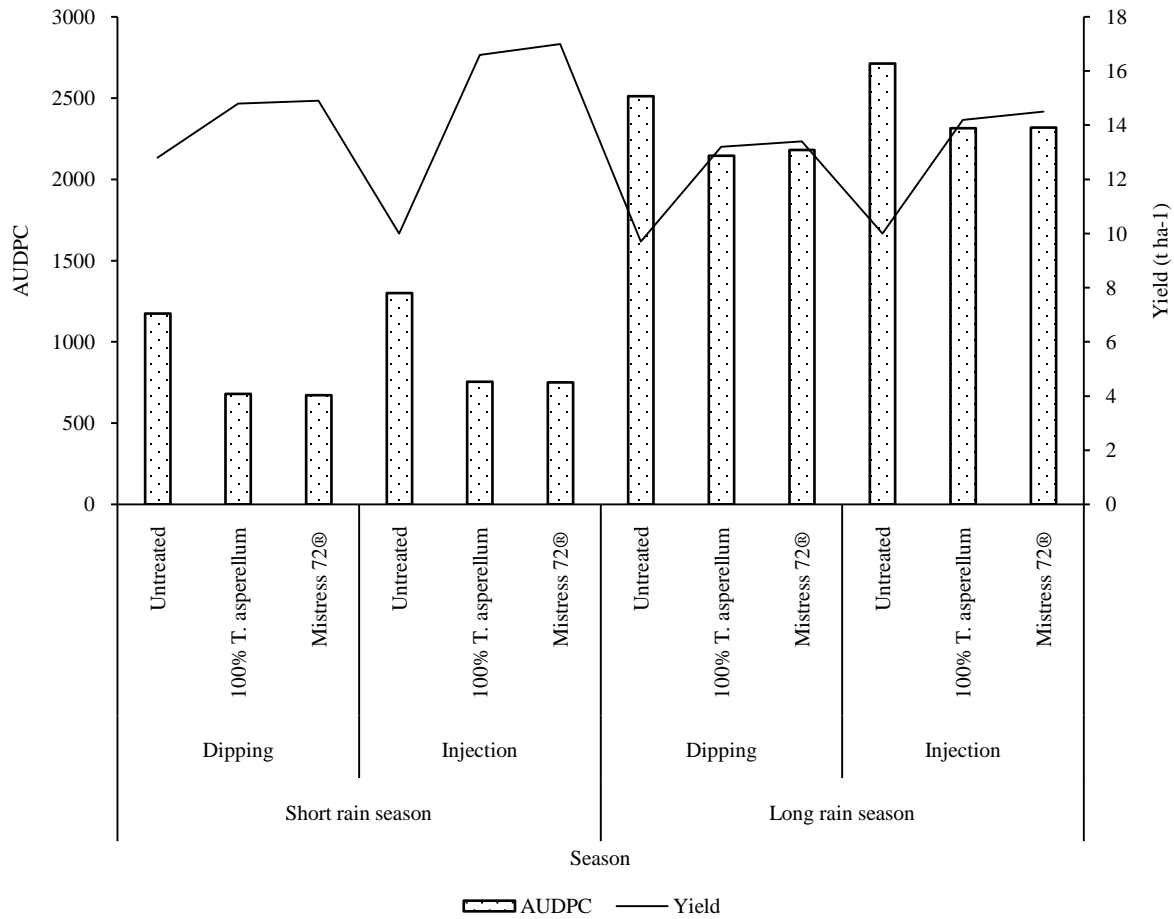


Figure 3. Effect of treatment method and seed treatment rate interaction on late blight severity and yield

Table 5. Effects of seed treatment and fungicide spray regime on late blight severity and yield

Season	<i>T. asperellum</i> rates	AUDPC Means ± Standard Error (SE)				Yield (t ha ⁻¹) Means ± Standard Error (SE)			
		unsprayed	21 days	14 days	7 days	Unsprayed	21 days	14 days	7 days interval
Short rain	untreated	1868±29.23a	1435±53.39b	1403±51.30b	1241±78.53c	0.73±0.06a	11.01±1.75b	20.9±0.85c	20.7±0.80c
	33%	1718±36.37a	1455±47.25b	1319±38.55c	1164±85.12d	0.86±0.06a	11.28±1.01b	20.9±0.69c	21.0±0.87c
	66%	1233±43.09a	780±22.47b	597±24.68c	540±55.34c	1.21±0.10a	15.98±0.49b	25.9±0.51c	25.9±0.45c
	100%	1120±14.15a	769±19.84b	578±23.87c	527±48.45c	1.28±0.12a	16.39±0.56b	26.1±0.48c	26.2±0.5c
	Mistress 72	1125±23.39a	781±27.22b	581±19.01c	516±65.43c	1.25±0.12a	16.19±0.56b	26.1±0.56c	26.3±0.4c
	CV%				7.19				6.92
Long rain	untreated	3568±42.97a	2905±56.46b	2527±83.84c	2450±115.67c	0.51±0.11a	7.74±0.37b	20.04±0.65c	19.51±0.54c
	33%	3511±45.03a	2859±77.50b	2436±86.63c	2361±86.65c	0.64±0.09a	8.00±0.28b	19.71±0.70c	19.84±0.54c
	66%	3049±44.76a	2542±65.25b	2024±115.15c	2027±109.64c	0.89±0.05a	9.26±0.17b	20.70±0.69c	20.54±0.56c
	100%	3055±39.77a	2521±67.93b	2008±123.72c	2015±107.11c	0.84±0.05a	9.46±0.28b	20.77±0.73c	20.90±0.50c
	Mistress 72	3026±40.25a	2522±64.05b	2012±120.56c	2017±116.38c	0.79±0.06a	9.59±0.28b	20.77±0.70c	20.96±0.57c
	CV%				4.09				11.85

Values followed by same letter within the same row indicate treatments do not differ significantly.

Table 6. Effect of spray regime and seed treatment method on late blight and yield of potato

Spray interval	Short rain season				Long rain season			
	AUDPC		Yield (t ha ⁻¹)		AUDPC		Yield	
	Dipping	Injection	Dipping	Injection	Dipping	Injection	Dipping	Injection
Unsprayed	1219.44 ± 67.65a	1341.89 ± 100.68a	1.35 ± 0.17a	0.96 ± 0.06b	2964.11 ± 50.30a	3104.22 ± 72.19b	0.98 ± 0.09a	0.76 ± 0.06b
21-day	856.89 ± 76.81a	903.44 ± 76.23a	8.95 ± 0.61a	10.83 ± 0.26b	2398.78 ± 58.70a	2592.78 ± 61.75b	6.94 ± 0.37a	9.48 ± 1.45b
14-day	665.00 ± 70.09a	785.00 ± 60.89a	17.87 ± 0.51a	21.36 ± 0.47b	1907.11 ± 72.94a	2100.56 ± 63.82b	21.30 ± 0.14a	23.67 ± 0.47b
7-day	647.00 ± 50.94a	716.22 ± 52.54a	16.47 ± 0.76a	21.16 ± 0.36b	1836.67 ± 82.53a	1998.44 ± 75.55b	21.16 ± 0.16a	23.77 ± 0.36b
CV%		6.01		8.27		8.25		5.95

Values followed by same letter within the same row indicate treatments do not differ significantly.

Table 7. Effect of seed inoculation on stem and branch count and height

Treatment	Branch count		Stem count		Height (cm)		No. of hills	
	21 DAE	45 DAE	21 DAE	45 DAE	21 DAE	45 DAE	S	L
Untreated	2.82a	1.67a	2.98a	3.17a	10.87a	31.71a	8a	6a
33% <i>T. asperellum</i>	2.40a	3.33a	4.26b	5.08b	15.50b	46.5b	8a	6a
66% <i>T. asperellum</i>	3.15a	4.17b	4.29b	6.67b	16.22b	55.17c	13b	12b
100% <i>T. asperellum</i>	3.81a	4.17b	4.79b	6.67b	17.05b	56.04c	12b	12b
Mistress 72 [®]	3.08a	4.42b	4.80b	6.42b	17.00b	55.96c	13b	13b
HSD (p=0.05)	1.979	0.875	0.782	0.898	2.145	3.394	1.245	1.145
Unsprayed	2.81a	3.13a	4.51a	3.93a	16.32a	36.43a	8a	8a
7 days interval	3.46a	3.73b	4.07b	5.87b	15.61a	52.97b	13b	13b
14 days interval	3.74a	3.74b	4.21b	6.13b	14.60a	52.60b	12b	13b
21 days interval	2.20a	3.33b	4.13b	6.47b	14.79a	52.30b	10c	9a
HSD (p=0.05)	0.418	0.733	0.656	0.753	1.802	2.853	1.335	1.230
Apical cuttings	3.0	3.4	-	-	14.72a	48.83a	12a	8a
Tuber seed	-	-	4.1	5.6	15.94b	57.32b	13b	12b
HSD (p=0.05)					0.967	1.529	1.842	1.753
CV%	22.98	15.68	22.98	15.68	17.32	10.23	10.23	12.30

Values in the same column followed by similar letters indicate treatments do not differ significantly. L and S represent long and short rain respectively while HSD represents Tukeys Honest significant difference.

3.4. Effect of Spray Regime and Seed Treatment on Crops from Seed Tuber and Rooted Apical Cuttings

Seed treatment and spray regime influenced branch and stem count in rooted apical cuttings and seed tuber crops. Higher stem and branch count was observed in treatments sprayed at 7 and 14 days at 45 DAE. There was a decline in branch count in the untreated check and crop treated with *T. asperellum* at 33% concentration at 45 DAE compared to what was recorded at 21 DAE. Seed treatment with *T. asperellum* at 100% and 66% concentration resulted in higher branch and stem count relative to untreated seed. Crop from seed tuber had a higher mean height of 57.32 cm compared to rooted apical cutting whose height was 48.83 cm. There was no significant difference ($P=0.05$) among spray regimes in terms of height but they differed significantly in branch number. Unsprayed treatments had the lowest stem count whilst higher stem count was observed at 7-, 14- and 21-day spray intervals in that order compared to unsprayed plots. The number of plants (number of hills) was also higher in crops from seed tuber (13) than one from rooted apical cutting (12) at harvesting (Table 7). Seed treatment contributed to increase in stem count by double and increased branch count by four fold compared with the negative control. Untreated check had the lowest number of stems and mean plant height.

3.5. Effect of Seed Inoculation on Seed Tuber Emergence and Rooted Apical Cuttings Survival

Seed tuber took 18 days to emerge after planting but in 5th week they were about the same height demonstrating vigorous growth in tuber seed. However, apical cuttings flowered 15 days earlier than crop from seed tuber. Seed tuber inoculation with *P. infestans* caused foliage infection appearing first on the base of the stems before leaves were infected about 7 DAE with untreated plots scoring the highest disease incidence. The symptoms afterwards

progressed up the crop canopy. Emergence in seed tuber was affected significantly by *P. infestans* inoculation in untreated check resulting in reduced plant population. The lowest emergence score was observed in untreated treatments scoring 68% followed by *T. asperellum* at 33% concentration with 79% emergence. Emergence was not significantly different ($P=0.05$) among *T. asperellum* at 66% (96%) and 100% (100%) concentrations and Mistress 72[®] (97%) treated seed. Decaying of seed tubers was observed on planting holes where plant failed to emerge while some sprouts died shortly after emergence in the untreated check. There was no significant difference in survival rate among rooted apical cutting plots inoculated with *P. infestans* only (83%) (Untreated check), *T. asperellum* at 33% (92%), 66% (96%), 100% (100%) concentration and Mistress 72[®] (96%). Plants from seed treated with *T. asperellum* at 66% and 100% concentration were dark green and remained green while the other treatments were yellowish-brown at harvesting.

3.6. Effects of Late Blight on Tuber Quality (Tuber Infection)

Tuber infection was only observed in the long rain season in the unsprayed plots. Mycelial growth of *P. infestans* was observed on the infected tubers after incubation. Higher tuber infection was observed on untreated followed by plots with seed treated with *T. asperellum* at 33% concentration plots than one treated with 66% and 100% *T. asperellum* concentrations and Mistress 72[®]. This contributed to reduced potato yield from the allied plots.

4. Discussion

Interest in the use of beneficial micro-organisms has continued to increase over time. Of all the described beneficial fungi, only few have been progressed to commercial application as a result of limited selection and efficacy evaluation strategies. Whereas efficacy studies on

biocontrol agents in general have been conducted *in vitro*, field screening under conditions with diverse soil microbial communities could provide an effective and reliable predictor. Field based screening of several *Trichoderma* spp. isolates for late blight control showed antagonistic activity against *P. infestans* albeit inefficient reduction of disease severity [21]. In the present study, efficacy of *T. asperellum* when used in combination with metalaxyl based fungicide formulation (Ridomil®) was evaluated under field conditions to estimate the accruing biological efficiency.

A higher amount of disease observed on crops from seed tuber as compared to crops from rooted apical cuttings in the short rain season could be attributed to latent infection as primary source of inoculum in asymptomatic seed tubers. Indeed, disease free materials including rooted apical cuttings have been found to contribute to reduction in disease epidemics [37]. Contrariwise, rooted apical cuttings were more vulnerable to late blight than crops from seed tuber in the long rain season. Although the study did not aim to establish the relative vulnerability of rooted apical cuttings, we speculate that dense canopy of crops from rooted apical cuttings could have predisposed the crop to late blight. Lack of food reserve unlike in seed tuber and weak rooting system at establishment phase may have also slowed adaptation to field conditions in rooted apical cuttings contributing to their vulnerability. Moreover, a strong rooting system influences nutrient acquisition including calcium and potassium which are vital in disease resistance [38,39].

Optimal use of *T. asperellum* in seed treatment reduced seed decay and sprout infection thereby improving crop emergence. In cases of sprout death, this occurred shortly after emergence in untreated plots, suggesting transmission of *P. infestans* from latently infected seed to developing sprouts [40,41]. Disease reduction meant more healthy plants as a result of escape as well as antifungal activities including mycoparasitism, antibiosis, enzymatic activities (chitinase, glucanase and proteinase) and induced plant defence instigated by *T. asperellum* against *P. infestans* are implicated [24,42,43]. Further, the apparent increase in plant height, stem and branch count can be explained by enhanced nutrients uptake [44,45,46,47,48]. The inability of the *T. asperellum* at 33% concentration to suppress the *P. infestans* implies that the bio-control was out-competed at this low concentration [49,50]. A higher starting concentration ensures quick and profuse sporulation that contributes to competition against the pathogen [51].

In regards to deployment of the metalaxyl based synthetic fungicide against *P. infestans*, closer spray intervals of 7-and 14-day intervals provided satisfactory late blight management. At 21 day spray intervals, fungicide sprays were insufficient to manage late blight especially during prolonged wet weather conditions. It is our view that in longer spray intervals, degradation of the fungicide's formulation compromised the efficacy [52,53]. In addition, when the product dosage is below optimum, then the pathogen establishes faster to produce more disease [54,55].

A delineation of the effects due to each factor or interaction of factors assessed in disease reduction under

this study is as follows. Firstly, during prolonged wet weather conditions, rooted apical cuttings, fungicide and seed treatment as essential disease measures singly provided unsatisfactory control of late blight. In contrast, use of either optimal fungicide spray intervals, cropping using seed tuber or seed tuber treatment individually reduced the amount of disease. Secondly, when considered separately, seed tuber pre-treatment with *T. asperellum* without crop spray with Ridomil® was ineffectual against late blight. Furthermore, crops from in control plots planted with seed pre-treated with *T. asperellum* yet unsprayed with the fungicide, were completely destroyed prior to tuberization. The effect of *T. asperellum* on the late blight could have been temporal to protect the potato crop from late blight [26]. Therefore, simultaneous use of the *T. asperellum* at optimal concentration with 7- or 14-day fungicide spray interval provided the most suppression of the disease. The synergistic activity achieved by this combination ensured that the biocontrol reduced infection of late blight early in the cropping season while Ridomil® protected the foliar or killed *P. infestans* mycelia already attached on the leaves [31,56].

The technique of treating seed with the biocontrol influenced disease control. Seed treatment by dipping produced better control of late blight compared to treatment by pericardial injection. We proffer that dipping seed in the bio-control suspension assures coating of the tuber surface and therefore increases the chance to counteract *P. infestans* [57]. As *P. infestans* infects host plant by establishing penetration peg into the host plant tissues [58], an intact seed tuber provides a natural barrier to *P. infestans* haustorial penetration during infection. Also, by virtue of precedence, dipping allows *T. asperellum* to penetrate and colonize internal tuber tissues and/or triggering induced disease resistance to hinder the pathogen from establishing [59]. Considering routine application of the biocontrol agent on a commercial scale, seed treatment by dipping is more efficient and cost effective compared to pericardial injection. The need for large scale seed tuber treatment can be resolved by exploring automated dipping as a method of treatment. The practical needs for smallholder farming systems devoid of technical expertise and large capital investment can be addressed through availing pre-treated packages of seed ready for planting. This eliminates the challenges of determining the appropriate suspension concentration if seed treatment were to be done on-farm.

In view of the foregoing, the study shows that fungicide application cannot be avoided. However, fungicide usage could be reduced sustainably by combining with biocontrols to offer prior protection against late blight infection early in the cropping season in an integrated disease management approach.

5. Conclusion and Recommendations

The findings of this study support the following conclusions; First, the rapid establishment and quick canopy development of rooted apical cuttings used as seed provides an opportunity for the crops to escape blight

attack, more so in the short rain season than in the long rain season. Secondly, to effective blight suppression through seed treatment is achieved at optimal *T. asperellum* suspension concentrations of 66% and 100%. Thirdly, there is synergistic benefit of combining *T. asperellum* optimal concentrations with 7-or 14-day spray interval with metalaxyl based fungicide to control late blight severity. Lastly, we conclude that seed treatment through dipping could be used prior to planting with subsequent fungicide usage on emerging crops. This approach reverses risks of latently infected yet symptomless seed tubers that are common source of primary *P. infestans* inoculum responsible for late blight epidemics early in the cropping season. Future studies could be conducted to establish possible roles and mechanisms of *T. asperellum* in potato growth and yield promotion.

Conflict of Interest

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

Acknowledgements

We would like to express our sincere gratitude to Centre for Sustainable Agriculture and Agribusiness (CESAAM) Egerton University for funding this study and Kenya Agricultural and Livestock Research Organization (KALRO) Tigoni Centre Director (Dr. Moses Nyongesa) for offering laboratory facilities and field for conducting this project. We are also grateful to Real IPM, Kenya for providing pure *Trichoderma asperellum* spores.

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