Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

EVALUATION OF *IN VITRO* EFFECTIVENESS OF SELECTED FUNGICIDES FOR THE MANAGEMENT OF EARLY BLIGHT OF TOMATO CAUSED BY *ALTERNARIA SOLANI* IN ETHIOPIA

Getachew Ayana, Endriyas Gabrekiristos
Ethiopian Institute of Agricultural Research, Melkassa Agricultural Research Center, P.O. BOX 436, Adama, Ethiopia.

ARTICLE INFO

Article history
Received: 10th February, 2022
Revised: 21st March, 2022
Accepted: 24th March, 2022

Keywords
Tomato
Early blight
Alternaria solani
Fungicide
Radial growth
Ethiopia

ABSTRACT

Tomato is one of the most popular vegetables grown throughout the world in general and in Ethiopia in particular. Production of the crop is constrained by different diseases and the disease caused by the early blight pathogen, *Alternaria solani*, is among the major ones. Early blight has been considered as commonly occurring and causing a significant economic impact on the yield and quality of the crops. Major tomato diseases in general and early blight, in particular, could be best managed by integrating a number of control practices and the application of fungicides. The application of fungicides is the most convenient and predominant way for disease control in the absence of resistant varieties. The present study was conducted to evaluate the efficacy of some selected fungicides with the objectives to assess *in vitro* efficacy of fungicides on the growth and sensitivity of pathogens, *in vitro* tolerance of pathogens to fungicides, and their effectiveness as potential foliar sprays against *A. solani*. *A. solani* was isolated from tomatoes showing typical early blight symptoms. Five fungicides viz. URGI 75% WP, Carbancholar 50%, Orozole 25 EC, Agro Laxyl MZ 63.5 WP, and Mancodex Super 72 WP were tested against the pathogen using poisoned food technique. The highest percentage reduction in the growth of *A. solani* was recorded with Orozole 25 EC followed by Agro Laxyl MZ 63.5 WP resulting in 100% and 56.5% inhibition in mycelial growth respectively. On the other hand, Carbancholar 50% was found the least effective in reducing the mycelial growth of *A. Solani*. Fungicides that showed better *in vitro* efficacy could be further tested for their efficacies under field conditions and can be verified for economic validity. The study also showed the potential effectiveness of fungicides for the management of early blight on tomatoes.

Corresponding Author: Endriyas Gabrekiristos
Email: endriasgabre@gmail.com

INTRODUCTION

Tomato is an important vegetable crop grown around the world and is second crop in production next to potato (Mutschler et al., 2006). Small scale farmers and commercial growers grow the crop for its fruits in different regions of Ethiopia. It is produced both during the rainy and dry seasons under supplemental irrigation (Lemma, 2002). Nutritionally, tomato is a rich source of Vitamin A, C, E and a good source of antioxidants and contains 95.3% of water, 0.07% calcium, and niacin, which play important role in metabolic activities and maintain good human health (Sgherri et al., 2008).

The average yield of tomato in Ethiopia is low, ranging from 6.5-24 Mt/ha (Gemechis et al., 2012). Under this circumstance, the total area under tomato production in Ethiopia reached to 9767.78 ha and in main production season, production is estimated to be over 913,013.42 tons with an average productivity of 93.47 tons per hectare (CSA, 2016). This is incomparable with the average yield of other countries such as China, USA, Turkey, India, Egypt, Italy and Spain with average yields of 22.67, 80.61, 35.81, 18.61, 40.00 and 76.35 tons/ha in the order (FAOSTAT, 2010).

The average low yield of tomato in Ethiopia could be attributed to different production constrains where diseases caused by different pathogens are the major ones. Tomato crop is vulnerable to different fungal, bacterial and viral diseases. Early blight caused by *Alternaria solani* has been considered as the most commonly occurring and causes significant economic impact on yield and quality of the crops. Among the fungal diseases, early blight of tomato caused by *Alternaria solani* is the most damaging one that causes reduction in quantity and quality of tomato (Majeed et al., 2014). *A. solani* is an air-borne pathogen and is responsible for leaf blight, collar and fruit rot of tomato spread by fungal spores (Abada et al., 2008). Major tomato diseases in general and early blight in particular could be best managed by integrating a number of control practices that may include: selection of disease-tolerant or disease-resistant varieties, different cultural practices (crop rotation, time of planting, level of fertilization, micro-climate modification, sanitation etc.), and application of fungicides. Application of fungicides is the most convenient and predominant way for disease control in the absence of resistant varieties. The use of fungicides could be considered as an alternative method to reduce the yield losses due to different fungal diseases. Application of fungicides requires determination and identification of their efficacies. However, the efficacy of fungicides is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells (Reinprecht, 2010).

Efficacy of fungicides could be studied under field and *in-vitro* conditions. *In vitro* methods for fungicide efficacy testing are a group of analytical methods which reproduce a range of environmental factors under artificial, laboratory, conditions. *In-vitro* efficacy tests help for reduction of the factors that influence the results of treatments at the experimental stage, shortest period to obtain primary data and enable to test multiple fungicides and targets simultaneously. Therefore, evaluating the inhibitory activities of selected fungicides on the growth of *A. solani* is considered as the first test step to screen the potential fungicides as alternative management options. The objectives of the current study were to assess the *in vitro* efficacy of fungicides on growth of pathogens, *in vitro* sensitivity of pathogens to various fungicides, *in vitro* tolerance of pathogens to fungicides and eventually to find an alternate use of these fungicides as potential foliar spray against *Alternaria solani*.

MATERIAL AND METHODS

An *in vitro* efficacy test was conducted at Melkassa Agricultural Research Center in Plant Pathology Laboratory during September to November 2020 using five fungicides.

Isolation and identification of *Alternaria solani*

The infected plants, showing characteristic symptoms of disease were cut with healthy portion into 2 mm pieces, surface sterilized with 0.1 percent sodium hypochlorite solution, thrice rinsed with sterilized distilled water and then transferred aseptically onto PDA medium in Petri plates having 9 cm diameter. The Petri plates having half infected and half healthy cut pieces of sample were incubated at 27 °C. After 3 days, a whitish growth of mycelium was observed and a portion from the periphery having single hyphal tip was separated and transferred to other Petri plates having medium to get pure culture. The identification of the pathogen was confirmed by observing the morphological features of the mycelium and spore formation by Camera mounted compound microscope.

In vitro evaluation of fungicides

Five fungicides (Table 1) were evaluated under *in vitro* conditions against *A. solani* through Poisoned Food Technique using Potato dextrose agar medium (Gabrekiristos and Ayana, 2020). Accordingly, the *in vitro* antagonistic potentials of five fungicides (URGI 75% WP, Carbancholar 50%, Orozole 25 EC, Agro Laxyl MZ 63.5 WP and Mancodex Super 72 WP) were evaluated as described previously (Iqbal and Mukhtar, 2020; Irfan and Khalid, 2007). The replication of treatments was done four times and untreated suitable control was maintained. *In vitro* evaluation of fungicides at different rate was based on the amount of water used to dilute the medium and fungicide rate determination was also done based on the water in the medium (Gabrekiristos and Ayana, 2020). The radial growth of the fungal mycelium was recorded on 3rd, 11th, 18th and 25th days intervals.

Table 1. List of evaluated fungicide and their description.

No	Trade Name	Common name	Rate
1	Mancodex Super 72 WP	Metalaxyl + Mancozeb	3.0 x 10^3 μg/ml
2	Carbancholar 50%	Carbendazim + Chlorothalonil	3.3 x 10^3 μl/ml
3	Orozole 25 EC	Propiconazole 25% EC	3.25 x 10^2 μl/ml
4	URGI 75% WP	Carbendazim + Mancozeb	3.0 x 10^3 μg/ml
5	Agro Laxyl MZ 63.5 WP	Mancozeb + Metalaxyl	3.0 x 10^3 μg/ml
6	Mock	Control	water

Figure 1. Blight symptom on fruit and leaf of tomato caused by *Alternaria solani*; Picture Taken by Endriyas G., 2020.

The inhibitory effect of fungicides on the growth of *A. solani* isolate was evaluated by using Poisoned Food Technique. Tested chemicals were added to conical flasks containing sterilized PDA medium before solidification to obtain the proposed concentrations and rotated gently to ensure equal distribution of added fungicides within the medium (Gabrekiristos and Ayana, 2020). A separate PDA flask free of tested fungicides was used as mock (control) treatment. The supplemented media (approximately 15 ml) were poured into each sterilized 9 cm diameter Petri-dishes). Mycelial discs (6 mm) taken from the periphery of an actively growing PDA culture of test fungus *A. solani* were placed at the center of the amended petri dishes and incubated for twenty five days at 27℃. The average linear growth diameter of colonies was measured and reduction in fungal growth was calculated in relative to mock treatment. The mycelial growth inhibition by fungicides were observed and recorded at 3rd, 11th, 18th and 25th days intervals. The inhibition of *A. solani* mycelial growth was calculated by using the following formula;

$$I = \frac{C - T}{C} \times 100$$

Where I = percent inhibition of mycelial growth, C = radial growth of fungus in control, and T = radial growth of fungus in treatment.

Statistical Analysis

The experiment was set up in a completely randomized design with four replications and each treatment was repeated two times. One-way ANOVA was used to analyze the differences between antagonistic inhibitor effect and linear growth of pathogenic fungi *in vitro.* A general linear model option of the analysis system SAS 9.3 was used to perform the ANOVA. Duncan's multiple range tests at $P \leq 0.05$ level was used for means separation (Winer, 1971).

RESULTS AND DISCUSSION

The pathogen Alternaria has septate, dark coloured mycelium and produce short, simple, erect conidiophores that bear single and branched chains of

 DOI: 10.33804/pp.006.01.4057

conidia in acropetal chains (Figure 1).

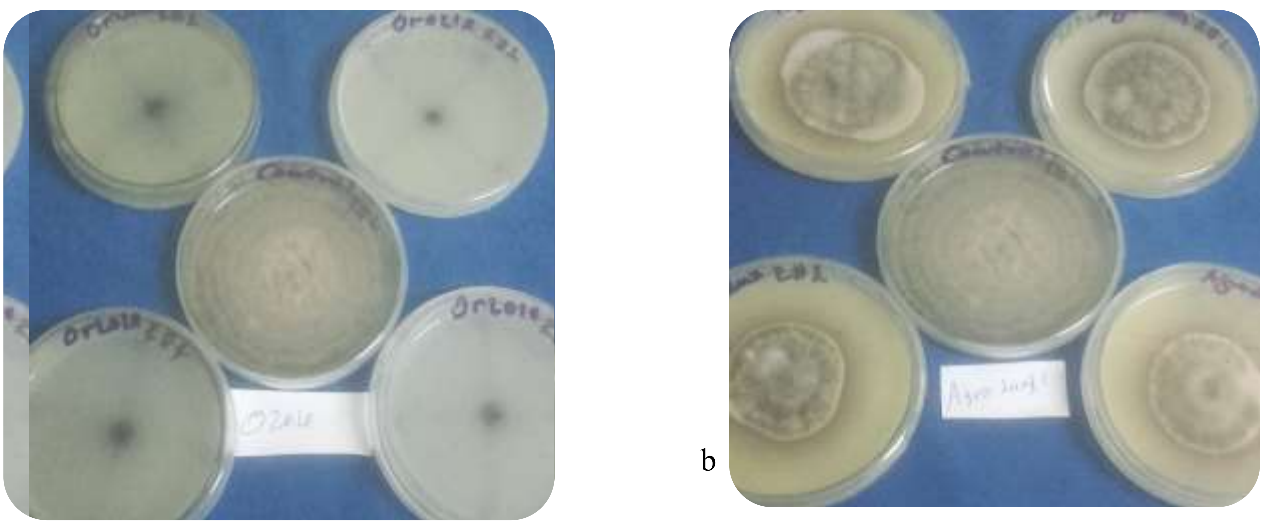
In vitro* efficacy of selected fungicides against *Alternaria solani

The results showed that all the tested fungicides significantly (*P*≤ 0.05) inhibited the mycelial growth of the pathogen with different magnitudescompared with control (Table 2).Orozole 25 EC and Agro Laxyl MZ 63.5 WP were among five fungicides, having both systemic and contact actions, inhibited the growth of the test fungus (Table 2 and Figure 2 a and b) and showed good toxic effect on the test pathogen. The effects of selected fungicides on *A. solani* started the inhibitory activities from the first date of incubation. From evaluated fungicides, fungicidal and fungistatic effects were evaluated and the use of protectant or eradicant fungicides to control fungal diseases depends on their ability to inhibit germination, growth and sporulation (Aggarwal and Mehrotra, 2003).

Table 2. Effect of fungicides on mycelial growth of early blight after twenty five days.

Treatments	Means of Radial growth	% Inhibition
URGI 75% WP	2.2[d]	48.9[c]
Carbancholar 50%	3.2[b]	23.1[e]
Orozole 25 EC	0.0[f]	100.0[a]
Agro Laxyl MZ 63.5 WP	1.8[e]	56.5[b]
Mancodex Super 72 WP	2.3[c]	44.6[d]
Mock	4.2[a]	0.0[f]
CV (%)	4.76	5.76
LSD(0.5)	0.16	3.95

Means followed by same letter indicate no significant difference between treatments LSD test (P ≤ 0.05; p= 0.05).



a b

Figure 2. Growth of *A. solani* isolate on PDA amended with a. Orozole 25 EC and b. Agro Laxyl MZ 63.5 WP

Data regarding mycelial growth revealed that Orozole 25 EC was the most effective fungicide in reducing the average linear mycelial growth of *A. solani* (0.0 mm) followed by Agro Laxyl MZ 63.5 WP (18.0 mm) after 25 days of incubation period (Figure 2a,b and Table 2). Similarly, Benlate achieved limited inhibition of growth, sporulation and germination of *A. solani* although it has been reported to be a highly effective and broad spectrum systemic fungicide against *Fusarium solani,* and *A.solani* (Iqbal and Mukhtar, 2020; Nawar, 2007). Of all the tested fungicides, complete inhibition was observed on the plate treated with Orozole 25 EC (Propiconazole 25% EC) and as a result, the fungicide exhibited the highest toxicity to *A. solani* (Figure 2a). The observed differences in the performance of tested fungicides could be due to detoxification before the site of action has been reached, lack of conversion of a compound into the fungi-toxic principal or the

 DOI: 10.33804/pp.006.01.4057

production of inhibitory factor (Cremlyn, 1991).
Orozole 25 EC was highly effective in reducing the radial mycelial growth of *A. solani*, no mycelial growth was observed (Figure 3 and 4). Similarly, in the experiment conducted on effect of some fungicides against early blight of tomato, the maximum disease management was obtained in the treatment carbendazim 12% + mancozeb 63% WP @ 0.2% concentration followed by propiconazole 25 EC @ 0.025% concentration (Sharma et al., 2018).

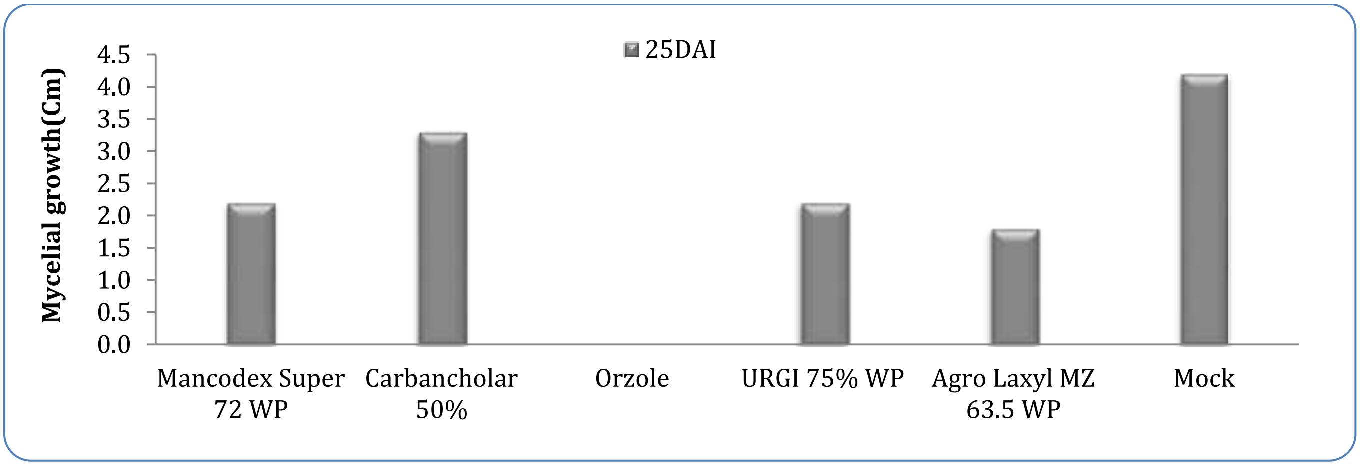


Figure 3. Mycelial growth of *A. solani* after 25 days post incubation on potato dextrose agar.

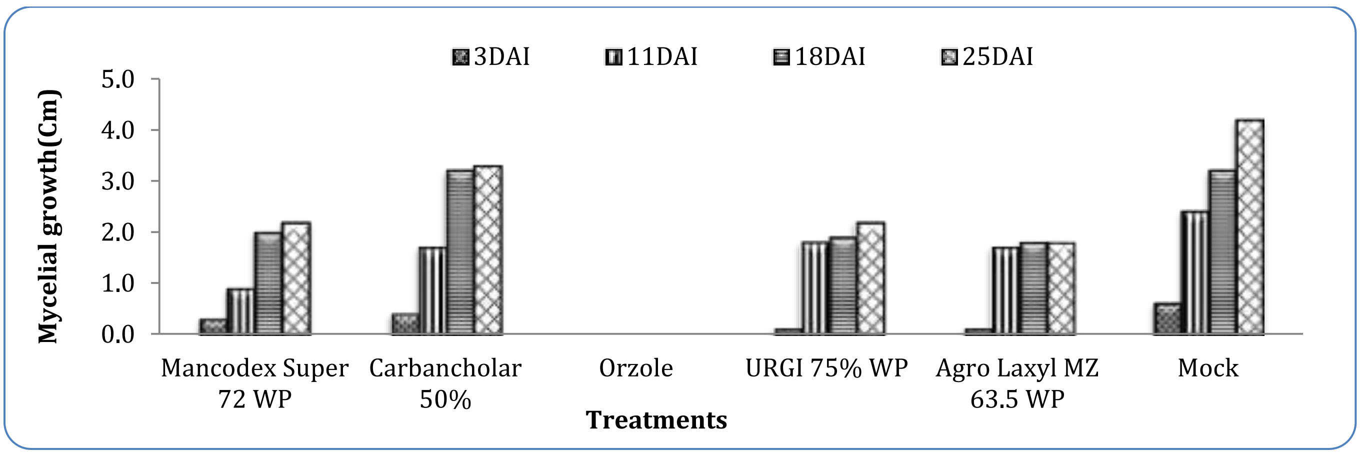


Figure 4. Mycelial growth of *Alternaria solani* of tomato treated by different fungicide.

Orozole 25 EC (Propiconazole 25% EC) performed top among evaluated fungicides by complete inhibition of *A. solani* without showing any mycelial growth starting from culture date. The mycelial inhibition recoded was 100, 56.5 and 48.9% on Orozole 25 EC, Agro Laxyl MZ 63.5 WP, Urgi 75% WP, respectively. However, the minimum inhibition in mycelial growth was recorded in Mancodex Super 72 WP and Carbancholor, 44.6 and 23.1 percent respectively that was significantly lower than rest of the treatments (Table 2; Figure 5a-f). Interestingly, Orozole 25 EC (Propiconazole 25% EC) was the best performing fungicide in this study. In a study by Chavan (2007) reported complete inhibition of the *F. solani* mycelial growth *in vitro* with carbendazim and carbendazim 12% + mancozeb 63%. Nikam et al. (2007) reported that carbendazim alone and in combination with thiram was the most effective against growth inhibition of *Fusarium oxysporum.*

CONCLUSION

Among various diseases of tomato caused by fungi, late blight and early blight caused by *Phytophtora infestans* and *Alternaria solani* is priority in Ethiopia. Now a day, bacterial leaf spot is also emerging in Ethiopia mainly in the central Rift valley. This pathogen is identified in most of tomato producing areas in Oromiya, South Nation Nationalities and peoples and Amhara regions. New approach was developed in Ethiopian Institute of Agricultural Research in Melkassa Research Center by Plant Protection Department. For immediate remediation, evaluating fungicides *in vitro* and availing for the users is the option to easily reduce the epidemics of the pathogen. In this experiment, five fungicides were tested *in vitro.* Of these, three (Orozole 25 EC, Agro Laxyl MZ 63.5 WP, and Urgi 75% WP) fungicides were effective *in vitro* mycelial growth inhibition and two (Mancodex Super 72 WP and Carbancholar) of them were not effective in controlling the test organism. The mycelial inhibition recoded was 100, 56.5 and 48.9% on Orozole 25 EC, Agro Laxyl MZ 63.5 WP, and Urgi 75% WP respectively. Therefore, from tested fungicides to manage tomato early blight, Orozole 25 EC and Agro Laxyl MZ 63.5 WP are recommended for further field evaluation and application. At the same time, the economic validity, these fungicides should also be further verified under field conditions.

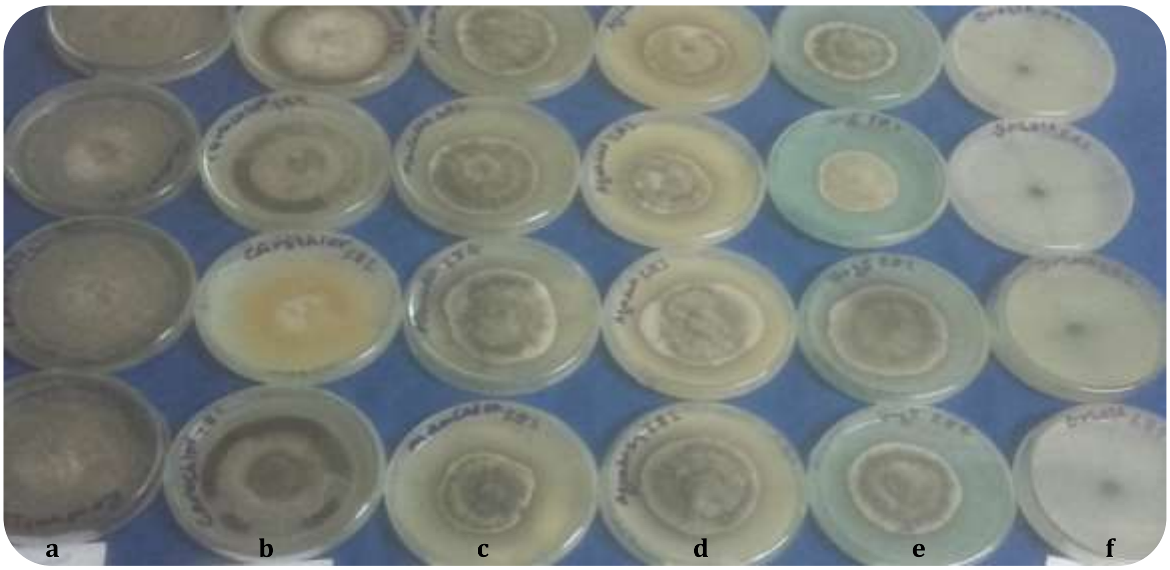


Figure 5. Mycelial growth inhibition of *A. Solani* of tomato on PDA medium *in vitro*condition (a. control; b. Carbancholar; c. Mancodex; d. Agro Laxyl; e. Urgi and f. Orozole).

AUTHORS' CONTRIBUTION

Both the authors designed the study, conducted surveys, performed the experiments, collected and analyzed the data, wrote the manuscript and proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCE

Abada, K.A., Mostafa, S.H., Mervat, R., Abada, K.A., Mostafa, A.H., Mervat, R., 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. Egyptian Journal of Applied Science 23, 47-58.

Aggarwal, A., Mehrotra, R.S., 2003. Plant Pathology 2nd ed. Tata McGRAW Hill Publishing Company Limited, New Delhi, p. 846.

Chavan, S.S., 2007. Studies on fungal diseases of patchouli with special reference to wilt caused by *Fusarium solani* (Mart.) Sacc, Universoty of Agricultural Sciences, Dharwad, p. 98.

Cremlyn, R.J.W., 1991. Agrochemicals: Preparation and Mode of Actions. John Wiley & Sons, Chichester, New York, p. 296.

CSA, 2016. Statistics agency agricultural sample survey 2015/2016. Statistical Bulletin Addis Ababa, Ethiopia.

FAOSTAT, 2010. Rom United Nations Food and Agriculture Organization.

Gabrekiristos, E., Ayana, G., 2020. *In vitro* evaluation of some fungicides against *Fusarium oxysporum* the causal of wilt disease of hot pepper *(Capsicum annum* L.) in Ethiopia. Advances in Crop Science and Technology 8, 443-449.

Gemechis, A.O., Struik, P.C., Emana, B., 2012. Tomato production in Ethiopia: constraints and opportunities. Resilience of Agricultural Systems Against Crises. Tropentag, 19-21.

Iqbal, U., Mukhtar, T., 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52, 709-715.

Irfan, Y.S., Khalid, A.N., 2007. *In vitro* biological controls of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. Mycopathology 5, 85-88.

Lemma, D., 2002. Tomatoes. Research experience and production prospects. EARO Report, 1-53.

Majeed, A., Ahmad, H., Ali, M., Khan, H., 2014. Effect of systemic and contact fungicides on late blight disease and tuber yield of potato. Journal of Agricultural Technology 10, 209-217.

Mutschler, M., Zitter, T., Bornt, C., 2006. Tomato lines for the northeast combining early blight and late blight resistance, 22nd Annual Tomato Disease Workshop, Vegetable Program. New York.

Nawar, L.S., 2007. Pathological and rhizospherical studies on root-rot disease of squash in Saudi Arabia and its control. African Journal of Biotechnology 6, 219-226.

Nikam, P.S., Jagtap, G.P., Sontakke, P.L., 2007. Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*. African Journal of Agricultural Research 2, 692-697.

Reinprecht, L., 2010. Fungicides for wood protection-World viewpoint and evaluation/testing in Slovakia. InTech, Rijeka, Croatia.

Sgherri, C., Kadlecová, Z., Pardossi, A., Navari-Izzo, F., Izzo, R., 2008. Irrigation with diluted seawater improves the nutritional value of cherry tomatoes. Journal of Agricultural and Food Chemistry 56, 3391-3397.

Sharma, R.K., Patel, D.R., Chaudhari, D.R., Kumar, V., Patel, M.M., 2018. Effect of some fungicides against early blight of tomato (*Lycopersicon esculentum* Mill.) caused by *Alternaria solani* (Ell. & Mart.) Jones and Grout and their impact on yield. International Journal of Current Microbiology and Applied Sciences 7, 1395-1401.

Winer, B.J., 1971. Statistical principles in experimental design. McGraw-Hill Kogakusha, Tokyo, Auckland, Bogota.