



Efficacy of foliar fungicides on controlling early blight disease of Eggplant, under laboratory and greenhouse conditions

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Abstract



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Eggplant (*Solanum melongena* L.) production has gone through increasing difficulties due to relatively low yields in the last few years in Iraq. *Alternaria solani*, the causal agent of eggplant early blight; attacks hybrid and local varieties either in open field or in the greenhouse, causing a serious damage that led to decrease in crop yield. The strategies employed to manage this disease by Iraqi farmers were the synthetic fungicides application. In this study, several assays were conducted such as poisoned food technique (*in vitro* assay) and greenhouse experiment (*in vivo* assay); to evaluate the inhibitory efficacy of 3 three synthetic fungicides including; Topas® 100 (penconazole), Tilt 250 (propiconazole) and Leimay® (amisulbrom) on *A. solani* mycelial growth and disease intensity. In laboratory assays, Topas® 100 and Tilt 250 exhibited high inhibitory activities against *A. solani* as an airborne pathogen; recording mycelial inhibition rate above 94 % at a concentration of 1000 mg/l. Furthermore, these two fungicides when applied preventively in greenhouse assays reduced significantly the disease severity index (DSI) by 18.83 % and 26.16 %, respectively. Current results revealed that Topas® 100 and Tilt 250 caused the highest antifungal potential manifested through reduction rate of fresh weight (9.62 and 8.58 g, respectively), and dry weight (4.61 and 4.60 g, respectively). Moreover, both fungicides recorded the highest peroxidase activities of 4.128 units/g/ml/min. and 3.038 units/g/ml/min., respectively. Current findings can be used to assist the eggplant growers to improve the control of early blight disease, and increase the marketable yields of this crop.

Keywords: *Alternaria solani*, Early blight, Eggplant, Fungicides

1. Introduction

Early blight caused by *Alternaria solani* is a common and widespread foliar disease of eggplant ([Salih and Abdul Ridha, 2019](#)). Early blight disease, which in severe cases can lead to complete defoliation, is more harmful in areas with high humidity; heavy rainfall, and fairly high temperatures (24-29 °C). Epidemics can also start in semi-arid and arid climates when nightly dews are prolonged ([Sempere and Santamarina, 2007](#); [El-Debaiky, 2018](#)). Crop losses due to this disease were reported from different regions of Iraq at 60-80 % ([Magesh and Ahila-Devi, 2017](#); [El-Tanany et al., 2018](#); [Chohan et al., 2019](#); [Rhouma et al., 2019](#); [Shafique et al., 2021](#)). In recent years, the increasing importance of *A. solani* has led to several studies about the best way to control this disease ([Iram et al., 2018](#); [Chohan et al., 2019](#); [Shafique et al., 2021](#)). For cultivars susceptible to early blight, responses to fungicide treatment can be highly important and cause increases in crop yield above 127 % ([Teng and Bissonnette, 1985](#); [El-Debaiky, 2018](#)). Previous works conducted by [Shtienberg et al., \(1996\)](#); [Roy et al., \(2019\)](#) have suggested that the effective fungicides should be applied late in the growing season when the host sensitivity increases.

Spray programs that use two or more different groups of fungicides are recommended to reduce the development of fungicide resistant strains, as proposed by [Rosenzweig et al., \(2008a\)](#); [Rhouma et al., \(2016\)](#). Fungicides resistance management is a key consideration for early blight disease control. The most common way to prevent pathogens resistance to fungicides involve reducing the application numbers of the fungicides 'at-risk' per growing season; through applying them alternately and using fungicides with different modes of action ([Rosenzweig et al., 2008b](#); [Horsfield et al., 2010](#)). A previous study conducted by [Velazhahan and Vidhyasekaran, \(1994\)](#) revealed that foliar treatment of plants with fungicides against phytopathogens increased the peroxidase activity in

these plants, and this increase could be related to plant cell lignification. Consequently, the objective of this study was to determine the inhibitory efficacy of the tested fungicides on the causal fungus and/or on the disease severity index (DSI); under laboratory and greenhouse conditions.

2. Materials and methods

2.1. Fungal material

Alternaria solani used in the present research was obtained from the Laboratory of Plant Protection, College of Agriculture, Basra, Iraq; isolated from eggplant leaves collected from an experimental greenhouse in Basra, Iraq, during the period of January- March, 2020.

2.2. Evaluation of *in vitro* inhibitory potential of the fungicide against *A. solani*

The fungicide efficacy on mycelial growth inhibition of *A. solani* was studied *in vitro* using the poisoned food technique. In this assay, three synthetic foliar fungicides were used to test their effectiveness including; Topas® 100 (penconazole), Tilt 250 (propiconazole) and Leimay® (amisulbrom), as presented in Table (1). These fungicides were provided by the Laboratory of Plant Protection, College of Agriculture, Basra, Iraq. The requisite volume of each fungicide was incorporated into Potato dextrose agar (PDA) medium to get different concentrations of; 250, 500, 750 and 1000 mg/l under aseptic conditions and then poured into Petri plates, according to [Rhouma et al., \(2016\)](#). One disc plug (3 mm) of *A. solani* was cut from a 5-7 d old culture using a sterile cork borer and then placed individually in the center of the treated PDA plates, and then the plates were incubated at 25± 2°C for 7 d. A plug of *A. solani* was placed in the center of non-treated PDA plate as a control. Three replicate plates were used for each treatment, and the assay was repeated twice. The

percentage reduction in mycelial radial growth of *A. solani* (I) was assessed according to the formula of [Matrood and Rhouma, \(2021\)](#):

$$I (\%) = ((D_o - D_n) / D_o) \times 100$$

Where; D_n represents the diameter of pathogen radial growth in the fungicide treated plate, whereas D_o represents the diameter of pathogen radial growth in the control plate.

Table 1. Fungicides evaluated for the control of *A. solani* under laboratory and greenhouse conditions

Trade name	Active ingredient	Action	Formulation	Application rate
Topas® 100	Penconazole (100 g/ l)	S	EC	0.5 l/ ha
Tilt 250	Propiconazole (250 g/ l)	S	EC	0.4-0.6 l/ ha
Leimay®	Amisulbrom (200 g/ l)	C	SC	0.2-0.5 l/ ha

Where; S: Systemic fungicide; C: Contact fungicide; EC: Emulsifiable Concentrate; SC: Suspension Concentrate

2.3. Detection of *in vivo* antifungal potency of the fungicide against *A. solani* in the greenhouse

The eggplant seedlings (cv. Barcelona) were planted in pots (50 cm diameter) containing peat at the rate of three seedlings per pot. The experiment was carried out in the greenhouse as a randomized complete block design. Treatment with the fungicide and inoculation with the pathogen were applied after one month of growing the eggplant seedlings. Each fungicide (at recommended dose shown in Table 1) was applied separately using 30 eggplants seedlings per treatment and per block. Five fungicide treatments were used including; T1: Topas® 100; T2: Tilt 250; T3: Leimay®; T4: Positive control and T5: Negative control per replicate (3 replicates) and per block (3 blocks). Eggplant leaves were sprayed individually with 40 ml of each fungicide per seedling. After 3 d, the eggplant seedlings were sprayed with 10 ml of conidial suspension (10^6 cfu/ ml) of *A. solani*. Immediately after treatment, eggplant seedlings were enclosed in plastic bags for 24 h to optimize infection conditions. Two controls were performed for each block; positive control plants inoculated with the pathogen only, and negative control plants treated with sterilized dist. water only, in reference to Rhouma *et al.*, (2016); [Rhouma *et al.*, \(2018\)](#).

Early blight disease assessments were conducted one month after inoculation. Symptoms of the observed areas of disease index (DI) were scored using a scale from 0 to 4; 0 = no spots; 1 = number of spots covering leaf about 1-3 spots, with yellowing of 1-25 % of the leaf area; 2 = number of spots covering leaf about 4-6 spots, with yellowing of 26-50 % of the leaf area; 3 = number of spots covering leaf about 7-9 spots, with yellowing of 51-75 % of the leaf area; 4 = spots covering the totality of leaf, with yellowing of 75-100 % of the leaf area according to [Matrood *et al.*, \(2021\)](#). The disease severity index (DSI) was evaluated using McKinney's formula proposed by [McKinney, \(1923\)](#); [Matrood *et al.*, \(2021\)](#):

$$DSI (\%) = (\sum vn) / (30 \times V) \times 100$$

Where; v represents the numeric value of DI, n represents the number of plants assigned to DI, and V is the numeric value of the highest DI. The numerical value of 30 represents the number of eggplant seedlings for each individual treatment and for each block.

After determination of the fresh weight of the eggplant seedlings, the samples were dried in an oven at 60°C for 48 h to determine the dry weight, according to [Rhouma *et al.*, \(2018\)](#). For evaluating the enzyme activity, plant tissue extract was prepared by freezing 0.1 g of sample leaves in liquid nitrogen to

stop the proteolytic activity, and then homogenized with an extraction buffer (1: 5) (0.1 M phosphate buffer + 0.5 mM EDTA (pH = 7.5)), followed by centrifugation at $15000 \times g$ for 20 min. at 4°C. The supernatant was collected, transferred to new Eppendorf tubes and then assayed for enzyme activity.

2.4. Peroxidase activity of the treated eggplant leaves

The [Velazhahan and Vidhyasekaran, \(1994\)](#) assay was used to determine the peroxidase (POX) activity of the treated seedlings leaves. In an Eppendorf tube; 0.5 ml guaiacol, 1 ml phosphate buffer, 0.5 ml H₂O₂, 0.1 ml enzyme extract and 0.9 ml dist. water were combined in a final reaction volume of 3 ml. After 5 min. of the reaction, absorbance readings were recorded. One unit of peroxidase activity is defined as the change in absorbance per minute at 420 nm.

2.5. Statistical analysis

Statistical analysis was performed using the mean values of the replicates. The data were analysed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA). Homogeneity of variances and normality were checked by applying Duncan's Multiple Range Test. Differences between treatments were determined by Duncan's Multiple Range Test. All statistical tests were performed with a significance level of 5% ($p \leq 0.05$).

3. Results

3.1. *In vitro* detection of the fungicide efficiency against *A. solani*

Results of the *in vitro* effects of the different fungicides on percentage inhibition of radial growth of *A. solani* at different concentrations using poisoned food technique are presented in Table (2). The fungicides exerted high significant reduction ($p < 0.01$) on radial mycelial growth of *A. solani* after 7 d of incubation (Table 2). The different fungicide concentrations exhibited different percentages of growth inhibition which were concentrations-

dependent. In fact, fungicide treatment at 1000 mg\ l concentration showed a good ability to limit the mycelial growth of this tested pathogen. Indeed, results showed that the highest percentage inhibition was recorded by Topas® 100 (99.53 %), followed by Tilt 250 (93.68 %) at a concentration of 1000 mg\ l. At a concentration of 750 mg\ l, all fungicides also expressed significant inhibitory effects against mycelial growth of *A. solani* with percentages of inhibition ranging from 69.48 % (Leimay®) to 89.61 % (Topas® 100). However, *A. solani* demonstrated a good resistance against the tested 3 fungicides at a concentration of 250 mg\ l; with inhibition percentage below 40 % (Table 2).

3.2. Detection of the inhibitory potential of the fungicides against *A. solani* in the greenhouse

The tested foliar fungicides were used in *in vivo* assay against *A. solani* at the recommended doses. Indeed, foliar applications of the fungicides reduced the disease severity index (DSI), and improved several agronomic parameters such as fresh and dry weights of the treated eggplant seedlings; results were statistically highly significant ($p < 0.01$) (Fig.'s 1, 2 and 3).

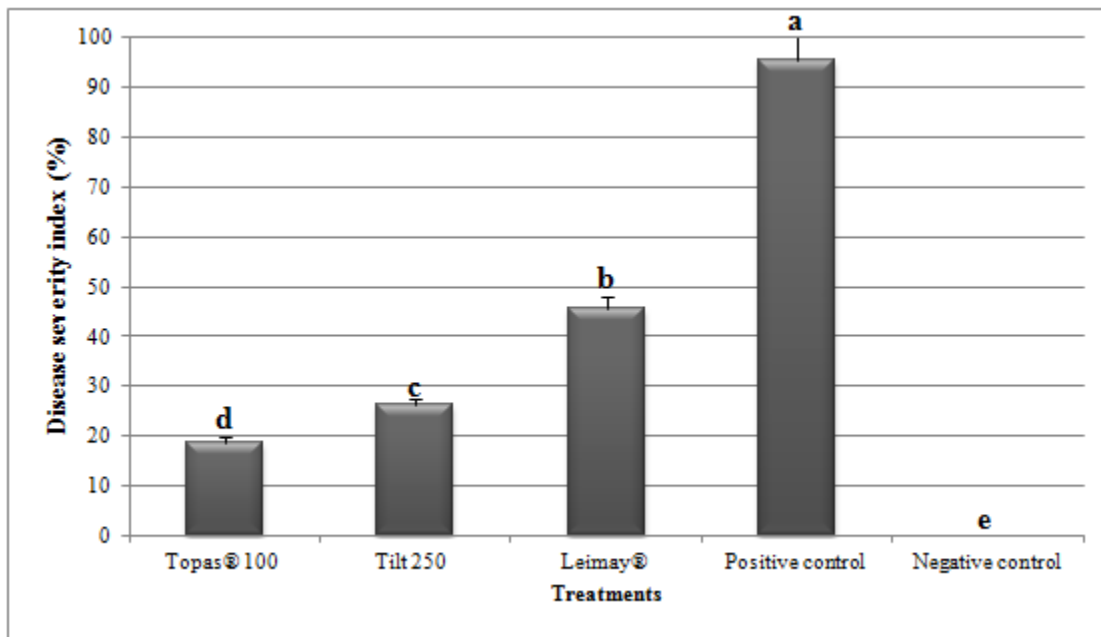
Results showed that eggplant seedlings treated preventively with the systemic fungicides (Topas® 100 and Tilt 250) showed less disease symptoms on the vegetative parts, through decreasing the disease severity index to 18.83 % and 26.16 %, respectively. However, the contact fungicide Leimay® was less efficient; recording DSI of 45.66 % (Fig. 1).

The effectiveness of fungicides applied preventively on the growth parameters of eggplant seedlings was also studied under greenhouse conditions. Current results revealed that Topas® 100 and Tilt 250 significantly increased the fresh weight (9.62 g and 8.58 g, respectively) and dry weight (4.61 g and 4.60 g, respectively) of the treated eggplant seedlings. However, the lowest values were noted on plants treated with Leimay® recording 6.75 g for fresh weight and 3.48 g for dry weight; respectively, as demonstrated in Fig. (2).

Table 2. *In vitro* effects of the 3 fungicides on percentage (%) of mycelial growth inhibition of *A. solani*; at different concentrations after 7 d of incubation at 25°C

Fungicide Treatments	Topas® 100	Tilt 250	Leimay®
250 mg\ l	40.65 ^d	38.62 ^d	28.65 ^d
500 mg\ l	78.78 ^c	61.54 ^c	67.74 ^c
750 mg\ l	89.61 ^b	79.64 ^b	69.48 ^b
1000 mg\ l	99.53 ^a	93.68 ^a	89.33 ^a
p-value	< 0.01	< 0.01	< 0.01
Interactions	Concentrations	< 0.01	
	Fungicides	< 0.01	
	Concentrations x fungicides	< 0.01	

Where; ^aMycelial growth inhibition percentage (%) = $((D_o - D_n) / D_o) \times 100$. D_n represents the diameter (mm) of pathogen radial growth in the fungicide treated plate; whereas D_o represents the diameter of pathogen radial growth in the control plate. p -value: represents probabilities associated with individual F tests. Small superscript letters are for comparison of means of results in the same column. Data are average of three replicates, with five Petri plates per each replicate.

**Fig. 1.** Effect of preventive treatments with three fungicides (Topas® 100, Tilt 250 and Leimay®) on disease severity index of eggplants seedlings inoculated with *A. solani* under experimental greenhouse condition. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to the Duncan's multiple range tests. Data are the average of 10 eggplant seedlings per treatment, per block and per replicate (with 3 replicates)

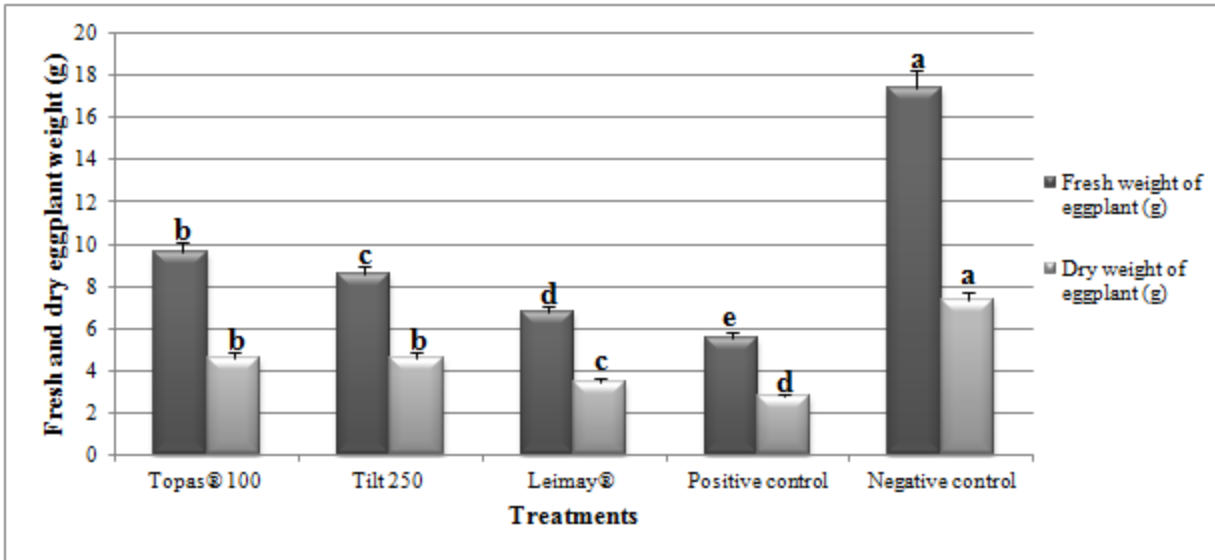


Fig. 2. Effect of preventive treatments of three fungicides (Topas® 100, Tilt 250 and Leimay®) on fresh and dry weights of eggplant seedlings inoculated with *A. solani* under experimental greenhouse condition. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to the Duncan's multiple range tests. Data are the average of 10 eggplant seedlings per treatment, per block and per replicate (with 3 replicates)

3.3. Peroxidase activity induced by the fungicides

Topas® 100 and Tilt 250 synthetic fungicides exhibited higher peroxidase activity in the presence of *A. solani*; recording 4.128 units/g/ml/min. for Topas® 100 and 3.038 units/g/ml/min. for Tilt 250, as presented in Fig. (3). On the other hand, the lowest peroxidase activity was recorded in seedling leaves treated with Leimay® (2.47 units/g/ml/min.).

4. Discussion

This study showed that Topas® 100 (penconazole) and Tilt 250 (propiconazole) systemic fungicides have significant inhibitory effects on radial growth of *A. solani* in PDA plates. Several previous reports have documented the effects of penconazole and propiconazole on controlling the phytopathogenic fungi. In accordance with the current results, [Amaresh and Nargund, \(2004\)](#); [Bavaji et al., \(2012\)](#); [Taware et al., \(2014\)](#) reported that penconazole, propiconazole, difenconazole, thiophanate methyl and carbendazim at different concentrations; inhibited significantly the mycelial growth of *Alternaria* sp. under laboratory

conditions. Moreover, [Murmu et al., \(2017\)](#) highlighted that foliar treatment of potato plant with systemic fungicides indicated strong inhibition of mycelial growth of *A. solani*. A previous study conducted by [Singh and Chowdhary, \(2008\)](#) revealed that the foliar treatment of chilli with both systemic fungicides (in combination) had the highest inhibitory potential on growth of *A. solani*; at concentrations of 500, 750 and 1000 mg/l, recording inhibitory percentages of; 83.93 %, 94.27 % and 97.64 %, respectively. Similarly, a previous work of [Sreenivasulu et al., \(2019\)](#) revealed that propiconazole provided critically important inhibition of conidium germination and mycelial growth of *A. solani*. A recent study conducted by [Madadi et al., \(2021\)](#) pointed out that penconazole showed near-complete inhibition of mycelial growth of *A. alternata* at 3 testes concentrations of; 100, 300 and 500 mg/l. Furthermore, [Sharma et al., \(2018\)](#); [Sreenivasulu et al., \(2019\)](#) reported that less disease severity was observed in tomato plants treated with propiconazole against the pathogenic *A. solani*.

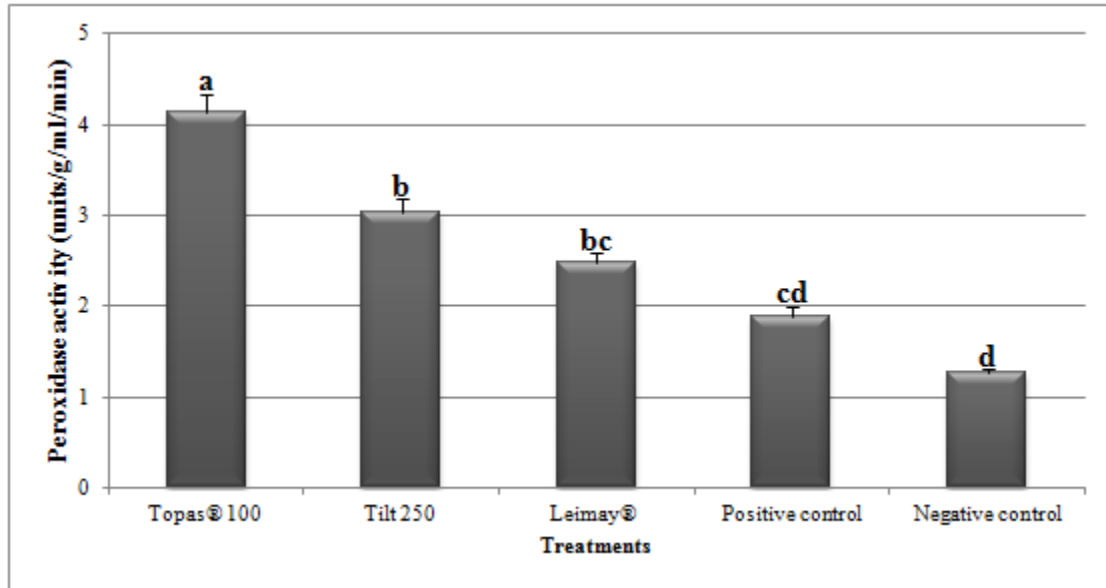


Fig. 3. Effect of preventive treatments of three fungicides (Topas® 100, Tilt 250 and Leimay®) on peroxidase activity in eggplant seedlings inoculated with *A. solani* under experimental greenhouse condition. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to the Duncan's multiple range tests. Data are the average of 10 eggplant seedlings per treatment, per block and per replicate (with 3 replicates)

[Ashour, \(2009\)](#) recorded that fenomen + propamocarb, difenoconazole, trifloxystrobin, mancozeb and mancozeb + fenamidone caused a significant reduction in linear growth of *A. solani* under laboratory, greenhouse and fields conditions. Early studies of [Mantecón, \(2009\)](#); [Issiakhem and Bouznad, \(2010\)](#) documented that preventive treatment of potato using strobilurin and difenoconazole showed a complete control of early blight symptoms under field conditions.

[Dahmen and Staub, \(1992\)](#) pointed out that foliar treatments of wheat, peanut and tomato with the systemic fungicides was highly effective against early blight pathogen. Fungicides spray programs rely solely on protective fungicides, which were less effective than those included in several applications of systemic fungicides. There are likely several reasons for this; such as: (i) the protective fungicides do not enter into the leaf and may be washed by the rain fall or

overhead sprinkler irrigation; (ii) treatment before and after infection with systemic/ translaminar fungicides are effective ([Shtienberg et al., 1996](#); [Zitter and Drennan, 2005](#)).

In a pathogenic fungal cell, many systemic fungicides such as site specific fungicides prevent the fungal mitochondrial respiration; by blocking the electrons transport and inhibiting the oxidation site of coenzyme Q situated on the outer face of cytochrome b, thus hindering ATP production and consequently cause cell death ([Rhouma et al., 2016](#); [Manasa et al., 2018](#); [Vielba-Fernández et al., 2020](#)). Therefore, pathogenic fungi suffer from spores death, mycelium disintegration and other developmental problems ([Vielba-Fernández et al., 2020](#)). Meanwhile, the contact fungicides possess multi action sites (multi-site inhibitors) by having several effects on the Krebs cycle, the respiratory chain, β -oxidation of fatty acids and glycolysis, which are essential processes for cells

multiplication and survival ([Rhouma et al., 2016](#); [Vielba-Fernández et al., 2020](#)).

In addition to disease control, systemic fungicides are acknowledged to have beneficial effects on growth-promotion and physiological activities of the treated plants including; increasing chlorophyll content ([Butkute et al., 2008](#)), delaying of leaf senescence ([Bertelsen et al., 2001](#)), tolerance to abiotic stresses ([Jabs et al., 2002](#)), and contribute to the increased yield of the plants ([Horsfield et al., 2010](#)).

Conclusion

Findings of this study documented that systemic fungicides with strong curative activity such as; penconazole (Topas® 100) and propiconazole (Tilt 250) were more effective than protective fungicide (Leimay® (amisulbrom)) against *A. solani* mycelial growth and early blight infections. This information can help the eggplant growers to improve early blight control and enhance marketable yields of this crop.

Conflict of Interests

The authors declare that there is no conflict of interests related to this article.

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Ethical approval

Non-applicable.

Both authors contributed equally in this work.

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