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# IN-VITROEVALUATION OF FUNGICIDES SENSITIVITY OF TOMATO LEAF BLIGHT PATHOGENS ISOLATES *Alternariasolani* and *Phytophthorainfestans*

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## ABTRACT

Tomato early and late blight diseases caused by *Alternaria solani*(Ellis & Martin) Sorauer and *Phytophthora infestans*respectively hinder tomato production globally. Use of fungicides in management of blight diseases has become a priority among farmers. However, indiscriminate or use of sub lethal doses negate disease management efforts. Despites the knowledge of possible occurrence of fungicide insensitive phyto-pathogens, farmers has continued using fungicides without periodic evaluation of their effectiveness. This study was carried out to evaluate an in-vitro sensitivity of tomato leaf blight pathogens (*Alternaria solani*and *Phytophthora infestans)* against selected fungicides mostly used by tomato farmers along River Ruguti in Tharaka Nithi County in Kenya.Fungicides evaluated included, Mancozeb (640 g $kg^{-1}$) + Metalaxyl (40 g $kg^{-1}$), mancozeb 640 g/kg + metalaxyl 80 g/kg, Mancozeb, Propineb700 g/kg + Cymoxanil 60g/kg, Carbendazim and Triticonazole at different concentration (25%, 50% and 75%). Poison food method was used for evaluation. Complete Randomise design was used for the experiment. Per cent data on mycelia growth inhibition by various fungicides at different concentration were analysed using General Linear Model ($\alpha$=.05) and significant means separated using Least significant difference (LSD) in Scientific Analysis System version 9.4. All the tested fungicides significantly ($P \leq 0.05$) inhibited the mycelial growth of tested pathogen. However, among tested fungicides, Carbendazim (89.83%) and Propineb700g/kg +Cymoxanil 60g/kg were superior over other fungicides with Carbendazim being the least. Mycelia growth inhibition increased with an increase in fungicide concentration. Mycelia growth inhibition of *Alternaria solani*was 81.78% as compared to *Phytophthora infestans at 69.78%*.
**Keyword:** In-vitro, fungicides sensitivity, *Alternariasolani*, *Phytophthorainfestans*

## INTRODUCTION

Tomato is attacked by fungal pathogen such as *Phytopthoraparasitica*, *Fusarium oxysporum spp. Lycopercisi*, *Phytophotherainfestans*, *Erysiphe* spp, *Alternaria altenata*, *Alternaria tomatophila and Alternaria solani.* These pathogens cause distinctive diseases such as buck eye rot, fusarium wilt, late blight, powdery mildew and early blights. Upto 79% of tomatoes loses are due to leaf blight disesaes (Singh *et al*., 2017; Gulzar *et al.,* 2018).Among the foliar blight pathogen, early blight caused by *Alternaria solani*and *Phytophotherainfestans*are associated with tomato early and late blight across the globe (Mengesha, 2017;Chastie*t al*., 2018; Saima Farooq *et al.,* 2019). *Alternaria solani*though airborn is a soil inhibitory pathogen that may overwinter in plant debris sustaininginfection in the subsequent planting seasons.Other than leaves the pearly blight symptoms may be observed stem and fruits (Roopa *et al.*, 2014). Distinctive feature of the symptom is formation of blight with concentric rings that appear brown to black usually appearing on mature lower leaves and progress to younger upper leaves. Infected leaves turn pale yellow prior to withering and fruit falls (Biovision, 2019). *Phytophotherainfestans*causes lateblight and attacks tomato at all growth stages (Biovision, 2019). It causes plant death arising from leaf and stem necrosis(Biovision,2019).Late blightsymptoms include water-soaked lesion which maybecircular or irregular and are near leaflet margins. Lesions may spread elsewhere on the leaves as disease progresses (Griffith *et al*., 1995).

Synthetic fungicide has gotten much use in management of fungal diseases due to ease of application andavailability to farmers (Akram *et al*., 2018; Vinay *et al*., 2020). Plant disease control begun in the mid nineteenth century when inorganic active ingredients such as sulphur, lime and copper sulphate were used(Fernández-Ortuño*et al.*, 2010). Advancement of plant protection against fungal diseases has since led to developing varied fungicides. Fungicides that range from protectants, systemic and eradicant are available for plant diseases (Bartlett *et al*., 2002, Russell, 2005; Fernández-Ortuño *et al.*, 2010). Example of protectant fungicide is mancozeb which is often used either alone or in combination with metalaxyl or other fungicides. Metalaxy is an example of both systemic and curative fungicidefungicide(Yang*etal.*,2019).Further,basedontheir modesofaction,fungicides maybegrouped assite-specificormultisitethosewhicharesitenon-specific(Yang*etal.*,2019).Sitespecificfungicidesare considered highly active and often systemic requiring low dose application with better disease cotrol. On the other side, multi-site fungicides double as pathogen preventive and as eradicant (Yang *et al.*, 2019).

Though handy, pathogen may become insensitive to fungicides due to improper dose or frequency of fungicide application (Namanda *et al.*, 2003; Akram *et al*., 2018). According to Nyankanga*et al.* (2004), some farmers in Kenya may only use fungicides upon development of symptoms which may lead to failure. Low sensitivity of *A. solani*to different fungicides may result from formation of melanin response (Bell and Wheeler, 1986). It istherefore necessary to periodically evaluate existing fungicides for their potency against pathogen and advicefarmers on appropriate fungiceds and concentrations. Other than development of resistance to fungicides, excessive application of fungicides is a health hazard to both human, non-target beneficial organisms due to associated risk of pollution (Masiello *et al.*, 2019).Such reality calls for continual evaluation of fungicides for their in vitro and in vivo efficancy.Continued evaluationoffungicidesensurecontinualuseoffungicidesattherightconcentrationsthat prevent development of resistant pathogen strains (Masiello *et al*., 2019).

The inhibitory effect of chemicals on fungus has been investigated by several researchers (Abada *et al*., 2008; Gomaa, 2001; Patil *et al.*, 2001). Esmeil (2005) tested two fungicides (Bifidan and Ivory M 72 WP) against early blight disease usingthree different concentrations. The disease variablydecreased byusingthe two fungicides at the three different concentrations with an increased tomato yield. Bifidan fungicide applied at the concentration of 0.375ml/L gave higher yield of 1.2 Ton. Whereas the recommended dose of 0.5 ml/l Bifidan was efficient in managing early blight, lower dose of the Ivory M 72 WP was effective (Esmeil, 2005).

Study by Akram *et al*. (2018) in Southern Punjab evaluated efficacies of different fungicides at different doses (25, 50, 75, 100 µg mL−1) against *A. solani*.The fungicide Bravo, Score, Camelot and Cabrio Top-not inhibitedmycelial growthat between6-35% at 100µg mL−1 concentration. Where as Bravo and Score at 25µgmL−1 did not reduce mycelial, Dithane Z-78, Dithane M-45, Cobox, Heritage and Maxim at 100 µg mL−1 concentration significantlyreduced (56-85%) mycelial growth. InParkistan,Hassan *etal.* (2014)reported higher inhibition(83 %) with 400 ppm of Helonil followed by clipper (64.70%) at 500 ppm and Antracol (46.66%) at 1000 ppm. Ridomil (7.74%)andDesomil(8.57%) withconcentrationof300ppmgavetheleast mycelia growthinhibition. Ghazanfar *et al*. (2016)observed that Dithane® M-45 80 WP with percent inhibition of 89.83% was significantly effective. Sarfraz *et al*. (2018) screened different systemic fungicides against *A. solani*at different concentrations where all fungicides gave maximum inhibition at 15 ppm after 172 hrs. Ganie *et al.* (2013) evaluated five non-systemic fungitoxicants and five systemic fungitoxicants at six concentrations each against *Alternaria solani*. Fungitoxicants mancozeb 75 WP was most effective among non-systemic fungicides while hexaconazole 5 EC fungicides performed better among systemic fungitoxicants (Ganie *et al.*, 2013).

Patel (2012)screened fungicides and pesticides and reported propiconazol and tebuconazole systemic fungicides to be effective in inhibiting the *A. solani*mycelia growth with 98.88 and 97.31 per cent growth inhibition respectively. Copper oxychloride performed better amongthe non-systemic fungicides evaluated followed bymancozeb resulting inpercentinhibitionof95.07and93.86respectively. Roopa*etal.*(2014)carriedanevaluationoftwelvefungicides, six bioagents and ten botanicals against *Alternaria solani*early blight pathogen of tomato.Contact fungicide mancozeb @0.2%,systemic fungicide, hexaconazole@0.1 %and thecombi fungicideHexazconazole4%+ Zineb 68% @ 0.2% had the highest per cent inhibition (87.21% 88.88%, 88.88%) ohmycelial growth respectively.

Saad *et al*. (2014) assessed inhibition activity of some antioxidants (ascorbic, benzoic, citric, salicylic acids andBion 50 WG®), a biofungicide Plant guard® (Trichoderma harzianum) 30 x106 spores/ml and fungicides (Ridomil gold MZ® 68%, Micronized soreil/Samark® 70% WP (Sulphur), Tridex® 80% and Vitavax 200® 75%) against *Alternaria solani*and *Fusarium solani*in vitro experiment. Both Tridex® and Ridomil gold MZ® resulted in maximum inhibition of *A. solani*at 100% of mycelial growth at concentration of 400 ppm as compared to other tested fungicides. Regardingthe $IC_{50}$ofthe testedfungicides,Tridex®exhibited the lowest$IC_{50}$value calculatedby

63.6ppmfollowedbyRidomilgoldMZ®(92.4ppm)whichshowsthesuperiorityasagrowthinhibitorof*A.solani* ascomparedwiththeotherfungicides.

Saima Farooq *et al.* (2019) assessed fungitoxic activity of different fungicides against *Alternaria solani*. All fungicides assesses significantly (P≤0.0001) inhibited the mycelial growth at different concentrations compared to control. Pyroclostrobin at 500 ppm gave higher percent (97.7%) inhibition of *A. solani*mycelial while Ridomil was hadleastinhibitioneffectonmycelialgrowth(42.2%).ThefindingsbySaimaFarooq*etal.*(2019)contracdicts

those ofKarima and Sayeda (2007) withreference to Ridomil. Mahantesh *et al.* (2017) observed different inhibition activity of different fungicides at 1000 ppm. Mphahlele (2017) tested sensitivity of *A. solani*isolates to chlorothalonil, copper oxychloride and mancozeb commonly used fungicides in Limpopo. Even at the lower concentrations,all fungicides testedreducedthe mycelial growthof *A.solani*isolates.Copperoxychloridefungicide however, exhibited better inhibition of mycelial growth. Fungicide evaluation by Yadav *et al.* (2018) reported efficiency of hexaconazole in managing *Alternaria solani*in Bharsar.In related studies, Kodemelwar*et al.* (1973) observed that copper based fungicides performed better in controling*A. solani*in vitro while. Lodha and Prasad (1975) reported Dithane Z-78 to effectively control the growth of *A. solani*in vitro. Choulwar*et al.* (1989) notedthat Mancozeb (0.2%) effectively inhibited *Alternaria solani*mycelial growth.

**METHODOLOGY**

The fungus pathogen used in this study were isolated from infected tomato leaves collected from Tharaka region of Tharaka Nithi County in Kenya. Tharaka Nithi County is located between longitudes 37° 19' and 37° 46' East and latitude00007'and00026'South.Thecountyhastwoagroecologicalzone namelyupperandlowerzones. Annual rainfallofoverage717mmisexperiencedinTharakaNithiCounty.AreasaroundChukaandChogoriawhichareon high altitude rceives reliable rainfall unlike lower regions around Tharaka area characterized bypoorly distributed low rainfall that are unreliable. The temperatures range in Tharaka Nithi County is from 14°C to 30°C in highlands and 22°Cto36°Cinlowlands. The soil pH inTharaka Sub Countyranges fromacid pH 5to alkaline pH 8.The soils are dark grey-brown, clay and sandy clay loam topsoil which are imperfectly drained 9 (Ministry of Agriculture, Livestock and Fisheries (MoALF., 2017)).

The study was conducted in a 3 x 5 factorial experimental laid out in Complete Randomized Design [CRD] with factor A being six levels of Fungicides (Mancozeb (640 g $kg^{-1}$) + Metalaxyl (40 g $kg^{-1}$), mancozeb640 g/kg + metalaxyl 80 g/kg, Mancozeb, Propineb700 g/kg + Cymoxanil 60g/kg, **Carbendazim** and Triticonazole) and factor B being days of fungicide concentration (25, 50 and 75).

**SampleCollectionandMediaPreparation**

Late and early blight symptomatic leaves were collected from tomato growing area along River Ruguti in Tharaka. The area was ideal for sample collection based on long history of tomato farming along the river. Blight symptoms were identified through physical examination of the leaves. Symptomatic leaves were randomly collected by aseptically cutting, wrapping in labeled ziplock bags, samples were then placed in cool box and transported toChuka University for pathogen isolation.

**PathogenIsolation**

The *Phythopthorainfestans*and *Alternaria solani*pathogen were isolated on the potato dextrose agar prepared following the manufacture procedure. The isolates were then purified on corn meal agar media. Media were Autoclaved at the temperature of 121°C, 15psi for 15 minutes prior to cooling at 50°C in water bath. Contamination of the media by the bacteria was prevented by in cooperating Antibiotic [25 mg/l] in all the media. Tomato leaves with early blight symptom were cleaned under running tap water to remove dust particles. Thin sections (4 mm) of diseased leaf were cut and places in 0.5% sodium hypochlorite solution for surface sterilization for 30 seconds. Surface sterilized leave sections were then 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 leave sections were incubated at room temperature of 25°C for fourteen days. Fungal colonies weresubculturedinCornMealAgar mediaforpurecultures.Purecultures wereusedforthepathogenicity test and evaluation of effect of different fungicides at different concentrations on mycelia growth.

**Pathogenicitytest**

Fifteen tomato seedlings (Five seedlings each for *Phythopthorainfestans*and *Alternaria solani*and for control experiment) wereused Pathogenicitytest. Commando F1 tomato varietyseedlings wereused for thestudysince itis grown from sample collection area. Five tomato seedlings were sprayed with 10 ml of a week old conidial suspension adjusted to $5 \times 10^6$ conidia/mL of *Phythopthorainfestans*and *Alternaria solani*and distilled water respectively. Tomato seedlings

were then covered with polythene bags for a day to favor pathogen establishmentand disease development. On the second day, inoculated tomato seedlings were transferred to the greenhouse and observed for diseasessymptomsafter two weeks.Pathogenre-isolation wasdoneonsymptomaticleavesand culture compared with the initial cultures.

**Pathogenselectionforfungicideassay**

The pathogen (*Phythopthorainfestans*and *Alternaria solani)* used in fungicide assay study was selected from other isolates based on their rapid growth rate.

**Fungicideassayusingpoisonedfoodtechnique:**

The efficacy of three fungicide treatments (Mancozeb, Mancozeb(640 g $kg^{-1}$) + Metalaxyl (40 g $kg^{-1}$) and mancozeb640 g/kg + metalaxyl 80 g/kg) at three concentrations 25 (Being the recommended concentration), 50% and 75% onthe growthof *A. solani*and *A. alternate* usingcorn meal agar medium. Corn meal agar was prepared by dissolving 17g of Corn Meal Agar in 250 ml of distilled water byboiling then topped up to 1000 ml. Sterilization of the media was done at 121°C and at of 15psi for 15 minutes and media cooled to 50°C in water bath, ampicillin (25 mg/l) was added thereafter, 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 *A. alternate* fungalisolates wereasepticallypicked and placed atthecenter oftreated cornmealagar.Measurementsof mycelia growth (Diameter cm) 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 theformula below:

$$\frac{a-b}{a} x100$$

wherea=myceliadiameterofcontrolplatesandb=myceliadiameteroffungicidetreatedplates. Table 12:

Details of fungicides used in the study

| Activeingredient | Trade name | Manufacturer | Chemicalgroup | Modeof action |
|---|---|---|---|---|
| Mancozeb (640gkg$^{-1}$) +Metalaxyl(40gkg$^{-1}$) | Ridomilgold MZ68W | SyngentaEast Africalimited | Dithiocarbamate Acylaminoacid | Disruptcellfunctions. Nucleicacidsynthesis |
| **Carbendazim** | Chariot | Greenlife cropprotectionAfrica | 500g/l | blockageofnucleardivision |
| Mancozeb | Oshothane 80WP | Oshochemical industrieslimited | 800 | Disruptcellfunctions. |
| Mancozeb640g/kg+ Metalaxyl80g/kg | Victory72WP | AmiranKenya Limited | mancozeb640g metalaxyl809 | Disruptcellfunctions. Nucleicacid |
| Propineb700g/kg Cymoxanil60g/kg | MilrazWP 76 | BayerCrop Science | Dithiocarbamate 700g/kg Cymoxanil60g/kg | |
| Triticonazole | TRINITY GOLD®452WP | Greenlife cropprotectionafrica ltd | Copperxychloride Mancozeb Cymoxanil | Demethylation inhibitor |

Hamel*etal.*(2011)andZhou*etal.*(2016)

**StatisticalAnalysis**

Per cent inhibition data collected were analyzed using general linear model (GLM). Significant means were compared using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.

**RESULTS**

**FungicidesmyceliagrowthInhibitioneffecton*Phythopthorainfestans*and*Alternariasolani***

*Phythopthorainfestans*and *Alternaria solani*growth inhibition by fungicides differed significantly ($p < .05$). Early blight pathogen (*Alternaria solani*) was inhibited more (80.42%) as compared to late blight pathogen *Phythopthorainfestans*at 69.51%(Figure1).The mean fungal myceliagrowthinhibition was74.96%withcoefficient variationof 17.804% and least significance difference (LSD) of 2.779 at $p<0.05$.

**MyceliagrowthInhibitioneffectofdifferentfungicides**

Myceliagrowthpercentinhibitiondifferedsignificantly($p<.05$)withdifferentfungicide.Mancozeb (640 gkg$^{-1}$) +Metalaxyl(40g kg$^{-1}$)andPropineb700g/kg +Cymoxanil60g/kgrecorded higherper centinhibition ofmycelia growth of 92.4% and 89.71% respectively. **Carbendazim** recorded lower per cent inhibition of 39.15% (Table 2)

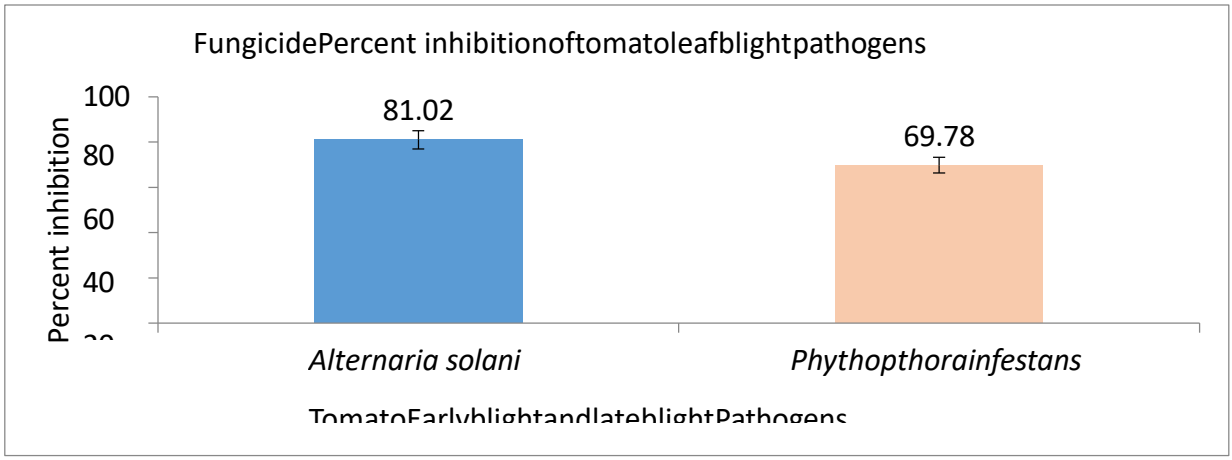


Figure8:Errorbarfor*invitro*percentmyceliainhibitionoftomatoleafblightpathogens Table 13:

Overal mycelia growth inhibition per cent inhibition of different fungicides

| Level of Fungicide | Fungalmyceliagrowth%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 CV | 74.97 |
| LSD[p<0.05] | 17.80 |
| | 5.0542 |

Meansfollowedbysamelettersineachcolumnarenotsignificantlydifferent.WhereMlz=Propineb700g/kg +Cymoxanil60g/kg,Trty=Triticonazole,Vty=mancozeb640g/kg+metalaxyl80g/kg,Ohn=Mancozeb,Rl= Mancozeb (640 g $kg^{-1}$) +Metalaxyl (40 g $kg^{-1}$), Crt= **Carbendazim.**

**Effect of fungicides rates on mycelia growth inhibition of *Alternaria solani*and *phythopthorainfestans***Different fungicides at different concentration differed significantly ($p < .05$) on their per cent inhibition effect ofmycelia growth of *Alternaria solani*and *Phythopthorainfestans.*Inhibition of myceliagrowth increasedwith an increaseinfungicideconcentration.Lowerinhibitionof71.78%wasobservedatthefarmersrecommended concentration. However, there was no significant difference of per cent fungal mycelia inhibition at 50% and 75% fungalconcentrationdespiterecordinghigherinhibitionzones(Figure2).Inoverall,fungalconcentrationhad 74.97% mean per cent inhibition, a CV of 17.80 and least significance difference of 3.573.

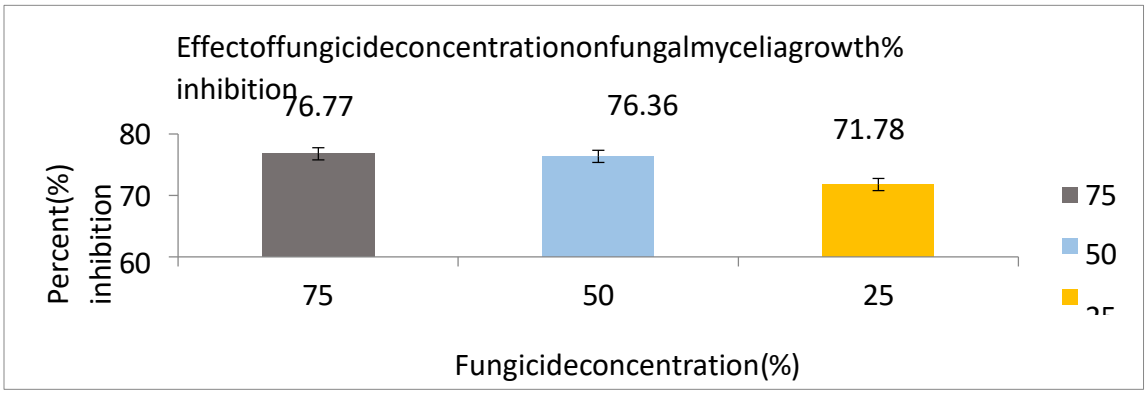


Figure9:Errorbarshowingtheeffectoffungicideconcentrationonfungalmyceliagrowth%inhibition

Individual fungicides at different concentrations differed significantly($p < .05$)onmycelia growthinhibition. At the concentrationof25%,50%and75%Mancozeb(640gkg$^{-1}$)+Metalaxyl(40gkg$^{-1}$)andPropineb700g/kg +Cymoxanil 60g/kg had higher per cent mycelia inhibition for both *Alternaria solani*and *Phythopthorainfestans*(Table 3). The per cent inhibition between Mancozeb(640 g kg$^{-1}$) + Metalaxyl (40 g kg$^{-1}$) and Propineb700g/kg + Cymoxanil 60g/kg at 25%, 50% and 75% fungicide concentration respectively for individual pathogens were not significantlydifferent. Mancozeb(640 g kg$^{-1}$) +Metalaxyl (40 g kg$^{-1}$)had the highest per cent inhibition of 90.56% and 89.63 at 50% and 75% fungicide concentrations respectively (Table 1). However, Propineb700g/kg +Cymoxanil 60g/kg recorded higher per cent mycelia inhibition of 89.71 at 25% fungicide concentration. **Carbendazim** had lowest mycelia growth per cent inhibition at all concentrations tested followed by Triticonazole (Table 3).

Table14:EffectofDifferentFungicidesonGrowthof*Alternariasolani*and*phythopthorainfestans*Mycelia Meancolonydiameter(cm)atdifferentfungicideconcentrations

| LevelofFungicide | LateBlight | | | EarlyBlight | | |
|---|---|---|---|---|---|---|
| | 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 |

Meansfollowedbysamelettersineachcolumnarenotsignificantlydifferent.WhereMlz=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$^{-1}$)

**EffectofIncubationPeriodonInhibitionActivityofDifferentFungicides**

Number of incubation days had significant ($p < .05$) effect on inhibitions activity of different fungicides on fungal mycelia growth. Fungal mycelia inhibition appeared to reduce with increase in incubation period. Higher mycelia growth inhibition of 77.41% was observed on the 3$^{rd}$ day while lower inhibition of 71.28% was observed on the 7$^{th}$ day (Figure 3). The mean per cent inhibition was 74.97% with coefficient variation of 17.803 and LSD of 3.588.

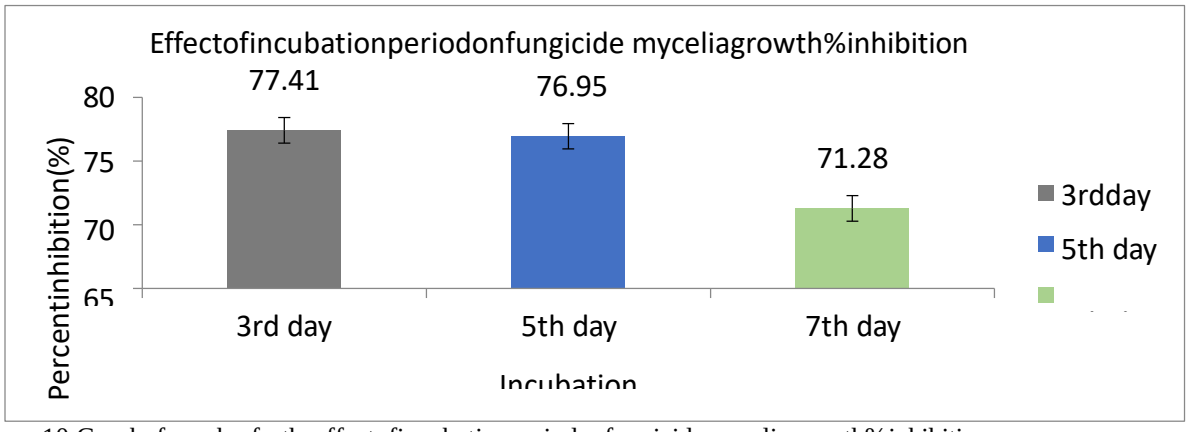


Figure10:Graphoferrorbarfortheeffectofincubationperiodonfungicidemyceliagrowth%inhibition

There was no significant difference on mycelia growth inhibition between Mancozeb(640 g kg$^{-1}$) + Metalaxyl (40 g kg$^{-1}$), Propineb700g/kg + Cymoxanil 60 g/kg, Mancozeb and mancozeb 640 g/kg + metalaxyl 80 g/kg. Mean difference of **Carbendazim**and Triticonazole fungicides were significantly different across incubation days (Figure 2). The inhibition zone for **Carbendazim** reduced progressively from 3$^{rd}$ to 7$^{th}$ day of incubation. The inhibition zone for **Carbendazim** on the 3$^{rd}$ day was 46.24%, 5$^{th}$ day 38.91% and on the 7$^{th}$ day was 31.47% (Figure 4).

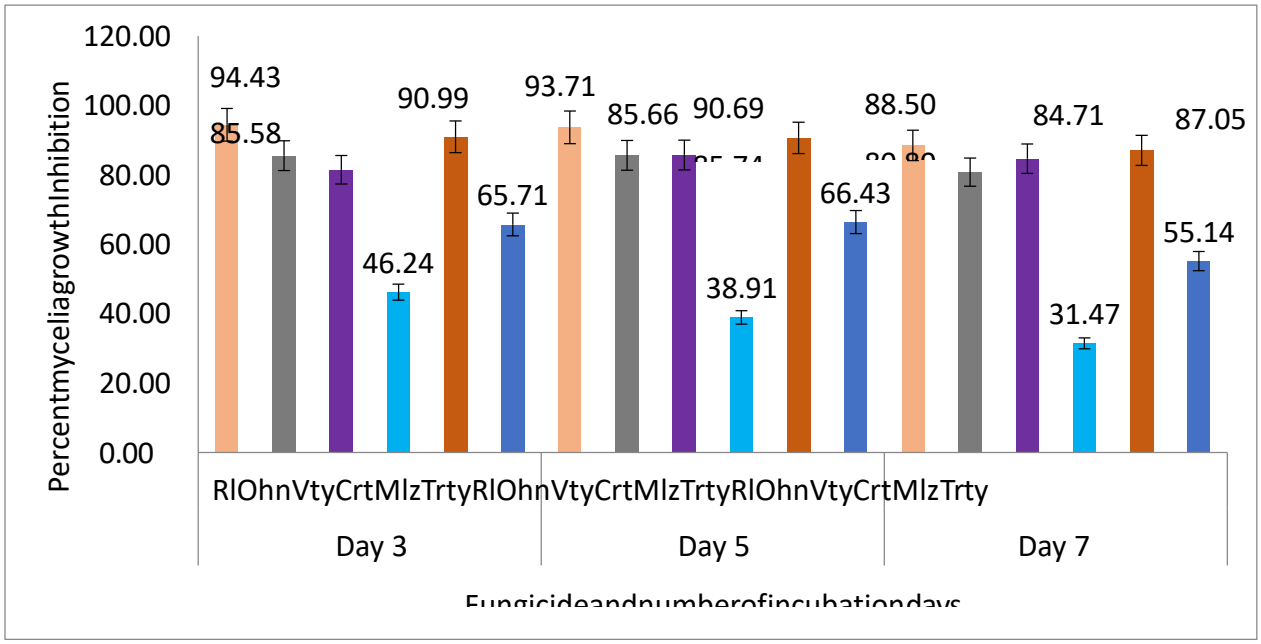


Figure11:Effectofincubationperiodoninhibitionactivityofdifferentfungicides.Wheremlz=Propineb700g/kg +Cymoxanil60g/kg,Trty=Triticonazole,Vty=mancozeb640g/kg+metalaxyl80g/kg,Crt=**Carbendazim**

**DISCUSSION**

**EffectofDifferentfungicidesonMycelialGrowthof*Alternariasolani*Isolates**

Fungicideresistanceisconsideredcrucialwhenitcomestolimitingtheefficacyandlifetimeoffungicides (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 studyreveals that the two tomato leaf blight pathogens *Alternaria solani*and *phythopthorainfestans*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 *phythopthorainfestans*have also been reported towards other chemicals (Mugao *et al.*, 2020). Growth of *Alternaria 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***. The finding in this study with regard to *Alternaria solani*and *Phythopthorainfestans*response towards fungicides is supported by other work (Mugao *et al.*, 2020)

In order of sensitivity, mycelia growth of *Alternaria solani was* highly inhibited by Mancozeb(640 g $kg^{-1}$) + Metalaxyl (40 g $kg^{-1}$) followed by Propineb700g/kg +Cymoxanil 60g/kg while **Carbendazim** had the lowestmycelia per cent inhibition. The percent inhibition observed in this study forMancozeb(640 g $kg^{-1}$) +Metalaxyl (40 g $kg^{-1}$) against *Phytopthorainfestans*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*Alternaria 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 ***Phythopthorainfestans***here with reference to other fungicides which contain dimethomorph, cymoxanil, zoxamide and mancozeb correlates to othert studies (Yadav and Dabbas, 2012; Rekanović*et al*., 2012). The*Phythopthorainfestans*and *A. solani*mycelia growth was highly inhinbited by Propineb700g/kg + Cymoxanil 60g/kg which contain cymoxanil. Cymoxanil has equally been reported to be effective against *Phythopthorainfestans 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 isdue to its constituents. Mancozeb isalow-resistance-risk fungicide(FungicideResistance Action Committee (FRAC,2010)).**Carbendazim**had thelowestpercentmyceliainhibition. Theperformanceof **Carbendazim**inthis study contradicts those of Kumar *et al.* (2017). Cabendazone (methyl-2-benzimidazole carbamate) is a benzimidazolesanditseffectivenessisduetoblockageofnucleardivision(Davidse,1975;Howard,1980;Zhou*et*

*al*.,2016).Benzimidazolesdisruptsthe functionsof microtubulesbuild (αβ-tubulinderivative) leadingto inhibition of DNA synthesis in fungi (Davidse, 1975; Howard, 1980; Zhou *et al*., 2016).Benzimidazole has a numerous biological activities that range from antihelminthic, anti-inflammatory, antiviral, antibacterial and antifungal (Tunçbilek*et al.*, 2009).The better performance of mancozeb 640 g/kg + metalaxyl 80 g/kg and Mancozeb (640 g $kg^{-1}$) +Metalaxyl (40 g $kg^{-1}$) maybe attributed to doifference in their ingradients 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 **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 was significantly different within the three levels of each fungicides ($p<0.05$). The fungal mycelia inhibition occurred at all the levels evaluated. The findings corroborates with earlier research finding (Ghazanfar *et al*., 2016; Mphahlele, 2017). The per cent inhibition increased with increase in fungicide level. Effect of increasing fungicide level on mycelia inhibition corresponds to other researchers (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. Thus, higher doses are recommended in such situations. According to Majeed *et al.* (2017) quantitative resistance showing less sensitivityto 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)

## CONCLUSIONANDRECOMMENDATION

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. Thus higher concentration of fungicide application is recommended in cases where there is low efficacy of fungicides. Our study suggests that 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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