

ALTERNARIA LEAF SPOT DISEASE CONTROL ON FABA BEAN IN EGYPT

Magda H. Behairy¹; H.M. Sobhy²; M. S. Abbas²; Kh. A. Abada³ and Medhat Y. Mourad¹

¹ Plant Pathology Research Institute, Agriculture Research Center, Egypt

² Institute of African Research and Studies, Cairo University, Egypt

³ Plant Pathology Department, Faculty of Agriculture, Cairo University, Egypt

* Corresponding author: magda.agriculture@hotmail.com

ABSTRACT

Faba bean (*Vicia fabae*) is considered the most important legume crop in Egypt. The varietal reaction of 16 different cultivars against Aggressive isolate of *Alternaria alternata* causing Alternaria leaf spot disease revealed that Wadi-I cultivar was the most susceptible cv while the other tested cultivars were varied in their susceptibility. The cultivars *i.e* Giza 429, Giza 3, Maser 1 and Sakha 1 were the most susceptible ones while Giza 2, Nobarria 2, Sakha 2 and Nobarria 1 were the most resistant ones. On the other hand the rest cultivars were moderate susceptible. In disease control trials using the biocides (Bio-ARC 6% and Bio-Zeid 2.5%) *Bacillus subtilis*, *Trichoderma harzianum*, *Bacillus megaterium*, *Trichoderma album*, *Bacillus lechniformis* and Ulva algae compared with chemical fungicide (Tridex 80% WP), significantly affected the causal pathogen *in vitro*, greenhouse, and under field conditions. Bio-Zeid product followed by the *B. subtilis* and Bio.ARC; respectively produced the best results and significant reduction of pathogen growth or infection severity. *B. lechniformis*, *T. harzianum* and *Ulva lactuca* were the less bio-agent against the pathogen. At the same time, period progress after using the tested antagonistic materials increased disease severity. Accordingly, disease severity was more aggressive after 7 days.

Keywords: Alternaria leaf spot, chemical fungicide, cultivars, disease control, faba bean.

INTRODUCTION

Faba bean (*Vicia faba* L.) is considered one of the most important legume crops due to its high nutritional value for both energy and as an excellent nitrogen fixer (Sahile *et al.*, 2008). In Egypt, it is grown mainly as food for human consumption. Seeds of faba bean are rich in protein (28%), carbohydrates (56%) and some other compounds thus; it is consider a source of food for both human and animals (Tewati and Virk, 1996).

In addition, faba bean helps to improve the soil fertility through nitrogen fixation. Therefore, improving the production of this crop is one of the main objectives in agriculture in many countries (Boubekeur *et al.*, 2012). Faba bean is belonging to family Fabaceae, which is native to North and Southwest of Africa.

Faba bean plants infected with many fungal pathogens which caused a considerable yield losses (Mahmoud, Nagwa, 1996). Also; it attacked by

many foliar diseases such as; chocolate spot (*Botrytis fabae* and *B. cinerea*), Alternaria leaf spot (*Alternaria alternata*), rust (*Uromyces fabae*), downy mildew (*Peronospora viciae*). As well as, root-rots and viral diseases, which are responsible to cause considerable losses in the yield and its component. In general; chocolate spot disease was the major problem of faba bean plants. However, Alternaria leaf spot disease comes predominant on faba bean during the last years as a consequence of global climate change especially temperature in Egypt (Reis *et al.*, 2007 and Juroszek 2011).

Different control methods including chemical, biological, cross protection, agricultural practices and resistant cultivars have been studied to control *A. alternate*, the causal pathogen of leaf spot disease on faba bean (Hiremath and Sundaresh, 1985). Sometimes, chemical control against this pathogen has offered good results but, improper use of fungicides mostly leads to environmental pollution hazards and resistance of *A. alternata* (Kamble *et al.*, 2000). Resistant cultivars are most significant method for Alternaria blight control (Smita *et al.* 1998; Kopack&Wagner, 2003; Reis *et al.*, 2006 and Dillard & Cobb 2008). Biological control is considered an important approach for controlling many fungal plant pathogens and exploration for new biological agents is increasing as potential biological control antagonists. (Porrás *et al.*, 2008, Deshmukh *et al.*, 2010 and Ryota *et al.*, 2010 Gveroska and Jovancev, (2011). *Trichoderma* spp. is most promising and effective biocontrol agent (Gan *et al.*, 2006 and Boubekeur *et al.*, 2012). Tran, (2010), Moreover, some strains may enhance plant growth. Also; there is data on antagonistic and inhibitory effect of *Bacillus* isolates (*in vitro*) was explained by (Mater *et al.*, 2009). Some biological, biocides products and bioagents compound products (*Ulva lactuca*, Bio-ARC and Bio-Zeid) were used to control Alternaria leaf spot disease caused by *Alternaria alternata* that contain antibiotics, useful plant nutrients and toxic products as ethylene and hydrogen Sulphide (Magda, 2009 and El-Metwally *et al.*, 2010). This work aimed to study faba bean varieties reaction to infection by aggressive isolate of *Alternaria alternata*. Also, to detect, isolate and identify new antagonists from faba bean microorganisms as biological agents with a potential antagonistic effect against *Alternaria alternata* compared with biocide and chemical control.

MATERIALS AND METHODS

The following trials were carried out to determine the best methods for controlling Alternaria leaf spot disease on faba bean under the Egyptian environmental conditions. The main strategy of these implemented experiments *in vitro* and *in vivo* depends on the comparison between the effectiveness of biological and chemical methods to control Alternaria leaf spot disease on faba bean.

I. Laboratory experiment

a) Microorganism

The most aggressive pathogenic fungus of *Alternaria alternata* (Kafr-El Sheikh- isolate), was used throughout this study and isolated from

naturally infected bean plants, showing leaf spot symptoms in Kafr El-Sheikh governorate, Egypt. The isolated fungi were identified on the basis of cultural and microscopic morphological characters .

The bio-control agent (*Bacillus subtilis*), was isolated from faba bean phylloplane. Other bioagents of *Bacillus lechniformis*, *B. subtilis*, *B. megaterium*, *Trichoderma harzianum*, *T. album*, *Ulva lactuca* (green algae as dried powder) and biocides (Bio-Zeid and Bio-ARC) were obtained from Biological Resource Center, Plant Pathology Research Institute, Agriculture Research Center, Egypt.

b) Effect of different biological antagonists on *Alternaria alternata* growth

The virulent isolate of *Alternaria alternata* (Kafr El-Sheikh isolate) was grown on PDA medium as an equal disc (5mm in diameter) was plated onto 9 cm PDA plates with different antagonistic bioagents *Bacillus lechniformis*, *B. subtilis*, *B. megaterium*, *Trichoderma harzianum*, *T. album*. One cm from the periphery in opposite to another for *Ulva lactuca* (green algae powder) and 5mm discs for the fungus and individual streak for bacteria. The control plates were inoculated individually only with the pathogen *Alternaria alternata*. All the plates were incubated at 30°C until the growth completely covered the control plate (after 10 days), three plates were used as replicates for each treatment, then the linear growth of *Alternaria alternata* in the tested bio-control agents was measured to compare the tested bio-control agents with control by the following equation.

Reduction % = $C - T \times 100 / C$ where;

C = the growth of *A. alternata* in control treatment.

T = the growth of *A. alternata* in each treatment.

c) Varietal reaction test

Sixteen varieties (Maser-3, Maser-2, Maser-1, Nobaria-1, Nobaria2, Nobaria-3, Wadi-1, Giza-2, Giza-3, Giza-429, Giza-716, Giza-843, Sakha1, Sakha-2, Sakha-3 and Sakha-4) obtained from Legume Crops Research Depart., Agricultural Research Centre, at Giza, Egypt were tested in to evaluate their reaction against *Alternaria* leaf spot disease caused by *Alternaria alternata*. The method followed by Dereje, (2000) and Marwa, (2005) was applied and faba bean plant leaflets were obtained from the fifth node of 6-8 weeks old. The leaflets were incubated horizontally in polyethylene boxes on sponge grid overlaid with water soaked filter paper. One droplet of the spore suspensions (10^6 spore/ml) was placed on each of the leaflets (10^6 spore/ml) proper ten μ l of suspension were placed near the midrib. The trial were covered with polyethylene sheets to maintain high moisture and incubated at 30°C. Four replicates were used in test for each cultivar.

II. Greenhouse experiment

Effect of bioagents, biocide and chemical fungicide on infection with *Alternaria alternata*

Two biocides, three bioagents microorganisms and one chemical fungicide were tested to control *Alternaria* leaf spot on faba bean disease

caused by *Alternaria alternata* under greenhouse condition. Faba bean plants of Giza-40 cv. was grown in pots (15cm), each treatment was replicated in three pots under greenhouse conditions at 27- 30°C. After 45 days plants were sprayed individually with the suspension of each control treatment, one day before artificial inoculation with *Alternaria alternata* spore suspension (10^6 spore/ml) according to Marwa, (2005). The Biocides (Bio-Zeid and Bio-ARC) with active ingredients of *Trichoderma album* and *B. megaterium*, applied at rate of 250g per 100 liter of water as stated by B.R.C.S manufacturer (Biological Resource Center Standard), Agricultural Research Center A.R.C (El-Metwally *et al.*, 2010). Each of *Bacillus lechniformis*, *B. subtilis*, *B. megaterium* were applied at concentration of 10^8 cfu and 6×10^6 spore/ml for *Trichoderma album* and *Trichoderma harzianum* microorganisms and *Ulva lactuca* (green algae powder) (Ghoneimy *et al.*, 2011). Tridex fungicide, active ingredient Mancozeb (80% WP); rate of application per 100 liters of water 250g for Tridex and *Ulva lactuca*. Control plants were sprayed with sterilized distilled water followed by *Alternaria alternata* only. All pots kept in polyethylene bags in moist chamber of 27- 30°C for one day. Disease severity was recorded 2, 3 and 7 days after treatment. Data of disease severity were recorded according to the scale applied by Abou Zeid (1978).

Effect of biocides (Bio-ARC and Bio-Zeid) on infection with *Alternaria alternata*

Sixteen Cultivars named Maser-3, Maser-2, Maser-1, Noubaria-Noubaria- 2, Noubaria-3, Wadi-1, Giza-2, Giza-3, Giza-429, Giza-716, Giza-843, Sakha1, Sakha-2, Sakha-3, and Sakha-4 were inoculated with the pathogen after 24 hrs from sprayed by the two biocides. Disease severity values were determined after 14 days from inoculation.

III. Field experiment

This experiment was carried out under field condition at (Kafer El-Sheikh). Faba bean seeds (Giza- 429) were sown on first week of November during season 2011/2012. The fungicide (Tridex 80% WP) were sprayed 4 times started at the mid January (flowering stage), 15 days intervals (Teshome *et al.*, 2013). On the other hand, the bio-agent (Bio-Zeid 2.5%, Bio-ARC 6%), *T. harzianum* and *B. lechniformis* were sprayed on faba bean plants 4 times beginning at the flowering stage (mid January), 15 day intervals.

RESULTS

I. Laboratory experiment

a) Microorganisms isolation

Alternaria alternata (Kafr- El Sheikh- isolate), was used throughout this study and isolated from naturally infected bean plants, showing leaf spot symptoms in Kafr El-Sheikh governorate, Egypt. The isolated fungi were identified on the basis of cultural and microscopic morphological characters Also; the bio-control agent (*Bacillus subtilis*), was isolated from faba bean phylloplane.

b) Effect of different biological antagonists on *Alternaria alternata* growth

Data in Table (1) were collected after 10 days from incubation of the inoculated plats with the antagonistic bio-microorganisms and the pathogen (*A. alternata*). Control was inoculated with the pathogen only. All the tested bio-microorganisms showed clear significant effect compared with control treatment. Reduction percentage was differed from bio-agent to another. The two tested *Trichoderma* (*T. album* and *T. harzianum*) gave the best effect against *A. alternata* and decreased the linear growth from 8.9 to 3.3 and 4.2cm, respectively.

Table 1: Effect of different antagonists on linear growth of *Alternaria alternata* on PDA plates.

Bioagents	linear growth (cm) after 10 days	Reduction %
<i>B. subtilis</i>	4.4	50.56
<i>B. megaterium</i>	5.4	39.32
<i>Trichoderma album</i>	3.3	62.92
<i>B. lechniforms</i>	5.8	35.5
<i>T. harzianum</i>	4.2	53.3
<i>Ulva lactuca</i>	6.5	27.7
Control	8.9	0
L.S.D 0.05%:	1.03	

Data in Table (1) showed that effect of antagonistic bacteria (*Bacillus subtilis*, *B. megaterium* and *B. lechniforms*) ranked the second position after *Trichoderma* spp antagonists against *A. alternata*. It caused gradual reduction in pathogen linear growth percentage *i.e.* 50.56, 39.32 and 35.5%; respectively. The algae named *Ulva* produced the lowest effect, it decrease the linear growth from 8.9cm to 6.5cm with reduction percentage of 27.7%.

c) Cultivar Reaction:

Faba bean susceptibility to leaf spot disease caused by *A. alternata* was tested by detached leaves technique. Sixteen cultivars were inoculated with *A. alternata* spore suspension on detached leaves. Data obtained were recorded on interval of 3, 5 and 9 days.

Results in Fig (1) revealed that all tested faba bean cultivars were found susceptible to *Alternaria* leaf spot disease while disease severity was in variable degrees in these tested cultivars. Also, prolonging incubation period increased disease severity. The most susceptible cvs are ranked as Giza 429, Wadi 1, Giza 3, Sakha3, Giza 843, Maser 1 and Sakha 1 while; Giza 2, Nobaria 2, and Sakha 2 while Nobaria 1 was the most resistant cv. The other cultivars were moderately susceptible.

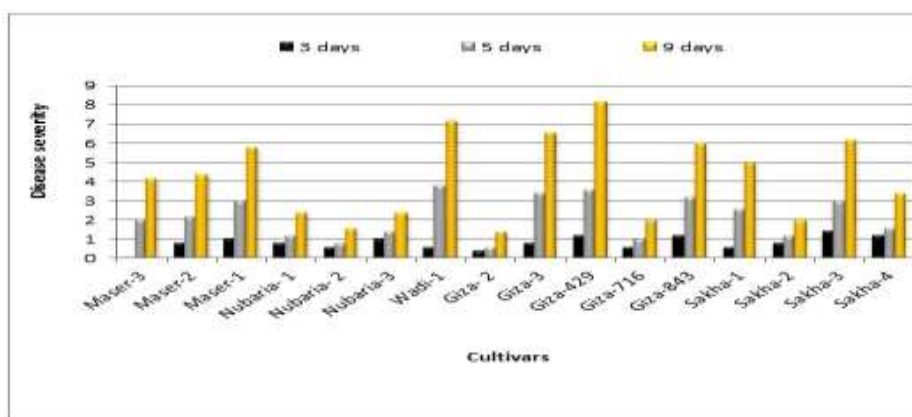


Fig.1: Disease severity of *Alternaria alternata* (Kafr El- Sheikh isolate) on sixteen faba bean cultivars using detached leaves technique scale (0-9)

II. Greenhouse experiment

a) Effect of bioagents, biocide and chemical fungicide on infection with *Alternaria alternata*

Data in Table (2) illustrated that all tested materials showed significantly differences compared with control. Chemical fungicide (Tridex 80% WP) produced the best effect in disease control after three disease severity assessment periods more than all other the tested treatments. Bio-Zeid product was the best biocide followed by *Bacillus subtilis* and Bio-ARC, respectively. *B. lechnifom* and *Ulva* were the less effect bioagents in against *A. alternata*. *Ulva* produced the lowest effect in this respect. Also, it was observed in obtained data that the greatest disease severity was resulted after 7 days for all tested materials. On the other hand, the least values of disease severity were obtained after two days.

Table.2: Effect of fungicide, two biocides, and bioagents on faba bean leaf spot disease compared with Tridex under greenhouse conditions at Giza

Treatments	Disease severity % after (days)			
	2	3	7	Mean
<i>B. lechniformis</i>	15	17	17	16.3
<i>B. subtilis</i>	9	10	10	9.6
Bio-ARC 6%	11	13	13	12.3
<i>T. harazianum</i>	18	20	19	19
Bio-Zeid 2.5%	9	9	9	9
<i>Ulva lactuca</i>	28	30	29.6	29.2
Tridex-80 %	7	7	6.6	6.8
Control	80	80	90	83.3
Mean	22.1	23.2	24.2	
L.S.D. 0.05% = Bio-agent : 2.9 = Period : 1.9 = Interaction : 5.1				

b) Evaluation of biocides Bio-ARC and Bio-Zeid against *A.alternata*

This experiment was carried out to evaluate the efficiency of the two biocides *i.e.* Bio-ARC and Bio-zeid. Sixteen cultivars were inoculated with the pathogen after the 2 biocides were sprayed before inoculation. Table (3) showed disease severity values recorded after 14 days. Results obtained indicated the effect of two biocides that produced significant differences after 14 days of inoculation compared with control treatment.

As observed before in the other trails of previous trend. Also, current results showed that progress of inoculation periods affected disease severity significantly. On the other hand, reaction of tested cultivars played an important role on disease control. Bio-ARC and Bio-Zeid proved their control efficiency compared with control treatment. Also, Bio-ARC and Bio-ARC showed non-significant differences in leaf spot disease control. On the other hand, the cultivar reaction of the tested ones was clear. Giza 2 was the less susceptible one under the prevailed environmental conditions in this trial while Wadi-1 showed the higher susceptibility determined as disease severity with both tested biocides. The rest cultivars showed various degrees of susceptibility and resistance.

Table.3: Evaluation of two biocides against *Alternaria alternata* pathogen of faba bean leaf spot disease

Cultivers	Treatments				
	Bio-ARC		Bio-Zeid		
	Disease severity	Efficiency %	Disease severity	Efficiency %	
Maser -3	38.8	33.9	24.2	58.8	58.7
Maser -2	41	36.9	47.3	27.2	65
Maser -1	47	31.2	52.5	23.1	68.3
Nubaria -1	31.5	45.2	42	26.9	57.5
Nubaria -2	26.3	44.04	35.5	24.5	47
Nubaria -3	35.5	32.3	46	12.2	52.4
Wadi -1	35	60.04	41	53.2	87.6
Giza -2	21	16.7	25.2	0.0	25.2
Giza -3	50	29.6	48	32.4	71
Giza -459	52.3	29.8	50.2	32.6	74.5
Giza -716	27.2	43.3	31.5	34.4	48
Giza -843	50.2	27.6	34.5	50.2	69.3
Sakha -1	43	34.8	36.7	44.4	66
Sakha -2	28.3	42.6	42	14.8	49.3
Sakha -3	51.2	30.2	48	34.5	73.3
Sakha -4	37.7	37.2	40	33.3	60
L.S.D. 0.05	2.57	--	2.05	--	2.53

4. Field trial:

Two biocides (Bio-ARC and Bio-Zeid) and bio-agents *Bacillus subtilis* and *T. harzianum* compared with one fungicide, related to Mancozeb (Tridex 80% WP) was used in this experiment under Kafr El-Sheikh governorate

conditions. Statistical analysis of data is shown in Table (4). Recorded results showed that effectiveness of the tested fungicide was more pronounced than bio-agents. Bio-Zeid was more effective than Bio-ARC but there were non-significant differences between them, followed by *B. subtilis* and *T. harzianum*. At the same time, there were no differences between the two tested bio-agents *B. subtilis* and *T. harzianum*.

Table.4: Effect of bioagents,two biocides comparing with one fungicide against *A. alternata* under field conditions, Kafer El-Sheikh governorate

Treatments	Disease severity	Efficiency%
<i>T. harzianum</i>	15.7	77
<i>Bacillus subtilis</i>	15.7	77
Tridex 80% WP	8.6	87.4
Bio-ARC 6%	11.6	83
Bio-Zied 2.5 %	10.8	84
Control	68.3	
L.S.D 0.05:	4.2	

DISCUSSION

Utilizing of some bio-microorganisms and biocides under greenhouse and field conditions compared with fungicide related to Mancozeb group revealed that fungicides is still had the superiority in controlling *A. alternata* the causal pathogen of leaf spot disease on faba bean (Hiremath and Sundaresh, 1985), and (Kamble *et al.*, 2000).

In laboratory, *T. harzianum* was more effective than *B. subtilis* due to its antagonistic effect against *A. alternata*. These results may be attributed to the reactions among the tested biocides products and bio-agent materials and the host pathogen relationship. These data were in harmony with Tran, (2010) who reported that certain strains of *Trichoderma spp* can induce systemic and localized resistance to several plant pathogens. Moreover, some strains may enhance plant growth and development. Also, data of antagonistic and inhibitory effect of *Bacillus* isolates (*in vitro*) was explained by Mater *et al.*, (2009). These data were in agreement with Gveroska and Jovancev, (2011) who found that biological control is an efficient, in contemporary way of protection to achieve a healthy and clean environment. They, also reported that *Trichoderma harzianum* is strong antagonist for control of brown spot disease on tobacco caused by *Alternaria alternata*. Also, El-Afifi, (2003) indicated that *Trichoderma spp* were the most effective than *Gliocladium spp* in controlling *Botrytis fabae* while, *Bacillus* isolates showed the highest value of relative power than the isolate of *Pseudomonas fluorescence*.

The active bio-agents tested in this trial were reacted with the causal pathogen through their biocidal uses while they were affected host plants through their analytical enzymes and excerpts materials. These data were accepted with Sahile *et al.*, (2011) and Babu *et al.*, (2000) who found that *Bacillus* and *Pantoea* had strong antifungal activity both in *in vitro* as well as *in vivo* conditions, Time progress caused increase of disease severity due to

the destine of the biocides material yielded by these tested bio-agents. *Ulva lactuca* decreased disease severity may refer to its products such as Hydroge Salphyade (toxic gas) or Mithane (Magda, 2009). On the contrary, Zbakh *et al.*, (2012) found that marine macro algae are the most interesting algae group because of their broad spectrum of biological activities such as antimicrobial (antifungal and antibacterial).

The chemical fungicide named Tridex 80% was the more effective among the tested materials. This was referred to its fast and chemical multisided action against the pathogen cells components. These conclusions may be attributed to the mode of action of Zinc and Manganese in mancozeb which had effect on poison action against the fungal cell (Wadiphasme *et al.* 1994). On the other hand, the mode of action of the tested bio microorganisms compared with biocide was referred to their secretions which concluded antibiotics, useful plant nutrients. These data were harmony with Sahile *et al.*, (2011) who stated that chocolate spot disease (*Botrytis fabae* Sard) is one of most yield limiting constraints of faba bean (*Vicia faba*) and there is promise in using biological control agents to control chocolate spot diseases.

Disease severity caused by *A. alternata* on sixteen faba bean cultivars was varied from cultivar to another due to their changes in morphological and anatomical characters such as cuticle layer, leaf thin, leaf secretion and chemical components. Similar results were obtained by Smita *et al.*, (1998) who evaluated ten varieties, lines of sunflower against leaf bright caused by *A. alternata* in pots experiments and they showed different degrees of susceptibility.

At the same time increasing of incubation period increased disease severity, may be due to increase of the toxin of secreted by the causal pathogen in the plant cell These data were harmony with Maskova *et al.*, (2012) who studied that *Alternaria* species produce more than 70 secondary metabolites which are toxic to plants.

The results showed the similar effect of the two tested biocides while they were varied significantly with control treatment. This similarity and non-significant results in both of them may be attributed to the prevailing conditions such as meteorological conditions, percentage of disease incidence, virulence of the used isolate to carry out inoculation and reaction of tested cultivars to evaluate the efficiency of some biological biocides under greenhouse conditions Bio-ARC and Bio-Zeid products (El-Metwally *et al.*, 2010). On the other hand, disease severity was varied on the used cultivars of faba bean due to their morphological and anatomical characters, their chemical components. As general trend, increasing inoculation periods led to increase disease severity.

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مكافحة التبقع الألترنارى على الفول فى مصر

ماجدة حسن على بحيرى¹، حسن محمد صبحي ، محمد سعيد عباس² ،

خيرى عبد المقصود عباده³ و مدحت يوسف مراد¹

1- وحدة تعريف الكائنات الدقيقة - معهد بحوث امراض النبات - مركز البحوث الزراعية - مصر

2- قسم الموارد الطبيعية - معهد البحوث والدراسات الإفريقية - جامعة القاهرة - مصر

3- قسم امراض النبات - كلية الزراعة جامعة القاهرة - مصر

يعتبر الفول من أهم المحاصيل البقولية فى مصر. وقد تم دراسة تفاعل أصناف ضد مرض التبقع الألترنارى باستخدام العزلة القوية من الفطر *Alternaria alternate* وقد وجد أن الصنف وادى 1 كان أكثر الأصناف حساسية أما بقية الأصناف فقد اختلفت فى درجة حساسيتها. وتعتبر الأصناف جيزة 429 وادى 1- جيزة 3- مصر 1 وسخا 1 من الأصناف الحساسة للتبقع الألترنارى بينما كانت الأصناف جيزة 2 نوبارية 2 سخا2 و نوبارية 1 من أكثر الأصناف مقاومة فى حين كانت بقية الأصناف متوسطة القابلية للإصابة .

وقد تم دراسة المقاومة لمرض التبقع الألترنارى باستخدام المبيدات الحيوية (بيوأرك -بيوزيد (وبعض العزلات الفعالة ، *Bacillus subtilis*, *Trichoderma harzianum*, *Bacillus megaterium*, *Bacillus lechniforms* و ألفا بالإضافة إلى المبيد الفطرى (Tridex 80% wp) والتي قد أثرت معنويا على المسبب المرضى تحت ظروف المعمل والصوبة والحقل. وقد أعطى إستخدام المبيد الحيوى بيوزيد أفضل معاملة يليه كلا من *Bacillus subtilis* والبيوأرك أفضل نتيجة على التوالي. وعلى الجانب الآخر أعطت *Trichoderma harzianum*, *Bacillus lechniforms* والألفا أقل نتيجة كعوامل مقاومة حيوية ضد المرض المختبر. زادت فترة التحضين بعد المعاملة بالعوامل السابقة من شدة الإصابة بالمرض والتي سجلت أعلى معدل لها بعد سبعة أيام. الكلمات الدالة: التبقع الألترنارى، المبيدات الفطرية ، الأصناف ، مقاومة المرض، الفول.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة

مركز البحوث الزراعية

أ.د/ محمد السيد عبدالله

أ.د/ عبد الفتاح الوكيل

