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In vitro and *In vivo* Evaluation of Certain Fungicides Against *Phytophthora Infestans* (Mont.) The Causal Pathogen of Late Blight Disease on Tomato

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ABSTRACT

Efficiency of fungicides; Shirlan 50% SC, Ranman 40% SC, Revus 25% SC, Leimay 20% SC, Vegeclean 15% WG and Evito 48% SC against tomato late blight disease was conducted *In vitro* and *In vivo* tests. The variation in the aggressiveness of the two isolates of *P. infestans* could be clearly differentiated to most aggressive one (PhK-2) and least aggressive (PhK-1). Medium effective concentrations (EC_{50}) of the candidate fungicides indicated that Shirlan 50% SC was the superior fungicides against mycelial growth of PhK-1 and PhK-2 isolates. Revus 25% SC and Ranman 40% SC were less efficiency against mycelial growth of PhK-1 and PhK-2 isolates, respectively. The tested fungicides gave excellent control of tomato late blight disease under greenhouse conditions especially Evito 48% SC and vegeclean 15% WG when applied as protective or curative. The candidate fungicides were used separately at their recommended rates of application against late blight disease on tomato plants during the season of 2015-2016 in two different locations (Fayoum and Sharkia Governorates). In Sharkia Governorate, all the candidate fungicides gave excellent control of late blight disease, which their efficiency ranged between 80.03 and 85.93% except Revus 25% SC gave 63.67% only. Efficiency of Leimay 20% SC and Shirlan fungicides against late blight disease was increased from 63.73 and 65.61% in Fayoum trial to 80.03 and 80.69% in Sharkia trial, respectively. Efficacy percentages of tested fungicides were highly correlated with area under the disease progress curve (AUDPC) values. All candidate fungicides when treated as curative were effective in controlling late blight disease except Revus 25% SC.

Keywords: *Tomato late blight disease, Fungicides, Mycelial growth, Greenhouse, Field trials.*

INTRODUCTION

In Egypt, tomato is cultivated during the year in many growing seasons, summer, fall (Nili) and winter in the period from the early of September to the end of February. Many factors can limit the tomato production. One of the most important factors is late blight disease that caused by the oomycete pathogen *Phytophthora infestans* (Mont.). Unfortunately the weather conditions allow the disease to start from the early of November and many outbreaks occur during December to the mid of April resulting severe yield losses (El-Sheikh *et al.* 2005). Large differences for aggressiveness were found to be present between *P. infestans* isolates. These factors are clearly associated with parasitic fitness, as they influence the relative ability of a genotype to persist successfully over time (Nelson 1979). Revelo *et al.* (2011) found that there were two separate populations of *P. infestans*, each with specific virulence towards two different cultivars of *Solanum betaceum*. Taleb-Hossenkhan and Ibrahim (2015) tested seven isolates of *P. infestans* collected from distinct geographical areas for aggressiveness on four potato and three tomato varieties. Results indicated that all seven isolates exhibit host preference, with higher aggressiveness on potato varieties compared to the tomato varieties. Although the aggressiveness characteristics of the seven isolates were very similar, but they were not identical. This indicates that the isolates have different pathogenic fitness properties.

Today, control of late blight disease conducting by a combination of sanitary measures, crop rotation, resistant varieties and chemical treatment. Although several biological control agents have been reported for late blight control, but they are not effective as fungicides **(**Olanya and Larkin 2006**)**. Commercial tomato production would hardly exist without routine use of fungicides. The use of protectant and systemic fungicides for managing late blight has perhaps been the most studied aspect of this disease management in temperate countries **(**Olanya *et al.* 2001**)**. Mitani *et al*. (2001) found that Cyazofamid was effective at low concentrations against late blight disease and has highly effective in protecting newly developing tomato leaves. Miyake *et al*. (2005) demonstrated that Benthiavalicarb-isopropyl was effectively controls potato and tomato late blight. Experiments *in vitro* indicated that benthiavalicarb-isopropyl was strongly inhibited mycelial growth, sporulation and the germination of sporangia and cystospores. Benthiavalicarb-isopropyl has not only a strong preventive, but also a curative effect against late blight disease in greenhouse experiments. Rekanović *et al*. (2011) studied sensitivity of twelve isolates of the *P. infestans* to fluazinam, fosetyl-Al and propamocarb-hydrochloride *in vitro*. All isolates were sensitive to the tested fungicides with resistance factor ranged from 1.0 to 2.8. Hence, the objectives of this study were to test, *In vitro and In vivo*, the antifungal activity of the recommended fungicides in Egypt; Shirlan 50% SC, Ranman 40% SC, Revus 25% SC, Leimay 20% SC, Vegeclean 15% WG and Evito 48% SC against mycelial growth of two isolates of *P. infestans* and their controlling tomato late blight disease under greenhouse and field conditions.

MATERIALS AND METHODS

1-Isolation, purification and identification of the pathogen

Samples of blighted tomato leaves were collected from Kafer El-Shaykh during year 2014 for isolation of the pathogen. The isolates of *P. infestans* were grown on pea meal agar medium in petri dishes for 10-14 days at 18°C in darkness. Healthy and fresh leaves of tomato cultivar Super Strain B were selected from the middle part of the stem, placed abaxial side up in moist plastic tray and inoculated with 5 mm mycelial of each isolates at 18°C under a photoperiod of 14 h light/10 h dark cycle for 24 h. The sporangia were harvested using 10 mL sterile water and put in a shaker for 10 min to release the zoospores, then the suspensions were filtered through four layers of cheesecloth to remove mycelial fragments and were adjust to 25X 10^3 sporangia/ml with a hemocytometer. The suspension was placed at 4°C up to 3 hours until use (Hodgson 1961). Two isolates of *P. infestans* (PhK-1 and PhK-2) were purified and identified according to their microscopical and morphological characters.

2. Pathogenicity test

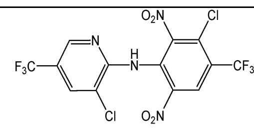
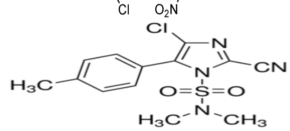
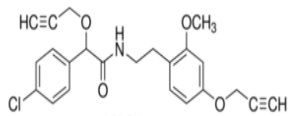
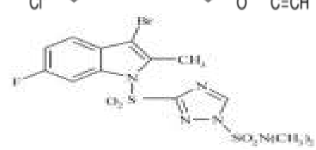
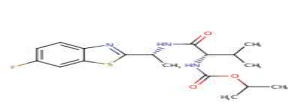
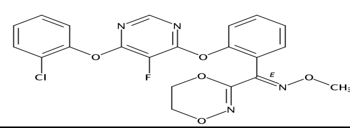
Tomato seeds (cultivar Super Strain B), obtained from the Vegetable Res. Dept., Hort. Res. Inst., A.R.C. were sown in ordinary cultivation trays filled with peat moss-vermiculate (1:1 w/w) for 30 days under greenhouse conditions. Growing seedlings were transplanted in pots (20-cm-diam.) filled with sterilized sandy-clay soil (1:1 w/w) at the rate of 3 seedlings/pot. Growing plants (50-day-old) were sprayed with the spore suspension (25X10^3 sporangia/ml) using a fine atomizer in laboratory and transported to greenhouse at 25±5°C and 75-80% R.H. Sensitivity of tomato plants to two isolates of *P. infestans* (PhK-1 and PhK-2) was evaluated after 7, 15 and 21 days of spray as disease severity according to scoring of El-Ganainy (2013) during season 2014/2015. Disease severity (%) was calculated using Townsend and Heuberger equation (1943).

3. Fungicidal applied

a. Tested fungicides

Five registered fungicides in addition to one recommended compound (Evito 48% SC) represent different chemical groups, were selected for conducting this study. The registered fungicides; Vegeclean 15% WG, Shirlan 50% SC, Leimay 20% SC, Revus 25% SC and Ranman 40% SC were selected on the basis of their widely commercial use in Egypt for controlling late blight disease. Commercial formulation of fungicides were used in this study. Evito 48% SC formulation was provided by Arysta LifeScience Egypt Company. Each fungicide was diluted into a set of stock solution with sterile distilled water. These fungicides are listed in Table (1).

Table 1. Trade names, common names, chemical groups and rates of application of the tested fungicides.

Trade name	Common name	Chemical group	Rate/ 100 L water
Shirlan 50% SC	Fluazinam		50 ml
Ranman 40% SC	Cyazofamid		30 ml
Revus 25% SC	Mandipropamid		50 ml
Leimay 20% SC	Amisulbrom		40 ml
Vegeclean 15% WG	Benthiavalicarb-isopropyl		50 g
Evito 48% SC	Fluoxastrobin		50 ml

b. Growth inhibition measurement

The inhibitory effect of the tested fungicides on the mycelial growth of *P. infestans* isolates (PhK-1 and PhK-2) was estimated on Pea Meal Agar medium (PMA). A serial of concentrations for each tested fungicide were prepared using sterilized distilled water. Poison Food Technique (PFT) of Togeston (1957) was used for evaluation of the tested fungicides against mycelial growth of *P. infestans* isolates (PhK-1 and PhK-2). Different quantities of the fungicides were mixed with the sterilized PMA medium after cooling at 45ºC before pouring. After mixing the fungicide, rotated gently to ensure equal distribution of fungicide and solidification of the medium. The isolates were seeded in the center of each Petri dish using 5 mm agar disc having active mycelial growth of the fungus. Each treatment was replicated three times. One treatment was maintained as check control (free from any fungicide).

All inoculated plates were incubated at 18°C for 12-15 days (until the fungal growth was completely filled the fungicide-free plates). The inhibition of mycelial growth in the different concentrations was determined in relation to that of the control treatment using Abbot's formula (1925). The data were subjected to statistical analysis by applying the Software (Bakr 2007) to calculate probit analyses to calculate the regression equation, slope of regression lines, EC_{50} and EC_{90} values of the tested fungicides. The toxicity index (TI) of each fungicide was determined according to Sun (1950).

c. Greenhouse experiments

This experiment was carried out in the greenhouse of Vegetable Disease Research Department, Plant Pathology Institute, ARC. Tomato seedlings of cultivar super Strain B were used in this study. Three seedlings (30-days-old) were transplanted in each pot (20-cm-diam.) filled with sandy-clay soil (1:1 w/w). Growing seedlings were divided into two groups and applied according to the method of Bodker and Nielsen (2000). In the first group, growing tomato plants (٥٠ days after transplanting) were applied with tested fungicides at their rates of application using hand atomizer, then after 24h sprayed with a suspension (٥X10^4 sporangia/ml) of *P. infestans* isolates (PhK-1 and PhK-2). On the other hand, growing tomato plants were sprayed with a suspension (٥X10^4 sporangia/ml) of *P. infestans* isolates, then after 24h applied with the tested fungicides (second group). Three replicates of tomato plants inoculated with the tested pathogen and sprayed with water only were served as a check treatment. Treated and untreated plants were kept under controlled greenhouse conditions (20°C and 75-80% R.H.). Data were scored after 10 days of inoculation time as disease severity. The efficiency of each treatment was calculated using the equation of Derbalah *et al*. (2011).

d. Field experiment

Two field trials were conducted to evaluate the efficiency of the tested fungicides against late blight disease on tomato during the agricultural season 2015/2016. The first field trial was carried out in Fayoum Governorate representing mid of Egypt. The second evaluation was conducted in Sharkia Governorate representing North of Egypt. Apparently, healthy seedlings (30-days-old) of tomato cultivars; Super Strain B and Oreit were transplanted at end of October and mid of August, 2015 in Fayoum and Sharkia Governorates, respectively. All cultural methods and fertilizers were followed as commonly practiced. The experimental area was divided according to the complete randomized block design including four replicates for each treatment. Each replicate was 6X7 m^2 (1/100 feddan). A Knapsack sprayer was used to apply the tested fungicides, diluted with water, as foliar treatment. Tomato plants were observed from transplanting until appearance of the initial late blight symptoms. Four sprays, 15 days intervals, were applied. The first spray was carried out in Fayoum Governorate at November12, 2015, while it was conducted in Sharkia Governorate at January 12, 2016. Four replicates free of fungicides were served as a check treaatment. Tested fungicides were sprayed at their recommended rates of application against natural infection of tomato late blight disease. The average of disease severity was calculated one day before spraying and 14 days after each spray according to Cooke *et al*. (2006). Area under the disease progress curve (AUDPC) was calculated according to equation of Louwes *et al*. (1996).

RESULTS AND DISCUSSION

1. Pathogenicity of *P. infestans* isolates on tomato c.v. Super Strain B

The pathogenic potency of two isolates of *P. infestans*, the causal of late disease was evaluated 7, 15 and 21 days after inoculation of tomato cv. Super Strain B under greenhouse conditions (Table 2). The first incubation period (7 days) of inoculated plants with the both isolates were enough for appearance disease symptoms. Data indicated that disease severity percentages of two isolates were increased with elapse the incubation period from 7 to reach the maximum after 21 days. Percentages of disease severity significantly differed between the two isolates at the same trend after 7, 15 and 21 days of inoculation. The variation in the aggressiveness of the two isolates of *P. infestans* could be clearly differentiated during the experimental period. Data presented in Table (2) showed that isolate PhK-2 was the most aggressive one, which caused disease severity reached to 26.76, 54.94 and 72.40% after 7, 15 and 21 days of inoculation, respectively. On contrary, the least aggressiveness isolate PhK-1 gave disease severity percentages 19.37, 36.68 and 58.55 after 7, 15 and 21 days of inoculation, respectively. Similar results were reported by Flier and Turkensteen (1999) who mentioned that large variation in aggressiveness was present among isolates for each regional *P. infestans* population. Also, Harbaoui *et al*. (2011) found that the virulence spectrum of Tunisian *P. infestans* isolates was highly variable. Of 31 isolates tested, all isolates were virulent depending on the number of virulence factors except three isolates were non virulent.

Table 2. Disease severity (%) of *P. infestans* isolates on tomato cv. Super Strain B after 7, 15 and 21 of inoculation under greenhouse conditions.

Tested isolate	Disease severity (%) at indicated days after inoculation			Mean
	7	15	21	
PhK-1	19.37b	36.68b	58.55b	38.20
PhK-2	26.76a	54.94a	72.40a	51.37
Check	00.00c	00.00c	00.00c	00.00

-Each figure represents the mean of 3 replicates (9 plants).

- Figures within the same column having the same letter are not significantly different (P< 0.05).

2- *In vitro* evaluation

a. Effect on the mycelial growth

Medium effective concentration (EC_{50}) in addition to values of EC_{25} and EC_{90} were calculated for each tested fungicide against two isolates of *P. infestans* according to Lpd Line program (Table 3).

Inhibitory activity of tested fungicides against mycelial growth differed significantly between the two isolates at values of EC_{25}, EC_{50} and EC_{90}. A survey of six fungicides revealed computable reaction in susceptibility of the tested two isolates considering of their aggressiveness. Accordingly, the least aggressive isolate PhK-1 ranked as the most susceptible isolate,while the most aggressive isolate PhK-2 was the least susceptible isolate to the tested fungicides.

Medium effective concentrations of the candidate fungicides; Shirlan 50% SC, Ranman 40%SC, Revus 25% SC, Leimay 20% SC, Vegeclean 15% WG and Evito 48% SC to PhK-1 isolate were 2.35, 359.20, 391.26, 298.12, 135.13 and 122.86 ppm, respectively. Data indicated that Shirlan 50% SC was the superior fungicides against the PhK-1 isolate, while Revus 25% SC was the inferior one. The toxicity indices of the tested fungicides; Evito 48% SC, Vegeclean 15% WG, Leimay 20% SC, Ranman 40% SC and Revus 25% SC were 1.91, 1.74, 0.79, 0.65 and 0.60% as toxic as Shirlan 50% SC. So, the treatments were arranged according to the EC_{50} values in the following descending order; Shirlan 50% SC, Evito 48% SC, Vegeclean 15% WG, Leimay 20% SC, Ranman 40% SC and Revus 25% SC.

The same efficiency trend of the tested fungicides against mycelial growth of the PhK-1 isolate was found when EC_{25} was calculated. Concerning the slope values of the toxicity lines for the tested fungicides, Revus 25% SC showed the steeper toxicity line with slope value 5.09 followed by Leimay 20% SC (4.02), Vegeclean 15% WG (3.96), Ranman 40% SC (2.80), Evito 48% SC (1.81) and Shirlan 50% SC (0.44).

Values of EC_{50} for the tested fungicides against mycelial growth of the PhK-2 isolate was more than those to the phK1 isolate. Data indicated that Shirlan 50% SC was the superior fungicides against the PhK-2 isolate, while Ranman 40% SC was the inferior one. The toxicity indices of the tested pesticides; Vegeclean 15% WG, Evito 48% SC, ,Leimay 20% SC, Revus 25% SC and Ranman 40% SC were 7.79, 5.43, 3.03, 2.68 and 2.22 as toxic as Shirlan 50% SC. So, the treatments were arranged according to the EC_{50} values in the following descending order; Shirlan 50% SC, Vegeclean 15% WG, Evito 48% SC, Leimay 20% SC, Revus 25% SC and Ranman 40% SC. Concerning the slope values of the toxicity lines for the tested fungicides, Revus 25% SC showed the steeper toxicity line with slope value 7.03 followed by Leimay 20% SC (5.69), Ranman 40% SC (3.35), Vegeclean 15% WG (3.30), Evito 48% SC (1.44) and Shirlan 50% SC (0.71). Mostly, slope of toxicity lines of each tested fungicide against mycelial growth of the two isolates of *P. infestans* was identical.

Table 3. Inhibitory activity of tested fungicides against mycelial growth of *P. infestans* isolates (PhK-1 and PhK-2).

Tested fungicide	Isolate	EC level (ppm)			Slope	Toxicity index*
		EC_{25}	EC_{50}	EC_{90}		
Shirlan 50% SC	phk1	0.06	2.35	754.63	0.44± 0.11	100.0
	phk2	1.33	11.84	1927.88	0.71± 0.11	100.0
Ranman 40% SC	phk1	206.70	359.30	1027.32	2.80± 1.85	0.656
	phk2	334.40	531.56	1282.34	3.35± 1.86	2.228
Revus 25% SC	phk1	288.51	391.26	697.97	5.09± 1.87	0.602
	phk2	353.39	440.72	670.51	7.03± 1.86	2.687
Leimay 20% SC	phk1	202.72	298.12	620.38	4.02± 0.46	0.791
	phk2	296.8	389.99	654.89	5.69± 0.85	3.037
Vegeclean 15% WG	phk1	91.31	135.13	284.56	3.96± 0.27	1.744
	phk2	94.99	151.94	370.88	3.30± 0.27	7.795
Evito 48% SC	phk1	52.18	122.86	625.26	1.81± 0.24	1.918
	phk2	74.59	217.87	1669.86	1.44± 0.23	5.436

***Toxicity index= (EC_{50} of the most effective pesticides / EC_{50} of the least effective pesticides) X 100.**

b. Evaluation in greenhouse

This experiment was conducted under artificial inoculation with the two isolates of *P. infestans* to evaluate protective and curative activities of the tested fungicides for controlling tomato late blight disease under greenhouse conditions (Table 4). All the candidate fungicides when treated as protective or curative were effective in reducing the disease severity of tomato late blight. Disease severity in check treatments produced from the least and most aggressive isolates (PhK-1 and PhK-2) reached to 56.18 and 73.48%, respectively. Late blight disease was developed extensively on tomato plants in the check treatment comparing with fungicide treatments. Although there were significant differences between disease incidences of late blight in all treatments but slight differences in the efficiency between the tested fungicides were deduced. Disease severity percentages in treatments of the most aggressive isolate (PhK-2) were more than those in the least aggressive isolate (PhK-1) except the treatment of Vegeclean 15% WG. In protective test, efficiency of the tested fungicides ranged between 87.20 and 94.39% against the isolate PhK-1, while was from 89.89 to 95.44% against the isolate PhK-2. When the fungicides were applied 12 h after inoculation, efficiency of the tested fungicides averaged between 81.67 and 85.10% against the isolate PhK-1, while was from 85.60 to 91.41% against the isolate PhK-2.

Table 4. Protective and curative effects of tested fungicides against tomato late blight disease that produced from the two isolates of *P. infestans* under greenhouse conditions.

Tested fungicide	Disease severity (%) after 21 days of spray							
	Spraying before inoculation (Protective)				Spraying after inoculation (Curative)			
	PhK-1		PhK-2		PhK-1		PhK-2	
	DS%	Efficiency	DS%	Efficiency	DS%	Efficiency	DS%	Efficiency
Shirlan 50%SC	6.35d	88.70	6.60d	91.02	9.64d	82.84	10.08d	86.28
Ranman 50%SC	5.40e	90.39	5.79f	92.12	8.90f	84.16	9.15f	87.55
Revus 25%	7.19b	87.20	7.43b	89.89	10.30b	81.67	10.58b	85.60
Leimay 20% SC	6.85c	87.81	7.11c	90.32	9.85c	82.47	10.27c	86.02
Vegeclean 15% WG	6.27d	88.84	5.94e	91.92	9.21e	83.61	6.31e	91.41
Evito 48% SC	3.15f	94.39	3.35g	95.44	8.37g	85.10	8.89g	87.90
Check	56.18a	---	73.48a	---	56.18a	---	73.48a	---

- Each figure represents the mean of 3 replicates. -

- Values within the same column with the same letter are not significantly different (P< 0.05).

Generally, efficiency of the tested fungicides against late blight disease that produced from the isolate PhK-2 was more than that conducted against the isolate PhK-1. All candidate fungicides were more effective when applied 24 h before inoculation. The tested fungicides gave excellent control of late blight disease especially Evito 48% SC, Ranman 50% SC and vegeclean 15% WG when applied as protective or curative. Generally, it could be noticed that the candidate fungicides have protective and curative effects against tomato late blight disease under greenhouse conditions

Inhibitory activity of tested fungicides against mycelial growth of *P. infestans* isolates (PhK-1 and PhK-2) in the present study indicated that both tested isolates were highly sensitive to Shirlan 50% SC (fluazinam). The obtained data were similar to Matheron and Porchas (2000) who reported that all isolates of *Phytophthora parasitica* were sensitive to fluazinam. It is the only commercially available product from the class of pyridinamines for the control of potato late blight in world-wide. The biological mode of action is uncouples mitochondrial oxidative phosphorylation (Stein and Kirk 2002). Miyake *et al.* (2005) found that Benthiavalicarb-isopropyl strongly inhibited the mycelial growth of *Phytophthora* species. Values of I_{50} of Vegeclean against *P. infestans*, *P. capsici*, *P. palmivora*, *P. cactorum*, *P. nicotianae*, *P. porri*, *P. katsurae*, and *P. megasperma* were within the range of 0.01–0.05 mg/l.

The current study of greenhouse experiments indicated that all the candidate fungicides when treated as protective or curative were effective in reducing the disease severity of tomato late blight. Bodker and Nielsn (2000) reported that preventive treatment for all tested fungicides had nearly 100 percent control for over three weeks. When the fungicides were applied curatively, only products containing systemic active ingredients gave efficient control. Translaminar products had moderate curative effect. Thompson and Cooke (2008) mentioned that Mandipropamid prevented infection by either zoospores or sporangia of *P. infestans* through both the adaxial and abaxial leaf surfaces of potato. Mandipropamid showed translaminar activity against *P. infestans,* preventing development of visible lesions when it was applied to the adaxial surface only and the abaxial surface was inoculated with *P. infestans*. Mitani *et al*. (2001) reported that cyazofamid at 0.4- 1.6 mg/L^{-1} gave excellent preventive activity against *P. infestans* on tomato. Minimum inhibitory concentrations of cyazofamid against *P. infestans* were over 63 times lower than those mancozeb and at least 16 times lower than those of metalaxyl. Cyazofamid at 1.6 – 25 mg/L^{-1} exhibited not only preventive activity but also curative activity. Cyazofamid at 6.3 mg/L^{-1} reduced zoosporangia formation of *P. infestans* on host plants.

- *In vivo* evaluation

-Field trial in Fayoum

This experiment was carried out in Fayoum Governorate where the weather conditions are favorable for tomato late blight diseases, which resulted in a significantly higher value for check treatment by natural infection. The results in Table (5) present the disease severity percentages of late blight disease after 14 days of each spray with the candidate fungicides in addition to the efficacy percentage for the last recorded disease severity and area under disease progress curve (AUDPC) values. The disease severity of late blight ranged from 6.1 and 7.7% one day before beginning the spraying. There was harmony in disease severity between all the treatments before application. After 14 days of the first, second, third and fourth spray, disease severity reached 27.1, 41.4, 53.1 and 69.2% in the check treatment,respectively. Generally, the tested fungicides were significantly decreased disease severity comparing with the check treatment during the experimental period. Spraying tomato plants with Evito 48% SC, Ranman 40% SC and Vegeclean 15% WG led to the highest significant decrease in disease severity during the experimental period. Based on the data of disease severity percentages after 14 days of the fourth spray, the tested fungicides can be classified significantly into two groups. The first group contains Evito 48% SC, Ranman 40% SC and Vegeclean 15% WG, which gave the lowest disease severity percentages. The second group contains Revus 25% SC, Leimay 20% SC and Shirlan 50% SC, which gave intermediate decrease in disease,severity. There were significant differences between the fungicides belong to the first and second groups. In contrary, there were no significant differences between the fungicides belong to each group.

Table 5. Efficiency of tested fungicides against late blight disease on tomato growing under field conditions (Fayoum Governorate).

Tested fungicide	Disease severity (%) after 14 days of each spray					Efficiency	AUDPC
	0*	1st spray	2nd spray	3rd spray	4th spray		
Shirlan 50% SC	7.1b	10.4d	15.6d	19.8d	23.8b	65.61	922.050
Ranman 40% SC	7.0c	9.7f	10.8f	11.6f	12.4c	82.08	629.475
Revus 25% SC	6.1f	12.1b	16.9b	21.5b	26.2b	62.14	1017.225
Leimay 20% SC	7.7a	11.7c	16.1c	20.2c	25.1b	63.73	968.475
Vegeclean 15% WG	6.4de	10.0e	11.4e	12.0e	14.3c	79.34	658.275
Evito 48% SC	6.4d	9.1g	10.0g	11.3g	11.9c	82.80	596.325
Check	6.2ef	27.1a	41.4a	53.1a	69.2a	---	2392.275

***Results were recorded 1day before spraying the tested fungicides.**
-Each figure represents the mean of 3 replicates. -Values within the same column having the same letter are not significantly different according to Duncan test (P< 0.05).

The tested fungicides can be arranged according to their efficiency against late blight disease in the following descending order; Evito 48% SC, Ranman 40% SC, Vegeclean 15% WG and then Shirlan 50% SC, Leimay 20% SC and Revus 25% SC. Area under the disease progress curve (AUDPC) was calculated and illustrated in Table (5). The non-sprayed control plots had the highest AUDPC value for disease severity. Efficacy percentages of tested fungicides were highly correlated with AUDPC values.

-Field trial in Sharkia

The percentage of late blight disease severity through natural infection ranged between 3.1 and 3.3% one day before beginning the spraying (Table 6). Although the disease severity percentages before spraying in Sharkia trial were approximately half of those occurred in Fayoum trial but there was harmony in disease severity between all the treatments. Disease severity percentages in check treatment reached to 23.3, 34.6, 48.5 and 61.1% after 14 days of the first, second, third and fourth spray, respectively.

Table 6. Efficiency of tested fungicides against late blight disease on tomato growing under field conditions (Sharkia Governorate).

Tested fungicide	Disease severity (%) after 14 days of each spray					Efficacy	AUDPC
	0*	1st spray	2nd spray	3rd spray	4th spray		
Shirlan 50% SC	3.1c	6.1bc	8.1d	10.7d	11.8d	80.69	487.275
Ranman 40% SC	3.1bc	4.8d	7.1f	8.8f	9.9f	84.80	410.925
Revus 25% SC	3.2ab	6.4b	12.1b	15.4b	22.2b	63.67	702.600
Leimay 20% SC	3.3a	6.3b	8.3c	10.9c	12.2c	80.03	502.050
Vegeclean 15% WG	3.2ab	5.9c	7.2e	9.0e	10.0e	83.63	434.625
Evito 48% SC	3.1bc	4.5d	6.9g	7.2g	8.6g	85.93	369.825
Check	3.3a	23.3a	34.6a	48.5a	61.1a	---	1715.85

***Results were recorded 1day before spraying with the tested fungicides.**
-Each figure represents the mean of 3 replicates.-Values within the same column having the same letter are not significantly different according to Duncan test (P< 0.05).

Generally, all the candidate fungicides gave excellent control of late blight disease, which their efficiency ranged between 80.03 and 85.93% except Revus 25% SC gave 63.67% only. Data in the both field trials confirmed that Revus 25% SC was the least efficiency against late blight disease on tomato. This finding is compatible with its efficiency in *in vitro* studies. Efficiency of Leimay 20% SC and Shirlan fungicides against late blight disease was increased from 63.73 and % 65.61 in Fayoum trial to 80.03 and 80.69% in Sharkia trial, respectively.

Decreasing in the natural infection of late blight disease in Sharkia trial may be due to increase the efficiency of Leimay 20% SC and Shirlan fungicides than those in Fayium trial. The tested fungicides can be arranged according to their efficiency against late blight disease in the following descending order; Evito 48% SC (85.93%), Ranman 40% SC (84.80%), Vegeclean 15% WG (83.63%), Shirlan 50% SC (80.69%), Leimay 20% SC (80.03%) and then Revus 25% SC (63.67%). The non-sprayed control plots had the highest AUDPC value for disease severity. Efficacy percentages of tested fungicides were highly correlated with AUDPC values.

Data from the field trials indicated that all the candidate fungicides when treated as curative were effective in controlling late blight disease except Revus 25% SC. On the other hand, efficiency of tested fungicides against mycelial growth of *P. infestans* were not completely compatible with those in the greenhouse and field trials. These results of the present study agree with earlier finding of Issiakhem and Bouznad (2010) who reported that *in vitro* results do not always reflect what happens in the field. Kalkdijk *et al.* (2007) showed that the timing of the spraying moment is very important. The spraying moment depends primarily on the weather conditions, the disease pressure. Sameer and El-Tawil (2011) reported that the first treatment was the most powerful treatment in reducing the severity of the disease in addition to schedule spraying of the fungicides, 10- day intervals could reduce the incidence of late blight on tomato grown under field conditions.Honda *et al*. (2008) found that amisulbrom concentration on potato leaves in drop water remained at 0.1 ppm or more and was inhibited zoospore release significantly up to 28 days after the application. Amisulbrom quickly penetrated into wax layers of plant leaf, which is a strong reason why the effect of amisulbrom not affected by rainfall and weather. Sakai *et al*. (2010) mentioned that Benthiavalicarb-isopropyl was strongly inhibits mycelia growth, zoosporangia germination and cystospore germination. It is act as inhibitor of phospholipid biosynthesis and cell wall synthesis. The fungicidal and disease-controlling activities of Benthiavalicarb-isopropyl due to its protective and curative action. Latorse *et al*. (2007) reported that Mandipropamid does not have strong translaminar properties. This finding may be explain its low efficiency against late blight disease under field conditions in the current study. Evenhuis *et al*. (2006) found that protectants like Ranman, Shirlan and Curzate showed a reduction in number of lesions after multiple applications. A possible explanation for this observation may be the redistribution of the contact fungicides cyazofamid, fluazinam and mancozeb from lower leaf layers to the developing growing point by splash of rain droplets or vapor activity.

The adaptability of *P. infestans* is impressive. If we wish to grow potato or Tomato in the future we must use the scientific knowledge available in order to control the disease and continue our research to developing effective, environmental friendly late blight specific fungicides, cultivars with durable resistance and reliable forecasting systems. If all research on late blight were aimed at combating the disease and to obtain healthy tomato crops maybe we would be able to subdue *P. infestans* infection. Finally, there should be more support for research oriented toward alternative methods to control tomato and potato late blight. In the present study, it was found that the recent introduction of new chemical groups with different modes of action is highly effective against late blight disease. Development of a management program employing the different groups of fungicides would be a reasonable approach when they are used in appropriate rotations.

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فعالية بعض المبيدات الفطرية ضد الفطر *Phytophthora infestans* المسبب لمرض الندوة المتأخرة في الطماطم معمليا، في الصوبة وفي الحقل.

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تم اختبار فعالية المبيدات: شيرلان ٥٠%, رينمان ٤٠%, ريفاس ٢٥%, ليماي ٢٠%, فجيكلين ١٥% و أفيتو ٤٨% ضد الندوة المتأخرة في الطماطم معمليا, في الصوبة وحقليا. لوحظ فرو واضح في شراسة العزلتين PhK-1 و PhK-2 من الفطر *P. infestans* حيث كانت العزلة PhK-2 الأكثر شراسة والعزلة PhK-1 الأقل شراسة. بينت التركيزات النصفية القاتلة (EC_{50}) للمبيدات المختبرة بأن المبيد شرلان ٥٠%أكثرها فعالية ضد النمو الميسليومي للعزلتين PhK-1 و PhK-2 في حين كان المبيدين ريفاس وريمان أقلها فعالية على النمو الميسليومي للعزلتين PhK-1 و PhK-2 . كانت المبيدات المختبرة فعالة في مكافحة مرض الندوة المتأخرة في الطماطم تحت ظروف الصوبة خاصة المبيدين أفيتو ٣٨% وفجيكلين ١٥% سواء تم تطبيقهما وقائيا أوعلاجيا. تم اختبار المبيدات المذكورة في مكافحة مرض الندوة المتأخرة في الطماطم حقليا في محافظتي الشرقية والفيوم برشها بالتركيزات الموصى بها خلال موسم الزراعة ٢٠١٥-٢٠١٦. في محافظة الشرقية, كل المبيدات المختبرة كانت فعالة في مكافحة مرض الندوة المتأخرة في الطماطم وتراوحت فعاليتها مابين ٨٠.٠٣% و ٨٥.٩٣% ماعدا المبيد ريفاس ٢٥% كانت فعاليته ٦٣.٧٣% فقط. فعالية المبيدين ليماي ٢٥% وشرلان ٥٠% كانت ٦٣.٧٣% و ٦٥ز٦١% في محافظة الفيوم في حين وصلت فعاليتهما إلى ٨٠.٠٣ و ٨٠.٦٩% في محافظة الشرقية. ارتبطت النسبة المئوية لعالية المبيدات المختبرة بشكل كبير بقيمة area under the disease progress curve (AUDPC). كل المبيدات المختبرة فعالة في مكافحة مرض الندوة المتأخرة في الطماطم عند رشها علاجيا عدا المبيد ريفاس ٢٥%.