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In-vitro Evaluation of Fungicide Sensitivity of Tomato Leaf Blight Pathogens

Fredrick O. Ogolla[1*], Ruth Nyakinywa[1], Samson K. Chabari[2] and Benson O. Onyango[3]

[1] Chuka University, Department of Biological Sciences. Chuka, Kenya,
[2] Chuka University, Department of Environmental Resource Development. Chuka, Kenya,
[3.]Jaramogi Odinga University of Science and Technology, Department of Biological Sciences, Bondo, Kenya
*Corresponding author: ogolla.fredy@gmail.com

ABSTRACT

Tomato early and late blight diseases caused by *Alternaria solani* and *Phytophthora infestans* respectively are constraints to tomato production globally. Conventional use of commercial synthetic fungicides in management of tomato blight disease has become a key input for tomato production among farmers in Tharaka Nithi County. This study was carried out *in-vitro* to evaluate the efficacy of six synthetic commercial fungicides used by farmers around River Ruguti, against two tomato leaf blight pathogens; *Alternaria solani* and *Phytophthora infestans*. The poison food method was used to evaluate fungicides known by trade names and application levels; Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}), Mancozeb 640 g/kg + Metalaxyl 80 g/kg, Mancozeb, Propineb700 g/kg + Cymoxanil 60 g/kg, Carbendazim and Triticonazole at different concentration (25%, 50% and 75%). The *in-vitro* plate experiment was laid out in a Complete Randomized Design with 3 replicates, and data on mycelia growth inhibition analyzed through General Linear Model (α=.05) and significant means separated using Least significant difference (LSD) using Scientific Analysis System version 9.4. All the tested fungicides significantly ($p \leq 0.05$) inhibited mycelial growth of tested pathogen. Percentage inhibition for early blight pathogen (*Alternaria solani*) was 80.42% compared to late blight pathogen *Phytophthora infestans* at 69.51%. Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}) and Propineb700 g/kg + Cymoxanil 60 g/kg recorded higher per cent inhibition of mycelia growth of 92.4% and 89.71% respectively. Carbendazim recorded lower per cent inhibition of 39.15%. Mycelia growth inhibition increased with an increase in fungicide concentration. Lower inhibition of 71.78% was observed at 25% concentration as compared to 50% and 75% with 76.77% and 76.36% respectively. Fungicides screened varied in mycelia inhibition against *P. infestans* and *A. solani* isolates with Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}) and Propineb700g/kg +Cymoxanil 60g/kg giving significantly ($p \leq 0.05$) better inhibition while Carbendazim had the lowest inhibition effect. Increased fungicide concentration effectively inhibited mycelia growth.

Keyword: In-vitro, fungicides sensitivity, *Alternaria solani*, *Phytophthora infestans*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a nutritious dietary source of the antioxidant lycopene and vitamins (Dawid, 2016). The production of tomatoes is constrained by several fungal pathogens including *Phytophthora infestans* and *Alternaria solani* which cause early and late blight diseases respectively (Mengesha, 2017; Chasti *et al*., 2018; Saima Farooq *et al.*, 2019). Up to 79% of tomato loses are due to leaf blight disease (Singh *et al*., 2017; Gulzar *et al.*, 2018). *Alternaria solani* propagules may overwinter in plant debris and sustain infection in the subsequent planting seasons (Chaerani and Voorrips, 2006). Distinctive symptoms of *A. solani* infection is blight formation with concentric brown to black

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rings that appear on mature lower leaves which progress to younger upper leaves (Roopa *et al.*, 2014). Infected leaves turn pale yellow prior to withering and falling off (Biovision, 2019). *Phytophthora infestans* causes late blight of tomatoes at all growth stages (Keskse, 2019). It results in plant death arising from leaf and stem necrosis (Biovision, 2019). Late blight symptoms include water-soaked lesion on leaves which maybe circular or irregular and are near leaflet margins, and the lesions may spread elsewhere on the leaves as disease progresses (Griffith *et al*., 1995).

Synthetic fungicides have been used in management of crop fungal diseases due to ease of application and availability to farmers (Akram *et al*., 2018; Vinay *et al*., 2020). The fungicides range from protectants, systemic and eradicant of plant diseases (Bartlett *et al*., 2002; Russell, 2005; Fernández-Ortuño *et al.*, 2010). Mancozeb is a protectant fungicide used alone or in combination with other fungicides (Gullino *et al.*, 2010), while Metalaxy is both a systemic and curative fungicide (Yang *et al.*, 2019). Fungal pathogens may develop insensitivity to fungicides due to improper dosage or frequency of fungicide application (Namanda *et al.*, 2003; Akram *et al*., 2018). According to Nyankanga *et al.* (2004), some farmers in Kenya only use fungicides when the crop develops symptoms which may affect disease management efforts. Fungal attributes of *A. solani* that contribute to fungicide resistance include its inherent genetic capacity to form melanin which protect it from effects of the fungicide (Bell and Wheeler, 1986). Periodic quality evaluation of fungicides is a necessary pre-requisite for disease management, to increase efficacy and tomato production (Hassan *et al.*, 2014).

Efficacy of fungicides some of which are used in tomato farming in Tharaka Nithi County has previously been investigated for different patho-sytems giving varied results (Abada *et al*., 2008; Gomaa, 2001; Patil *et al.*, 2001; Karima and Sayeda, 2007; Patel, 2012; Roopa *et al.*, 2014; Ghazanfar *et al*., 2016; Sarfraz *et al*., 2018; Farooq *et al.*, 2019). Though farmers have continually applied fungicides in their tomato farms along River Ruguti in Tharaka area of Tharaka Nithi County, symptoms of early blight and late blight diseases are still persistent in the farm. Further, there exists scarce information on the response of fungal blight of tomato in Kenya, particularly along River Ruguti. This study was therefore conducted to determine *invitro* efficacy of different fungicides commonly used along River Ruguti to manage early blight and late blight tomato diseases.

MATERIALS AND METHOD

Study area

The fungus pathogen (*Alternaria solani* and *Phytophthora infestans)* used in this study were isolated from infected tomato leavescollected from Tharaka-Nithi County, upper eastern Kenya. The County is located between longitudes 37º 19’ and 37º 46’ East and latitude 000 07’ and 000 26’ South. The study area is divided into the upper and lower agro ecological zones. It experiences an average annual rainfall of 717 mm. Areas around Chuka and Chogoria which are on high altitude receives reliable rainfall compared to lower regions in Tharaka area which is characterized by unreliable, low and poorly distributed rainfalls. The temperatureranges from 14ºC to 30ºC in highlands and 22ºC to 36ºC in lowlands of the County. The soil pH in Tharaka Sub County ranges from a pH of 5 to a pH of 8. The soils are dark grey-brown, clay and sandy clay loam topsoil which are imperfectly drained (Ministry of Agriculture, Livestock and Fisheries (MoALF., 2017)).

Study design

The study was conducted in a 3 x 6 factorial laid out in a Complete Randomized Design (CRD) with factor A being six levels of Fungicides (Mancozeb (640 g kg^{-1})) + Metalaxyl (40 g kg^{-1}), mancozeb 640 g/kg + metalaxyl 80

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g/kg, Mancozeb, Propineb700 g/kg + Cymoxanil 60g/kg, Carbendazim and Triticonazole) and factor B being levels of fungicide concentration (25, 50 and 75). The experiment was replicated six times.

Sample collection, media preparation and pathogen isolation

Sample collection

Late and early blight symptomatic leaves were collected from tomato growing area along River Ruguti in Tharaka. Early blight lesions were identified by their characteristic concentric rings on leaves, stem and on fruits. On the other hand, symptoms used to identify late blight included water soaked spots, appearing on the leaf at the margin or tips of lower leaves which were enlarging and were irregular in shape compared to early blight lesion. The area was ideal for sample collection based on long history of tomato farming along the river. Symptomatic leaves were randomly collected and aseptically cut using sterile scapels, wrapping in labeled ziplock bags, samples were then placed in cool box and transported to Chuka University laboratory for pathogen isolation.

Fungal pathogen isolation purification and identification

The *P. infestans* and *A. solani* pathogen were isolated on the Potato Dextrose Agar (PDA) prepared using the manufacturer's (OXOID, Thermo Fisher Scientific, United Kingdom) procedure. The isolates of *P. infestans* and *A. solani* were then purified on corn meal agar media. Media was autoclaved at 121°C, at 15 psi for 15 min prior to cooling at 50°C in water bath. Contamination by bacteria was prevented by incorporating antibiotic [25 mg/l] in all the media. Tomato leaves with early blight symptoms were cleaned under running tap water to remove dust particles. Thin sections (4 mm) of diseased leaves were cut and placed in 0.5% sodium hypochlorite solution for surface sterilization for 30 seconds. Surface sterilized leave sections were washed in a series of sterile distilled water to remove the disinfectant.

The pieces were dried using blotting paper in a petri dish placed on potato dextrose agar aseptically. Plates inoculated with diseased leaf sections were incubated at room temperature of 25°C for fourteen days. Fungal colonies were sub cultured in Corn Meal Agar media for pure cultures. Isolates were identified based on microscopy observation of the conidia where lactophenol cotton blue was used for staining. Pure cultures were used for the pathogenicity test and evaluation of effect of different fungicides at different concentrations on mycelia growth.

Pathogenicity test

Three weeks old Commando F1 tomato seedlings raised in seedling germination trays were used for pathogenicity test. Fifteen tomato seedlings, five seedlings each for *P. infestans* and *A. solani* were used for pathogenicity test. Commando F1 tomato variety was used for the study since it is grown in the sample collection area. Five tomato seedlings were sprayed with 10 ml of a week old conidial suspension 5×10^6 conidia/mL of *P. infestans* and *A. solani* and distilled water respectively. Tomato seedlings were then covered with polythene bags for a day to favor pathogen establishment and disease development. On the second day, inoculated tomato seedlings were transferred to the greenhouse and observed for diseases symptoms after two weeks. Pathogen re-isolation was done on symptomatic leaves and culture compared with the initial cultures to satisfy Koch's postulates (Agrios, 2005).

Fungicide assay using poisoned food technique:

The efficacy of six fungicides (Table 1) was tested at three concentrations 25, 50% and 75% against *A. solani* and *P. Infestans* on corn meal agar medium. Isolate PI-T1 (*P. infestans* -Tharaka) and AS-T10 (*A. solani*-Tharaka) used in fungicide assay study was selected due to their rapid growth rate. The media was prepared by dissolving 17 g of Corn Meal Agar in 250 ml of distilled water and was heated to completely dissolve the content then topped up to 1000 ml. Sterilization of the media was done at 121°C at 15 psi for 15 minutes and media cooled to 50°C in water bath, ampicillin (25 mg/l) was added thereafter to

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inhibit growth of bacteria contaminants, individual fungicides at different concentrations were added to individual media containers and dispensed in sterile plates. Using a sterile cork borer of 3 mm, pure cultures of *A. solani* and *P. infestans* fungal - isolates were aseptically picked and placed at the center of treated corn meal agar.

Measurements of the diameter of mycelia growth was taken at the 3rd, 5th and 7th day of incubation in two directions at 90° and subtracted from that of control plate. Percent inhibition (PI) values for each fungicide were calculated by the formula below (Mannai *et al*., 2018):

$$\frac{a-b}{a} \times 100$$

Where a = mycelia diameter of control plates and b = mycelia diameter of fungicide treated plates.

Statistical analysis

Inhibition data (%) collected were analyzed using General Linear model (GLM). Significant means were compared using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.

Table 1: Details of fungicides used in the study

Active ingredient	Trade name	Acronym used in this study	Manufacturer	Chemical group
Mancozeb (640g kg^{-1}) + Metalaxyl (40g kg^{-1})	Ridomil gold MZ68W	Rl	Syngenta East Africa limited	Dithiocarbamate Acylamino acid
Carbendazim	Chariot	Crt	Greenlife crop protection Africa	Carbendazim
Mancozeb	Oshothane 80WP	Ohn	Osho chemical industries limited	Dithiocarbamate
Mancozeb 640g/kg + Metalaxyl 80g/kg	Victory 72WP	Vty	Amiran Kenya Limited	Mancozeb 640g Metalaxyl 809
Propineb700g/kg Cymoxanil 60g/kg	Milraz WP 76	Mlz	Bayer Crop Science	Dithiocarbamate 700 g/kg Cymoxanil 60 g/kg
Triticonazole	Trinity Gold® 452WP	Trty	Green life crop Protection	Copper xychloride Mancozeb Cymoxanil

Source: Hamel *et al.* (2011) and Zhou *et al.* (2016)

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RESULTS AND DISCUSSION

Microcopy identification of the *Alternaria solani* and *Phytophthora infestans*

Pathogenicity experiment for early blight and late blight produced symptoms which were comparable to those observed on the leaf samples from where the pathogens were isolated. Late blight symptoms in tomato begun as pale green water-soaked lesions and turned brown with time. The lesions spread both in the leaves and stem of inoculated tomatoes. Lesion margins in late Blight were irregular as compared to those of early blight which were slightly circular and formed small concentric rings. The early blight cultures had grey to greenish colour and darkened with time while those of late blight pathogen appeared whitish. When observed under the microscope, some of the conidia observed for *Alternaria solani* either had beaks or no beaks, had transverse and longitudinal segments (Figure 1 A). The conidia of *Alternaria* isolates were either straight or slightly curved. The conidia for the *Phytophthora infestans* were ovoid and lemon shaped with irregular swellings (Figure 1 B).

Inhibition effect of fungicides on *Phytophthora infestans* and *Alternaria solani*

Percentages of mycelia growth inhibition for pathogens (*P. infestans* and *A. solani*) was statistically significant ($p<0.05$). Pathogen had $F(1, 310) = 64.07$, $p < .00$. Early blight pathogen (*A. solani*) was inhibited more at 80.42% as compared to late blight pathogen *P. infestans* at 69.51% (Figure 2). The mean fungal mycelia growth inhibition was 74.96% with coefficient variation of 17.804% and least significance difference (LSD) of 2.779at $p<0.05$.

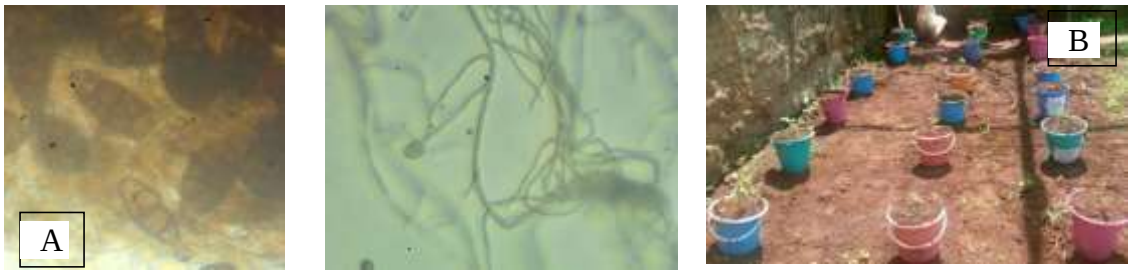

Figure 1: Microscope images of *Alternaria solani* (A), *Phytophthora infestan* (B) conidia, experiment set up for pathogenicity test (C)

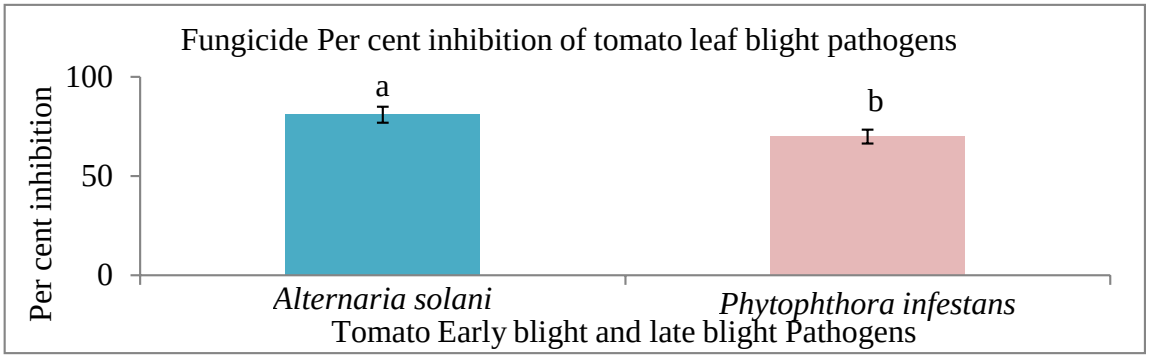

Figure 2: *In vitro* mycelia inhibition (%) of tomato leaf blight pathogens

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Mycelia growth inhibition effect of different fungicides

Effect of different fungicide on mycelia growth inhibition was statistically significant at 0.05 significant level. Fungicides had F (5, 310) = 142.03, $p < .0001$. Fungicide Rl and Mlz recorded higher per cent inhibition of mycelia growth of 92.4% and 89.71% respectively. Fungicide Crt recorded lower per cent inhibition of 39.15% (Table 2).

Table 2: Overall mycelia growth inhibition per cent inhibition of different fungicides

Fungicide	Fungal mycelia growth % inhibition
Rl	92.41[a]
Mlz	89.71[a]
Ohn	84.20[b]
Vty	84.02[b]
Trty	60.33[c]
Crt	39.15[d]
Mean	74.97
Cv	17.80
LSD [p<0.05]	5.0542

Means followed by same letters in each column are not significantly different based on analysis of variance (α=.05). Where Mlz = Propineb700g/kg +Cymoxanil 60g/kg, Trty= Triticonazole, Vty= mancozeb 640 g/kg + metalaxyl 80 g/kg, Ohn= Mancozeb, Rl= Mancozeb (640 g kg^{-1}) + Metalaxyl (40g kg^{-1}), Crt= Carbendazim.

Effect of three fungicides concentrations on mycelia growth inhibition of *Alternaria solani* and *Phytophthora infestans*

Effect of fungicide concentrations on mycelia growth inhibition was statistically significant at 0.05 significant level. Concentration had F (2, 310) = 6.57, $p = .0010$. Inhibition of mycelia growth increased with an increase in fungicide concentration. Lower inhibition of 71.78% was observed at the farmers recommended concentration. However, there was no significant difference of percentage fungal mycelia inhibition at 50% and 75% fungicide concentration despite recording higher inhibition zones (Figure 3). In overall, fungal concentration had 74.97% mean per cent inhibition, a CV of 17.80 and least significance difference of 3.573.

At 25%, 50% and 75%, Rl and Mlz fungicides had higher per cent mycelia inhibition for both *A. solani* and *P. infestans* (Table 3; Figure 4 and 5). The percentage inhibition between Rl fungicide and Mlz fungicide at 25%, 50% and 75% fungicide concentration respectively for individual pathogens were not significantly different. Fungicide Rl had the highest percentage inhibition of 90.56% and 89.63 at 50% and 75% fungicide concentrations respectively (Table 1). However, Mlz fungicide recorded higher percentage mycelia inhibition of 89.71 at 25% fungicide concentration. Fungicide Crt had lowest mycelia growth percentage inhibition at all concentrations tested followed by Trty fungicide (Table 3).

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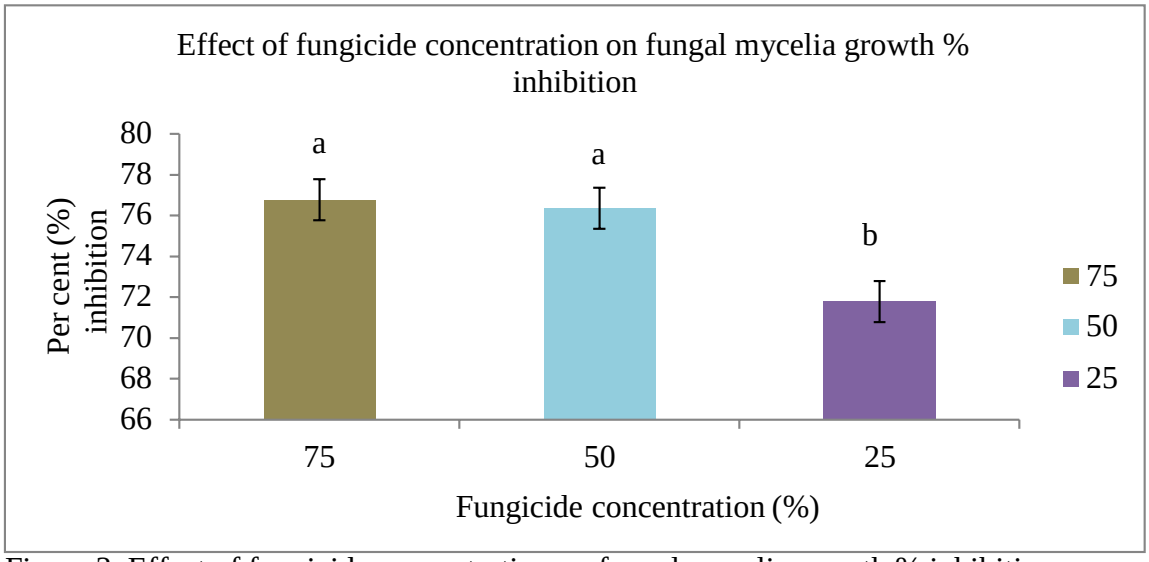

Figure 3. Effect of fungicide concentration on fungal mycelia growth % inhibition

Table 3. Effect of Fungicides on Mycelia Growth of *Alternaria solani* and *Phytophthora infestans* Mycelia

Level of Fungicide	Mean colony diameter (cm) at different fungicide concentrations					
	Late Blight pathogen			Early Blight pathogen		
	25%	50%	75%	25%	50%	75%
Rl	83.94^{ab}	90.56^{a}	89.63^{a}	97.33 ^{a}	90.56^{a}	98.59^{a}
Mlz	89.71^{a}	87.84^{a}	87.74^{a}	95.72 ^{a}	87.84^{a}	95.69^{a}
Ohn	69.27^{c}	74.08^{b}	83.72^{a}	88.89 ^{a}	74.08^{b}	96.65^{a}
Vty	69.73^{bc}	70.70^{b}	72.62^{a}	87.38 ^{a}	70.70^{b}	98.14^{a}
Trty	55.25^{d}	61.28^{c}	59.55^{c}	62.11 ^{b}	61.28^{c}	85.83^{b}
Crt	38.44^{e}	45.80^{d}	31.88^{c}	34.03^{c}	45.80^{d}	31.88^{c}
Mean	65.97	71.70	70.856	77.57	81.01	84.47
CV	15.76	10.82	20.17	15.4	12.94	8.77
LSD [p<0.05]	9.87	7.04	13.58	11.35	7.37	4.97

Means followed by same letters in each column are not significantly different based on analysis of variance (α=.05). Where Mlz = Propineb700g/kg +Cymoxanil 60g/kg, Trty= Triticonazole, Vty= mancozeb 640 g/kg + metalaxyl 80 g/kg, Ohn= Mancozeb, Ohn= Mancozeb, Rl= Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}).

Fungicide resistance is crucial on the basis of limiting the efficacy and lifetime of fungicides (Stević *et al*., 2017). Thus, timely evaluation and information on development and spread of resistant strains of pathogens is necessary to ensure success in disease management strategy (Stević *et al*., 2017). In vitro evaluation of available molecules in the market enables selection of most effective molecules against mycotoxigenic fungi (Masiello *et al.*, 2019). The study reveals that the two tomato leaf blight pathogens *A. solani*

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and *P. infestans* are inhibited by the fungicides evaluated. However, the two blight pathogen differed significantly ($p<0.05$) on their sensitivity to tested fungicides. Difference in response of *A. solani* and *P. infestans* have also been reported towards other chemicals (Mugao *et al.*, 2020). Growth of *A. solani* and *P. infestans* on media amended with different fungicides at different concentrations differed significantly ($p<0.05$). *Alternaria solani* was more sensitive to the fungicides tested than the *P. infestans*. Our findings on *A. solani* and *P. infestans* response of towards fungicides are supported by Mugao *et al.* (2020).

In order of sensitivity, mycelia growth of *A. solani* was highly inhibited by Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}) followed by Propineb700 g/kg +Cymoxanil 60 g/kg while Carbendazim had the lowest mycelia percentage inhibition. The percentage inhibition observed in this study for Mancozeb (640 g kg^{-1}) +Metalaxyl (40 g kg^{-1}) against *P. infestans* were higher than those reported by Zhu *et al*. (2008) but differed to the findings of Saima Farooq *et al.* (2019). Such conflicting results might be attributed to resistance development towards fungicides in a pathogen population. Higher percent *A. solani* mycelia inhibition by Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}) in this study corresponds to those of Saad *et al.* (2014). Further, the finding *P. infestans* here with reference to other fungicides which contain dimethomorph, cymoxanil, zoxamide and mancozeb correlates to other studies (Yadav and Dabbas, 2012; Rekanović *et al*., 2012).

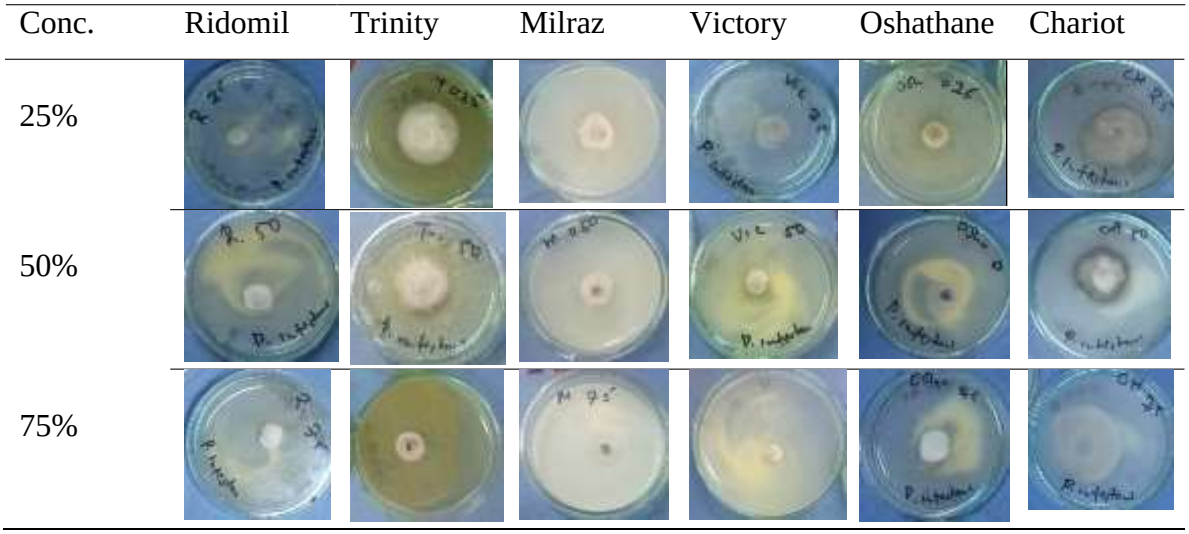

Figure 4. Figure 4. Selected images of mycelia inhibition (Late blight pathogen) for different fungicides and concentrations after one week of growth on corn meal agar.

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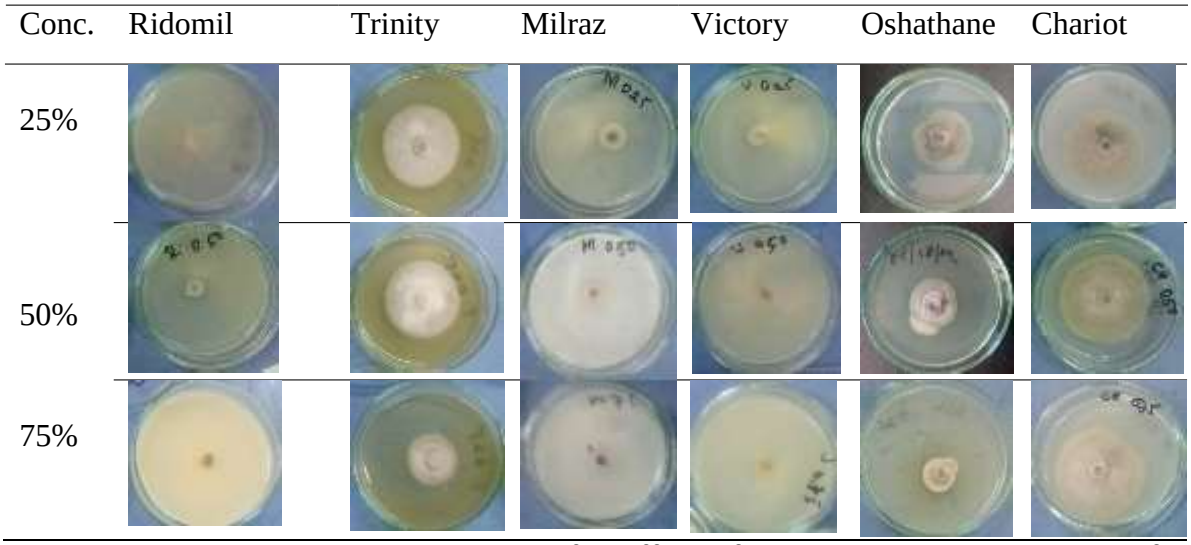

Figure 5. Inhibition (Early blight pathogen) for different fungicides and concentrations after one week of growth on corn meal agar

Effect of duration of incubation on fungicide inhibition activity

Effect of duration of incubation (in days) on mycelia growth inhibition was statistically significant at 0.05 significant levels. Days had F (2, 310) = 6.89, p = .0012. The duration of incubation (in days) had a significant ($p < .05$) effect on fungicide activity on fungal mycelia growth. Inhibition reduced with increase in incubation period. Higher mycelia growth inhibition of 77.41% was observed on the 3rd day while lower inhibition of 71.28% was observed on the 7th day (Figure 7). The mean percentage inhibition was 74.97% with coefficient variation of 17.803 and least significance difference of 3.588.

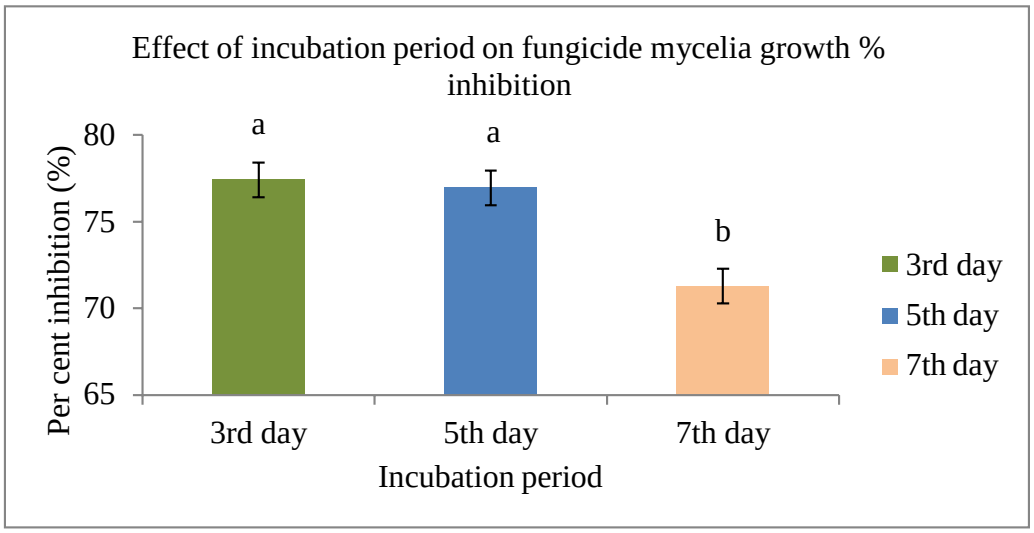

Figure 6. Effect of incubation period on fungicide mycelia growth % inhibition

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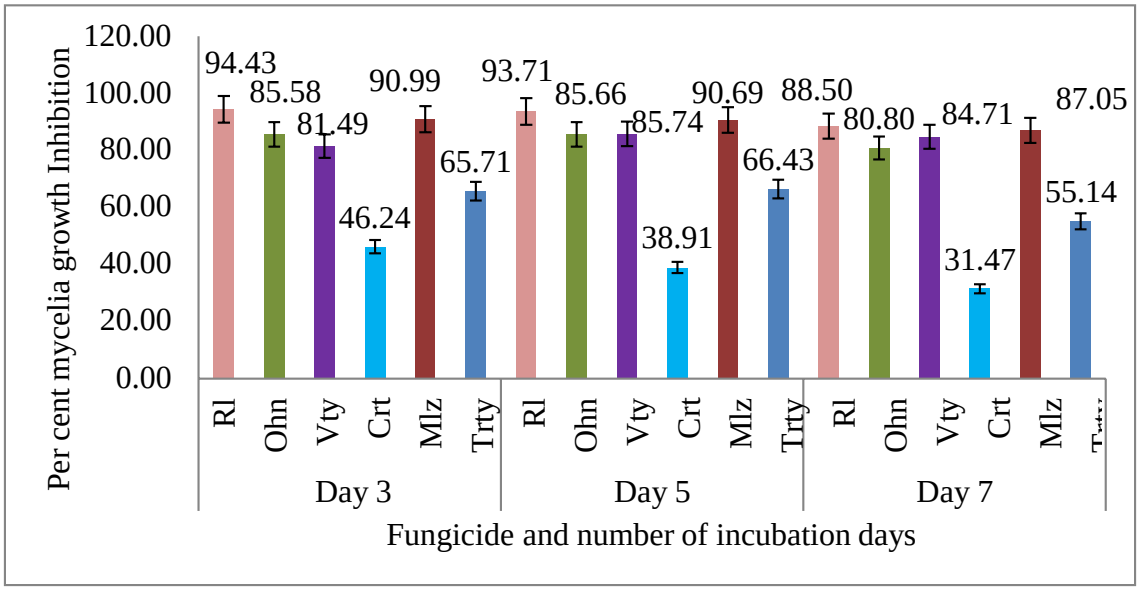

Figure 7. Effect of incubation period on inhibition activity of different fungicides based on analysis of variance (α=.05). Where mlz= Propineb 700 g/kg + Cymoxanil 60 g/kg, Trty = Triticonazole, Vty= mancozeb 640 g/kg + metalaxyl 80 g/kg, Crt = Carbendazim

There was no significant difference on per cent mycelia growth inhibition between Rl, Mlz, Ohn and Vty. Mean difference of Crt and Trty fungicides were significantly different across incubation days (Figure 7). The inhibition zone for Crt reduced progressively from 3rd to 7th dayof incubation. The inhibition zone for Crt on the 3rd day was 46.24%, 5th day 38.91% and on the 7th day was 31.47% (Figure 7).

The *P. infestans* and *A. solani* mycelia growth was highly inhibited by Propineb700g/kg + Cymoxanil 60g/kg which contain cymoxanil. Cymoxanil has equally been reported to be effective against *P. infestans in other related studies* (Gouot, 1994). However, other studies have reported resistance of *P. infestans* towards fungicides with Cymoxanil (Zhu *et al.,* 2008). Per cent fungal mycelia inhibition effect of mancozeb 640 g/kg + metalaxyl 80 g/kg fungicide was equally higher for both the fungi tested. The performance of mancozeb 640 g/kg + metalaxyl 80 g/kg is due to its constituents. Mancozeb is a low-resistance- risk fungicide (Fungicide Resistance Action Committee (FRAC, 2010)). Carbendazim had the lowest per cent mycelia inhibition. The performance of Carbendazim in this study contradicts those of Kumar *et al.* (2017). Cabendazone (methyl-2-benzimidazole carbamate) is a benzimidazoles and its effectiveness is due to blockage of nuclear division (Davidse, 1975; Howard, 1980; Zhou *et al*., 2016).

Benzimidazoles disrupts the functions of microtubules (αβ-tubulin derivative) leading to inhibition of DNA synthesis in fungi (Davidse, 1975; Howard, 1980; Zhou *et al*., 2016). Benzimidazole has numerous biological activities that range from antihelminthic, anti-inflammatory, antiviral, antibacterial and antifungal (Tunçbilek *et al.*, 2009). The better performance of mancozeb 640g/kg + metalaxyl 80 g/kg and Mancozeb (640 g kg^{-1}) +Metalaxyl (40 g kg^{-1}) may be attributed to difference in their ingredients concentrations (Hamel *et al.*, 2011; Zhou *et al*., 2016). Carbendazim which is constituted of Carbendazim had the lowest per cent mycelia inhibition. Low per cent inhibition of carbendazim has also been reported by Vanitha *et al.* (2013). The result here demonstrating that use of Carbendazim that is constituted with

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Carbendazim alone may not sufficiently offer significant advantage in managing early blight in tomato due to its lower per cent mycelia growth inhibition.

Mycelial growth percent inhibition was significantly different within the three concentrations of each fungicide ($p<0.05$). The fungal mycelia inhibition occurred in all the concentrations evaluated. The findings corroborate with earlier research finding (Ghazanfar *et al*., 2016; Mphahlele, 2017). The per cent inhibition increased with increase in fungicide concentration. Effect of increasing fungicide concentration on mycelia inhibition corresponds to other studies (Vanitha *et al.*,2013; Ghazanfar *et al*., 2016; Roy *et al.,* 2019; Peerzada *et al.,* 2020; Iqbal *et al.,* 2020). Increase per cent mycelia inhibitions correlating to increasing fungicide concentration indicate that lower doses may be sub lethal to the fungi when compared to high concentrated does. Thus, higher doses are recommended in such situations. According to Majeed *et al.* (2017) quantitative resistance showing less sensitivity to fungicides can be minimized by use of stronger dose of fungicides. Mycelia growth inhibition activity reduced with increase in number of incubation. This finding corroborates to those of Ghazanfar *et al*. (2016)

CONCLUSION AND SUGGESTION

The Fungicides screened in this study varied in their mycelia per cent inhibition against *P. infestans* and *A. solani* isolates. Mancozeb (640 g kg^{-1}) + Metalaxyl (40 g kg^{-1}) and Propineb700g/kg +Cymoxanil 60g/kg had better inhibition effect while Carbendazim had the lowest effect. Increased fungicide concentration effectively inhibited mycelia growth.

Continuous monitoring of efficacy of fungicides against *P. infestans* and *A. solani* both in the laboratory and field populations is necessary for different niches.

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