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مقاله پژوهشی

ارزیابی مقاومت به قارچ‌کش‌ها و واکنش جدایه‌های *Alternaria alternata* سیب زمینی در منطقه اصفهان

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چکیده

بلایت زودرس یا سوختگی سیب زمینی که با گونه‌های مختلف *Alternaria* ایجاد می‌شود، یکی از بیماری‌های مخرب و شایع سیب زمینی در دنیا می‌باشد. در سال‌های اخیر بیماری در مناطق تولید سیب زمینی کشور خصوصا استان اصفهان گسترش پیدا کرده است و کشاورزان غالبا برای کنترل بیماری از قارچ‌کش‌ها استفاده می‌کنند. با توجه به مشکل بروز مقاومت در برخی قارچ‌ها به قارچ‌کش‌ها، این مطالعه با هدف ارزیابی حساسیت/مقاومت نه جدایه *A. alternata* به قارچ‌کش‌های رایج (مانکوزب، کلروتالونیل، ایمین اکتادین تریس و کانسنتو) با استفاده از روش بازدارندگی رشد رویشی ریسه در شرایط آزمایشگاه و گلخانه انجام شد. نتایج نشان می‌دهد که جدایه‌های جمع‌آوری‌شده از مزارع سیب زمینی که به طور مرتب با قارچ‌کش‌هایی مانند مانکوزب سم‌پاشی شده‌اند، دارای کمترین حساسیت به مانکوزب بوده‌اند. بر عکس، در برابر کلروتالونیل در این جدایه‌ها کمترین اختلاف در حساسیت مشاهده گردید. در آزمایش‌های گلخانه‌ای بیشترین کنترل بیماری توسط کلروتالونیل و مانکوزب صورت گرفت. این دو قارچ‌کش در مقدار توصیه‌شده باعث کاهش شدت بیماری به کمتر از ۱۵٪ در یک دوره ۲۰ روزه پس از مایه‌زنی شدند. بر اساس نتایج بدست آمده با ملاحظه سطح مقاومت مشاهده‌شده در شرایط آزمایشگاهی، کاربرد مانکوزب در تناوب با سایر قارچ‌کش‌ها مانند کلروتالونیل و ایمین اکتادین تریس می‌تواند قابل توصیه باشد.

کلیدواژه: مقاومت، بازدارندگی رشد، کلروتالونیل، مانکوزب، ایمین اکتادین تریس

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Research Article

Evaluation of fungicide resistance in *Alternaria alternata* isolates of potato in Isfahan region

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Abstract

Early blight of potato caused by *Alternaria* species is one of the most destructive and prevalent diseases of potato in the world. In recent years, the disease has become important in Iranian potato-producing regions, especially in Isfahan province, and farmers often use fungicides to control the disease. Considering the problem of resistance to some common fungi, this study aimed to evaluate the susceptibility/resistance of nine isolates of *A. alternate* to conventional fungicides (Mancozeb, chlorothalonil, iminoctadine tris, Concento) by using vegetative growth inhibition method under *in vitro* and greenhouse conditions. The results revealed that most isolates collected from potato fields that were regularly treated with fungicides such as Mancozeb exhibited less sensitivity to Mancozeb. In contrast, against chlorothalonil, the least difference was observed in the susceptibility of different isolates. The greenhouse experiment showed that the highest control of the disease could be achieved by chlorothalonil and Mancozeb treatment. These two fungicides at their recommended concentrations reduced the severity of the disease to less than 15 percent for 20 days after inoculation. Based on the results, concerning the resistance levels observed in the laboratory conditions, Mancozeb application might be recommended in alternation with other fungicides such as chlorothalonil and iminoctadine tris.

Keywords: Resistance, Growth inhibition, Chlorothalonil, Mancozeb, Iminoctadine Tris

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Introduction

Early blight disease is one of the oldest known diseases in potato and tomato fields that are widely distributed in the world. It is one of the most important foliar diseases in favorable conditions (Ellis and Martin, 1882). About 38 to 78 percent of tomato and potato yield production in the world is lost each year due to this disease (Shtienberg *et al.*, 1990). The earliest symptom of the disease is the appearance of dark spots and necrotic spots on older leaves, and then the entire plant may be infected throughout the planting season (Sherf and MacNab, 1986). Since the disease usually appears early in the season, the disease is called early blight. The causal organism was isolated in 1882 by Ellis and Martin on potato dried leaves in the US, New Jersey (Ellis and Martin, 1882). The first report of this disease in Iran was made by Ershad in 1977 from Ahwaz, Khuzestan province (Ershad, 2009). The most effective way to control the disease is the application of fungicides along with some non-chemical methods including crop rotation, use of resistant varieties, disposing or covering crop residues, use of non-infected tubers, irrigation management, and harvesting at fully ripe stage.

It is recommended that the initial application of fungicide should be made when the lower leaves of potato plant show the first symptoms of the disease (Patel *et al.*, 2004; Pscheidt and Stevenson, 1988; Shtienberg *et al.*, 1990). Available fungicides belong to different chemical groups. Chlorothalonil and mancozeb were among the first effective fungicides in the control of early blight disease, but in recent years, due to the development of fungicide resistant isolates of the pathogen, they are not generally useful (Pasche *et al.*, 2005; Reuveni and Sheglov, 2002; Sujkowski *et al.*, 1995). Odibekov et al. 2019 found that fungicides affect the population structure of *A. solani* within a cropping season and the sensitivity to azoxystrobin tended to increase with time, thus control strategies should be adjusted to decrease the selection pressure for decreased sensitivity to fungicides. Recently, iminoctadine tris and fenamidone + propamocab-HCl, have also been introduced to control early blight disease in Iran (Nasr Esfahani and Ansari-pour, 2010). Trifenyltin hydroxide and azoxystrobin have also been recommended against the disease. Once introduced, azoxystrobin was effective in controlling the disease; however, similar to mancozeb and chlorothalonil, in the following years, its efficiency was gradually decreased due to the resistance (Barak and Edgington, 1984).

In Iran, many different fungicides have been evaluated against potato early blight in field trials. (Nasr Esfahani and Ansari-pour, 2010). However, no information is available on fungicide susceptibility/resistance in field isolates of *A. alternata*. Considering the importance of early blight disease in potato fields of Isfahan, Iran, the mycelial growth inhibition activity of several fungicides, including chlorothalonil, mancozeb, iminoctadine tris, and fenamidone + propamocab-HCl was determined against different isolates of *A. alternata* in laboratory conditions. In addition, greenhouse tests were also performed to evaluate the effectiveness of fungicides in controlling the disease.

Materials and methods

Sample collection

Twenty potato leaf samples showing early blight symptoms were collected during the growing seasons of 2018/2019 from several potato fields located at Daran, Damaneh, and Chadegan, Isfahan province, Iran (Table 1). The selected fields had a different history in fungicide applications; only two fields have been frequently sprayed with mancozeb. The early blight severity

Table 1. *Alternaria alternata* isolates obtained from potato fields of Isfahan province with early blight symptoms

Source	Latitude, Longitude	Isolate
Fields with no fungicide history		
Chadegan	32°46'06"N, 50°37'43"E	Ch-3
Chadegan	32°46'06"N, 50°37'43"E	Ch-22
Daran	32°59'19"N, 50°26'46"E	d-12
Daran	32°59'19"N, 50°26'46"E	d-21
Chadegan	32°46'06"N, 50°37'43"E	Ch-11
Damaneh	33° 04' 09"N, 50°28'58" E	F.B-2
Damaneh	33° 04' 09"N, 50°28'58" E	F.B-3
Field with fungicide history		
Chadegan	32°46'06"N, 50°37'43"E	Ch.B-17
Chadegan	32°46'06"N, 50°37'43"E	Ch.B-31

Table 2. Rating scale for evaluation of the severity of foliar damage in potato caused by *Alternaria alternata* (Rodriguez *et al.*, 2007)

Rating	Description of symptoms
10	Spots on lower leaves
20	Spots on most of the lower leaves
30	Spots on all lower and some of the middle leaves
40	Clearly developed blight lesions in lower leaves
50	Blight lesions in lower leaves spread to some middle leaves
60	Blight lesions developed in all inferior and most of the middle leaves
70	Blight lesions developed in all lower and middle leaves
80	Blight lesions developed in all lower and middle leaves and spread to upper leaves
100	Total blight (death of the plant)

in these fields was relatively high, with 30 to 60% defoliation. Samples were collected randomly from each field. Leaf samples were placed in plastic bags and were transferred in an icebox to the laboratory.

Isolation of A. alternata and production of single spore isolates

Alternaria isolates were collected from sections of potato leaves with early blight lesions. Leaf sections were excised with a sterile scalpel and washed thoroughly in tap water. Then, the surface was sterilized by soaking in disinfectant solution (10 ml of 0.05% commercial bleach + 35ml ethanol 80% + 55ml of distilled water) for 40 seconds and rinsed twice in sterile distilled water. Leaf sections were placed on sterile filter papers, allowed to dry inside the laminar flow cabinet, and were placed on potato dextrose agar (PDA) medium plates (Abu-El Samen *et al.*, 2016). Plates were incubated at room temperature (25 $^{\circ}$C ± 2) for 6- 8 days. From individual lesions, single conidia of spores were transferred onto potato carrot agar (PCA) plates to obtain pure cultures. Then, the mycelia originated from single spores that were hyphal-tipped and transferred to PCA plates. The identification of the fungal isolates was carried out using the Simmons' key (Simmons, 2007).

Pathogenicity tests

In order to determine the pathogenicity of the isolates, a pathogenicity test was conducted using selected isolate from a field with fungicide application history (Ch.B-17) and another selected isolate from a field without spraying history (FB-2). The pathogenicity test was performed through artificial inoculation of all aerial parts of potato plants (var. Agria), a sensitive cultivar (Personal observations). For each isolate, three pots were considered. The plants were grown under day/night temperature of 25/18 $^{\circ}$C and the light cycle of 13 hours of brightness and 11 hours of darkness. Six to seven-week-old plants were individually sprayed until run-off (approximately 2.0 ml per plant) with a conidial suspension of 3×10^5 spores ml^{-1} of each isolate of *A. alternata*. After inoculation, wetness was maintained on plants for 48 h by covering plants with clear polyethylene bags sprayed inside with distilled water. Control plants were sprayed with sterile distilled water and covered with clear polyethylene bags for 48 h. Disease severity was assessed three weeks after inoculation, using Rodriguez's approach (Table 2) (Rodriguez *et al.*, 2007).

In vitro sensitivity of A. alternata isolates to selected fungicides

Fungicide sensitivity of isolates was evaluated based on the inhibition of mycelial growth. The fungicides used are given in Table 3. The comercially formulated fungicides were dissolved in distilled water to prepare stock solutions of 5000 µg active ingredient (a.i.) of fungicide per ml (µg a.i ml^{-1}). Final concentrations of 0, 0.1, 1.0, 10, 50, 100, 500, 1000, and 2000 µg a.i ml^{-1} medium were prepared by appropriate dilution of the stock solution and were added to autoclaved PDA cooled to 50°C.

Each Petri plate received 20 ml of the amended PDA medium. Control plates (PDA without fungicide) were also prepared in the same manner (Tremblay 2003, Chang et al. 2007). Petri plates containing fungicide as well as control plates were

Table 3. Fungicides used to evaluate the sensitivity of *Alternaria alternata* isolates in laboratory and greenhouse conditions

Common name	Trade name	Chemical group	Recommended Concentration per hectare	Recommended Concentration per liter
Mancozeb	Mancozeb 80%	Dithiocarbamate	2 kg	5 g
Chlorothalonil	Daconil 72%	Chloronitril	2 l	5 ml
Fenamidone + Propamocarb-HCl	Consento 45%	Carbamate	2 l	5 ml
Iminoctadine tris	Belkute 40%	Guanidine	750 g	1.875 g

inoculated with a 5.0 mm diameter mycelial plug cut from the edges of the 6-7 day old actively growing culture of *A. alternata* using a cork borer. Each isolate was treated in three replicates from each of the eight fungicide concentrations. Petri plates inoculated with different isolates were placed in a dark incubator at 23 ± 2 $^{\circ}$C for seven days. After the incubation period, colony diameter (mm) in two directions perpendicular to each other was measured using a caliper. EC50 value, the concentration of fungicide, which inhibits 50% mycelia growth, for each isolate and fungicide combination was calculated using Graph Pad Prism 7 software (Graph pad software incorporation, CA, USA). The growth-inhibitory percent for each fungicidal concentration was calculated based on the following formula (Abu-El Samen et al. 2016):

Percentage of radial mycelial growth inhibition= [1- (diameter of colony on fungicide amended plate/ diameter of colony on control plate)] × 100

EC50 was estimated through inhibitory percent of the fungicide concentration logarithm. The dose curve against the inhibitory percent for each fungal isolate showed a direct relationship between the fungal concentration and inhibitory percent. The Graph pad prism seven software uses the following equation to calculate EC50:

$$Y= 100/[1 + 10(\text{Log EC50-X})]$$

Where Y is the percentage inhibition, and X is the logarithm of the concentration of the fungicide. To measure the resistance factor (RF) of each isolate, the EC50 value of an isolate was divided by the EC50 of the most sensitive isolate.

Evaluation of fungicides effect in greenhouse conditions

To evaluate the effect of fungicides on the disease, a factorial split experiment was conducted in a completely randomized design with three replications in a greenhouse with a temperature of 25 ± 5°C and an optical cycle of 13 hours of light and 11 hours of darkness. Potato cultivar Agria was used as a sensitive cultivar in this experiment. Fungicides were applied 24 h before inoculation of conidial suspension at the 8- to the 10-leaf stage. Each fungicide was tested at three concentrations, recommended, double, and half of the recommended concentration. Potato plants were inoculated with a spore suspension of 10^6 spores Ch.B-17 isolate per milliliter. To understand the effect of fungicides on the control of the disease, sterile distilled water treatments were considered as controls. In order to measure the severity of the disease, the symptoms were evaluated at 3, 6, 9, 12, and 15 days after inoculation, according to Rodriguez's approach (Rodriguez *et al.*, 2007). Two-way analysis of variance means comparisons were analyzed by SAS software version 9.4 (SAS v9.4; SAS Institute, Cary, NC).

Results

Isolates of *A. alternata* were obtained and identified from early blight lesions of potato leaf samples. In total, nine isolates were identified, seven isolates from fields without spraying, and two isolates from mancozeb sprayed fields. The results of the pathogenicity test using two isolates (one from each sprayed and not sprayed fields) showed that both isolates were pathogenic on the potato plants in the greenhouse conditions. The isolate Ch.B-17 from a sprayed field was found more aggressive than the isolate F.B-2 from an unsprayed field and thus was selected to study the effect of fungicides in greenhouse conditions.

The sensitivity of nine isolates to different fungicides is presented in Table 4. There was a significant difference between *A. alternata* isolates in terms of sensitivity to mancozeb. The EC50 values against mancozeb ranged from 9.06 to 82.91 µg ml-1. The isolates Ch.A-22 exhibited the

Table 4. In vitro sensitivity of Alternaria alternata isolates to mancozeb, chlorothalonil, consento, and Immunoctadintris expressed as EC50 values (µg active ingredient ml^{-1}) and resistance factors (RF).

Fungicide	Isolate	EC50 (fiducial limits) µg a.i/ml	Resistance factor
Consento	CH.A-22	121.2 (90.23 to 153.5)	1
	CH.B-31	464.3 (432.2 to 498.4)	3.83 (1.79 to 8.4)
	CH.B-17	464.9 (398.8 to 540.3)	3.84 (1.73 to 7.99)
	D-21	467.2 (429.8 to 507.6)	3.85 (1.92 to 8.2)
	FB-3	466.9 (399.1 to 544.5)	3.85 (1.84 to 7.88)
	FB-2	732.9 (507 to 556.2)	4.38 (2.18 to 9.08)
	CH.M-11	977.2 (840.6 to 1150)	8.06 (3.97 to 16.45)
	D-12	1063 (980.7 to 1156)	8.77 (4.22 to 17.9)
	CH.A-3	1075 (981.2 to 1183)	8.86 (4.43 to 18)
Chlorothalonil	CH.B-31	52.99 (47.5 to 59.09)	1
	FB-3	76.52 (55.64 to 106.6)	1.44 (0.9 to 2.16)
	CH.B-17	76.86 (45.2 to 134.3)	1.45 (0.88 to 2.19)
	CH.A-3	77.1 (44.06 to 15.08)	1.45 (0.76 to 2.8)
	CH.A-22	78.66 (66.26 to 93.64)	1.48 (0.96 to 2.3)
	FB-2	85.71 (51.34 to 148.1)	1.62 (0.98 to 2.43)
	D-21	89.34 (64.31 to 123.9)	1.69 (1.06 to 2.36)
	D-12	117.5 (81.99 to 177.4)	2.22 (1.34 to 3.79)
	CH.M-11	181.3 (104.6 to 388.6)	3.42 (2.06 to 5.73)
Mancozeb	CH.A-22	9.06 (6.88 to 11.75)	1
	D-21	17.17 (12.89 to 22.51)	1.89 (1.06 to 2.66)
	CH.A-3	20.51 (10.32 to 38.39)	2.26 (1.62 to 3.8)
	CH.M-11	21.24 (18.69 to 24.07)	2.34 (1.38 to 3.56)
	FB-3	22.4 (17.99 to 27.62)	2.47 (1.39 to 3.77)
	D-12	30.5 (21.67 to 42.09)	3.37 (1.96 to 4.9)
	FB-2	50.69 (41.24 to 61.77)	5.59 (3.43 to 7.86)
	CH.B-17	63.57 (55.1 to 72.96)	7.02 (4.33 to 9.62)
	CH.B-31	82.91 (66.99 to 102)	9.15 (5.69 to 12.96)
Immunoctadin tris	CH.A-22	0.19 (0.15 to 0.25)	1
	D-12	0.21 (0.15 to 0.29)	1.1 (0.9 to 3.6)
	FB-2	0.28 (0.14 to 0.52)	1.47 (1.4 to 5.26)
	D-21	0.28 (0.22 to 0.36)	1.47 (1.3 to 4.86)
	CH.A-3	0.41 (0.29 to 0.57)	2.16 (1.92 to 7.16)
	CH.B-31	0.52 (0.37 to 0.74)	2.74 (2.61 to 9.96)
	FB-3	0.54 (0.36 to 0.83)	2.84 (2.73 to 10.18)
	CH.B-17	0.65 (0.5 to 0.83)	3.42 (2.9 to 10.92)
	CH.M-11	0.69 (0.51 to 0.97)	3.63 (3.4 to 12.5)

lowest EC50 value (9.06 µg ml^{-1}) and considered as the most sensitive isolate. All tested isolates showed significantly different sensitivity to mancozeb. The EC50 values against chlorothalonil ranged from 52.99 to 181.3 µg ml^{-1}, indicating lower sensitivity of tested isolates to this fungicide. The most chlorothalonil sensitive isolate was Ch.B-31, with an EC50 value of 52.99 µg ml^{-1}.

The estimated EC50 values against Consento (fenamidone + propamocarb-HCl) ranged from 121.2 to 1075.91 µg ml-1 (Table 4). Eight isolates of *A. alternata* exhibited EC50 values that were significantly different from the most sensitive isolate (Ch.A-22; EC50 = 121.2 µg ml-1). The lowest LC50 values were estimated for immunoctadin tris, ranged from 0.19 to 0.69 µg ml^{-1} (Table 4).

Relatively high levels of resistance (RFs ≥ 5) were found only in three isolates against mancozeb and Consento. However, relatively moderate

Table 5. The results of the analysis of variance of the effects of mancozeb, chlorothalonil, immunoctadin tris, and Consento on the control of early blight caused by *Alternaria alternata* in greenhouse conditions at different fungicide concentrations (half, recommended and twice the recommended concentrations)

Source	df	Mean square
Replication	2	31.67^{ns}
Fungicide	3	1856.11**
Concentration	3	9000.55**
fungicide×concentration	9	700.93**
Error	30	40.55
Time	4	3095.42**
time×fungicide	12	63.75**
time×concentration	12	105.42**
time×concentration×fungicide	36	23.38^{ns}
Time×replication	8	44.17^{ns}
Total error	120	16.94
CV=13.31		

ns= not significant differences
**= significant differences at 1% level

degrees of resistance (RFs ≥ 2) were also observed in some isolates against chlorothalonil and immunoctadin tris.

The results of the ANOVA analysis of the effect of fungicide in the greenhouse tests are presented in Table 5. The effects of fungicide, concentration, time, and their interactions were significant (P< 1%). The effects of different fungicides on reducing the severity of disease showed significant differences between fungicide types and concentrations. Chlorothalonil and mancozeb caused the highest reduction in the disease severity at the recommended concentrations (4 L/ha) and were able to control the disease throughout the post-inoculation period. There was no significant difference between different concentrations of Consento and the effect of this fungicide at different times after inoculation. At the same time, immunoctadin tris was more effective in controlling the disease than Consento up to 15 days after inoculation (Fig. 1).

Discussion

Chemical control is an effective tool in the management of potato early blight. Under favorable conditions, without fungicide application, the pathogen can cause economic damage to the crop. The increased severity of early blight in many potato production areas of Isfahan sometimes necessitates fungicide applications. Thus, the primary objective of this study was to determine if *A. alternata* field isolates have developed reduced sensitivity to the currently used fungicides. Five isolates of *A. alternata* from Chadegan, two isolates from Daran, and two isolates from Feriedan were selected, and their fungicide susceptibility was studied using the vegetative growth inhibition method in the laboratory conditions. This method is widely used to estimate the susceptibility of plant pathogens to both protective and systemic fungicides (Al-Mughrabi, 2004; Myresiotis *et al.*, 2008; Pasche et *al.*, 2005; Reuveni and Sheglov, 2002; Shi *et al.*, 2015). Also, the fungicide concentration at which 50% of spore germination can be inhibited was estimated (Fairchild *et al.*, 2013; Holm *et al.*, 2003; Pasche *et al.*, 2005). Most studies have suggested that both methods are comparable in estimating EC50 values. Therefore, researchers have used either one of them or both (Avenot and Michailides, 2007; Myresiotis*et al.*, 2008). Myresiotis *et al.* (2008) compared the vegetative growth inhibition and spore germination inhibition methods in the assessment of *Botrytis cinerea* sensitivity to pyraclostrobin and boscalid. They stated that both methods provide the same results to calculate EC50 values. Comparing these methods showed the superiority of mycelium inhibition (Mostert *et al.*, 2017). The results of our study revealed that there is a significant difference between the isolates of *A. alternata* from potato fields of Isfahan province (Chadegan, Friedadan, and Daran) in terms of sensitivity to most tested fungicides.

The *in vitro* mancozeb sensitivity assay demonstrated significant differences among *A. alternata* isolates. Two isolates of *A. alternata* showed an EC50 value higher than 60 µg ml−1; thus, they were considered as resistant isolates to mancozeb, with 7-9 folds increase in insensitivity compared to the sensitive isolate. Al-Mughrabi (2004) stated that 16% of *A. solani* isolates were highly resistant to mancozeb from potato fields. The highest value of RF found in this study was 9.15, while in other studies (Wang *et al.*, 2010; Wiber and Hann 2011), resistance factors of 100 or higher have been reported. Additionally, the mechanisms of resistance against this group of fungicides have not been revealed at the molecular level. The mode of action of this group of

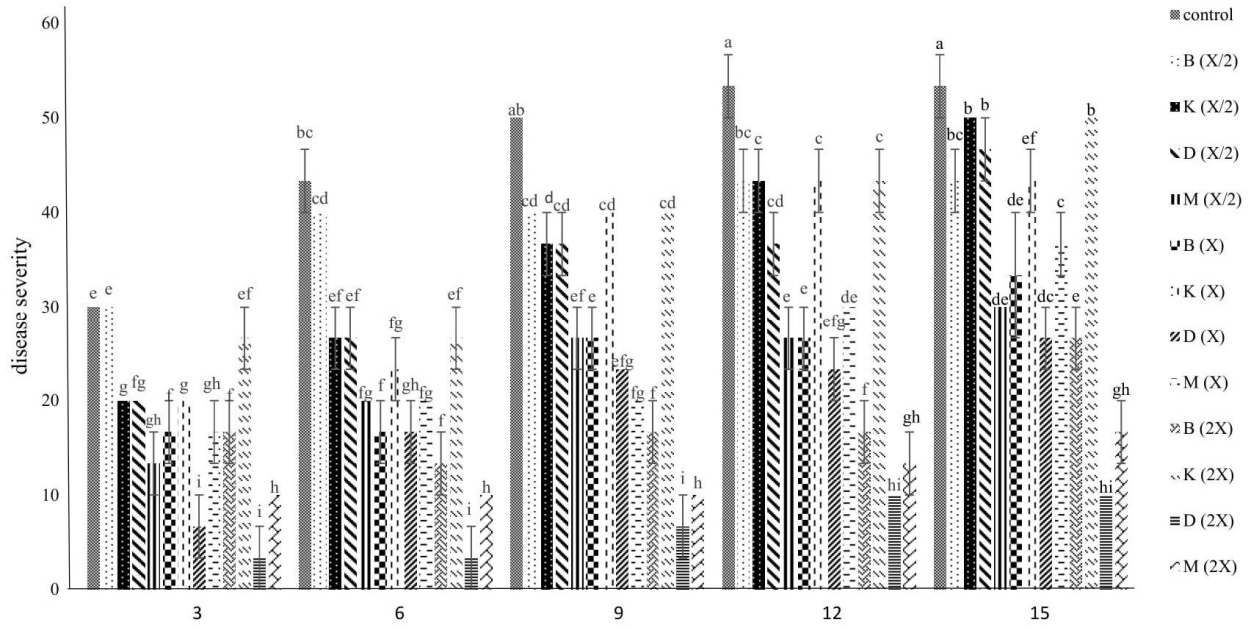

Figure 1. Effect of fungicides (Belkute (B), Consento (K), Daconil (D) and Mancozeb (M)) on controlling the early blight caused by *Alternaria alternata* in greenhouse conditions at different concentrations (half (X/2), recommended (X) and twice the recommended (2X) concentrations), at different times (3,6,9,12 and 15 days) after inoculation.

fungicides has been described as multi-site inhibitors. Consequently, theoretically, for the development of resistance, multiple mutations at several genomic locations have to occur simultaneously or to accumulate over time in order to produce resistance against these fungicides (Staub 1991).

Significant differences in sensitivity to chlorothalonil were detected among tested *A. alternata* isolates. Fifty percent of *A. alternata* isolates exhibited a reduction in sensitivity to chlorothalonil. Four isolates showed sensitivity reduction to chlorothalonil with EC50 values > 78 µg ml−1 with 1.48 to 3.42-fold increase in insensitivity compared to the most sensitive isolate.

Against Consento, there was a significant difference between the isolates, and one-third of isolates showed reduced susceptibility to Consento. EC50 values of these isolates were higher than 970 µg ml-1. Based on our knowledge, there is no report on the resistance to Consento in this fungus.

Chlorothalonil and immunoctadin tris, like mancozeb, have a multiplicity of functions, which may reduce the probability of resistance development in plant pathogenic agents against them. However, several pathogens have shown a reduction in the sensitivity or resistance to chlorothalonil. Helm et al. (2003) reported that isolates collected in two successive growing seasons varied considerably in sensitivity to chlorothalonil. They concluded that the decreased sensitivity was due to the chlorothalonil application and was not heritable.

Based on the results of the greenhouse experiment, fungicide foliar application reduced the severity of the disease significantly compared to the control. The highest disease severity (45%) was observed in control treatment (without spraying), and the lowest severity of disease (8.33%) was recorded with twice the recommended concentration of chlorothalonil. Besides chlorothalonil, mancozeb and Immunoctadin tris showed satisfactory control with disease severity of 15 and 30%, respectively. There is also a need to develop alternative disease control strategies that can be integrated into disease management programs and decrease the dependence on fungicides (Odilbekov et al. 2019). Yaug et al. 2019 concluded that the mode of action alone may not be the only suitable criterion to determine that components to use in the mixture

and/or rotation of fungicides, besides, use of evolutionary principles in closely monitoring populations and the use of suitable fungicide applications are important for effective use of fungicides. Nasr Esfahani and Ansari-pour 2010 and Abu-Al Samen 2016, also reported that chlorothalonil was effective on potato and tomato infected with *A. alternata* and *A. solani* in field and greenhouse conditions, respectively. It could be concluded that resistance to conventional fungicides is widespread among *A. alternata* isolates infecting potato fields of Isfahan, and an appropriate fungicide resistance management using fungicide rotations or mixtures should be considered.

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