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EFFICACY OF SOME RESISTANCE INDUCERS IN CONTROLLING CUMIN FUSARIUM WILT DISEASE

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Abstract: The present study was undertaken to evaluate the effect of some resistance inducers in controlling cumin wilt disease caused by *Fusarium oxysporum* f. sp. *cumini*. In *vitro*, three resistance inducers (salicylic acid, ascorbic acid and catechol) at ten concentrations (from 1 up to 10 mM) and two isolates of the pathogen were used in this experiment. All tested resistance inducers reduced the mycelial growth of the tested isolates at the higher concentrations. Salicylic acid gave the higher inhibition effect on the linear growth of the pathogen, while ascorbic acid exhibited the lowest effect.

Under greenhouse conditions, application of the resistance inducers at 1 and 2 mM with two isolates of the pathogen gave significant reduction in disease infection percentage. Cumin seeds treatment by salicylic acid and ascorbic acid at 2 mM concentration for 3 minutes before sowing proved to be very effective against the pathogen. Meanwhile, salicylic acid exhibited the highest effect in controlling the disease.

Key wards: induced resistance, cumin wilt, salicylic acid, ascorbic acid, and catechol.

Introduction

Cumin (*Cuminum cyminum* L.) is one of the most important condiments consumed in Egypt; it has a great importance in the pharmaceutical industry and food uses. Occurrence of fungal diseases on cumin is the principle factor limiting the production potential of this crop (Dange, 1995). Losses due to wilt alone may reach 40 % (Lodha *et al.*, 1986; Agnihotri, 1991; Dange *et al.*, 1992 and Mandavia *et al.*, 2000). Cumin is severely damaged by Fusarium wilt which is caused by the soil and seed borne fungus *Fusarium oxysporum* f. sp. *cumini* (Champawat, 1986; Prabha-Purohit and Purohit, 1999 and Ghasolia and Jain, 2004). The management of Fusarium wilt is difficult due to its soil-borne nature.

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The fungus is present in most of the soil types. In addition, infested soil with the pathogen may not be replanted with cumin for at least 10 years. In recent years, induced resistance has been extensively used for plant promotion and disease control. Induction of systemic resistance is a new approach in plant disease control (Kuc, 1987). Application of salicylic acid (SA) has been long known to induce pathogenesis-related-proteins (PRproteins) gene expression and acquired resistance against a variety of microbial pathogens (Word *et al.*, 1991 and Meena *et al.*, 2001). Catechol and salicylic acid were significantly inhibited fungal spore germination and mycelial growth of *F. oxysporum* f. sp. *cumini* (Mandavia *et al.*, 2000). Also, ascorbic acid caused protection against Fusarial diseases (Galal and Abdou, 1996).

The present study aimed to evaluate the efficacy of salicylic acid, ascorbic acid and catechol applied as seed treatments for controlling damping-off and wilt disease of cumin under greenhouse conditions.

Materials and Methods

Source of pathogen isolates and pathogenicity tests:

Two isolates of *Fusarium oxysporum* f. sp. *cumini*, used in the current study were isolated from naturally infected cumin plants, showing typical symptoms of Fusarium wilt and collected from different localities of Assiut Governorate. Identification procedures of the selected isolates were carried out, according to their mycelia and spores characteristics (Booth, 1971) then confirmed at Assiut University Mycological Center (AUMC). The fungus was identified as *Fusarium oxysporum* f. sp. *cumini.* The pathogenicity tests of the isolates were carried out under greenhouse conditions using Baladi cumin cv. Pots (25 cm) and soil were sterilized by autoclaving. Soil infestation by the pathogen isolates was done at rate 25 CFU/gm soil 7 days before sowing. Uninfested pots were used for control. Inocula were prepared by growing the tested isolates in 250 ml conical flasks each containing 100 ml of Czapek's liquid medium was inoculated with the fungus. The flasks were incubated at 25°C for 15 days. Resulting mycelial growth of fungus was decanted, washed with distilled water, suspended in 100 ml of distilled water and blended for 5 minutes using a warring Blender. 10 seeds were sown per pot and three pots were as replicate. After 20, 40 and 80 days from sowing pre-, post damping-off and wilt % were recorded.

Effect of resistance inducers on mycelial growth of *Fusarium oxysporum* f. sp. *cumini* in *vitro*:

The three resistance inducers (salicylic acid, ascorbic acid and catechol) in Czapek's agar medium

and the fungus isolates No. 1 and No. 19 were used in this study. Resistance inducers were added to the medium and mixed thoroughly before solidification at 50–60°C. The concentrations for each tested compound were 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mM. Petri dishes (9 cm in diameter) containing the medium were inoculated in the center with disks (6 m) of the fungus mycelium (7 day-old). Four replicates were used for each treatment. Untreated medium was used as control. Inoculated plates were incubated at 25°C, growth diameter was recorded when the control plates were completely covered by the fungal mycelium (Mandavia *et al.*, 2000 and El-Ganaieny *et al.*, 2002). Growth inhibition % was calculated according the following formula:

$$\text{Growth inhibition\%} = \frac{\text{Growth in control–growth in treatment}}{\text{Growth in control}} \times 100$$

Effect of soaking cumin seeds in resistance inducers on damping-off and wilt disease of cumin, under greenhouse conditions:

These resistance inducers (salicylic acid, ascorbic acid and catechol) were tested for controlling damping-off and cumin wilt disease under greenhouse conditions in the two successive seasons 2005/2006 and 2006/2007. For soil infestation, fungal suspensions were added to 25 cm pots filled with steam sterilized sandly loamy soil to give 25 CFU/gm soil, 7 days before sowing date. Surface-sterilized cumin seeds (Baladi cumin cultivar) were soaked in the tested resistance inducers concentrations (1 and 2 mM) for 3 minutes before sowing. Seeds were left to dry then sown in soil infested with the tested isolates of the fungus. Ten seeds were sown in each pot and three pots were used as replicates for each treatment. Untreated seeds were sown in infested pots as control. Also, three pots for each treatment contained non-infested soil planted with treated seeds were used as control. Percentage of pre, post–emergence damping–off and wilted plants were recorded after 20. 40 and 80 days respectively from sowing date.

Statistical analysis:

The obtained data were subjected to statistical analysis using MSTAT C software (Michigan Statistical Program Version C). Least significant difference (L.S.D.) value at 0.05 for comparison between means of treatments was used (Gomez and Gomez, 1984).

Results

1- Pathogenicity tests:

Data in Table (1) indicated that all isolates of *Fusarium oxysporum* f. sp. *cumini* were able to infect cumin plants and caused typical symptoms of Fusarium wilt, reduced plant height and showed foliar symptoms, with different degree of disease severity causing

Fusarium wilt disease. In general, isolates No. 1 and 19 caused the highest percentage of infection and reduced plant height compared to the control (32.22 and 31.11% respectively), fallowed by isolates No. 2, 3, 6, 7, 8, 9 and 15. However, isolates No. 4, 5, 10, 11, 12, 13, 14, 16, 17 and 18 gave the lowest infection percentage of cumin.

Data also indicated that isolates No. 1, 2, 7, 8 and 9 caused the highest percentage of infection and reduced plant height compared to the control in pre-emergence damping-off, while isolates No. 10, 11, 12, 14, 15, 17 and 18 gave the lowest infection percentage of cumin pre emergence damping-off. Data also showed that isolates No. 1, 2, 4, 6, 8, 9, 15, 17, 18 and 19 caused the highest percentage of infection in post emergence damping-off. While, isolates No. 4, 6, 8, 9, 15 and 16 gave the lowest percentage of infection in post emergence damping-off. Isolates No. 1, 3, 6, 11, 12, 15 and 19 gave the highest percentage of wilt. While, the other isolates gave the lowest percentage of infection.

Table (1): Pathogenicity tests of *Fusarium oxysporum* f. sp. *cumini* isolates on Baladi cumin variety under greenhouse conditions in 2004/05 season.

Isolates No.	Damping -off %		Wilt %	Mean
	Pre-emergence	Post-emergence		
1	40.00	23.33	33.33	32.22
2	33.33	30.00	10.00	24.44
3	20.00	16.67	23.33	20.00
4	13.33	26.67	13.33	17.78
5	30.00	10.00	20.00	20.00
6	20.00	23.33	23.33	22.22
7	33.33	6.67	16.67	18.89
8	36.67	23.33	16.67	25.56
9	16.67	30.00	16.67	21.11
10	10.00	16.67	16.67	14.45
11	6.67	6.67	20.00	11.11
12	3.33	16.67	20.00	13.33
13	20.00	13.33	10.00	14.44
14	10.00	16.67	16.67	14.45
15	3.33	30.00	23.33	18.89
16	20.00	20.00	10.00	16.67
17	10.00	30.00	10.00	16.67
18	10.00	23.33	16.67	16.67
19	33.33	20.00	40.00	31.11
Control	3.33	3.33	6.67	4.44
Mean	18.67	19.33	18.17	18.72

L.S.D. at 5 % for: Isolates (A) = 11.50
Infection (B) = 4.45 Interaction (AxB) = 19.92

Table (2): In *vitro*, effect of some resistance inducers on growth reduction % of *Fusarium oxysporum* f. sp. *cumini*.

Conc.(mM) / Resistance inducers	Growth reduction %											Mean
	0	1	2	3	4	5	6	7	8	9	10	
Salicylic acid (Iso. No. 1)	0.00	38.52	43.70	50.37	55.18	61.48	71.85	80.37	88.15	92.96	100	62.05
Salicylic acid (Iso. No.19)	0.00	41.48	44.44	51.48	55.18	62.22	72.59	81.85	87.41	91.85	100	62.59
Ascorbic acid (Iso. No. 1)	0.00	2.96	5.19	9.63	12.59	15.93	18.85	20.37	22.59	23.70	27.04	14.44
Ascorbic acid(Iso. No.19)	0.00	5.19	8.52	11.11	14.07	16.63	18.52	20.37	24.07	26.30	27.78	15.69
Catechol (Iso. No. 1)	0.00	21.85	34.56	45.18	55.19	60.74	71.85	79.26	84.94	100	100	59.42
Catechol (Iso. No. 19)	0.00	19.88	36.05	41.36	54.07	63.70	70.37	77.41	82.96	100	100	58.71
Mean	0.00	21.65	28.74	34.85	41.05	46.78	54.01	59.94	65.02	72.47	75.80	45.48

L.S.D. at 5% for:
Inducers (A) = 0.37
Concentration (B) = 0.39
Interaction (AxB) = 0.95

2- Effect of some resistance inducers on the linear growth of *Fusarium oxysporum* f. sp. *cumini*. in *vitro*:

Data presented in Table (2) indicated that all tested resistance inducers reduced the mycelial growth of the tested isolates. The increase in inducers concentration resulted in an obvious decrease in the mycelial growth of the pathogen. Salicylic acid caused higher reduction % of growth of the tested isolates of the pathogen than the other tested resistance inducers, followed by catechol then ascorbic acid.

3- Effect of seeds soaking in different concentrations of resistance inducers on controlling damping-off disease, under greenhouse conditions:

Data presented in Tables (3 and 4) showed that all tested treatments with resistance inducers signify-cantly reduced the percentage of damping-off disease compared to the control in both tested seasons.

Table(3): Effect of soaking cumin seeds in different concentrations of resistance inducers on controlling damping-off disease in cumin in 2005/06 season.

Isolates No. / Inducers	Damping-off %					
	Pre-emergence %			Post-emergence %		
	Iso. No.1	Iso. No.19	Mean	Iso. No.1	Iso. No.19	Mean
Salicylic acid (1 mM)	10.00	6.67	8.33	16.67	6.67	11.67
Salicylic acid (2 mM)	13.33	13.33	13.33	13.33	13.33	13.33
Ascorbic acid (1 mM)	3.33	23.33	18.33	10.00	10.00	10.00
Ascorbic acid (2 mM)	10.00	20.00	15.00	10.00	13.33	11.67
Catechol (1 mM)	6.67	16.67	11.67	13.33	16.67	15.00
Catechol (2 mM)	23.33	16.67	20.00	13.33	16.67	15.00
Control	26.67	43.33	35.00	20.00	23.33	21.67
Mean	14.76	20.00	17.38	13.81	14.29	14.05

L. S. D. at 5 % for:

Inducers (A) = 6.41	Inducers (A) = 7.27
Isolates (B) = 3.42	Isolates (B) = 3.89
Interaction (AxB) = 9.06	Interaction (AxB) = 10.29

Table (4): Effect of soaking cumin seeds in different concentrations of resistance inducers on controlling damping-off disease in cumin in 2006/07 season.

Isolates No. / Inducers	Damping-off %					
	Pre-emergence %			Post-emergence %		
	Iso. No.1	Iso. No.19	Mean	Iso. No.1	Iso. No.19	Mean
Salicylic acid (1 mM)	26.27	3.33	15.00	10.00	3.33	6.67
Salicylic acid (2 mM)	20.00	16.67	18.33	10.00	10.00	10.00
Ascorbic acid (1 mM)	13.33	16.67	15.00	13.33	13.33	13.33
Ascorbic acid (2 mM)	16.67	26.67	21.67	16.67	13.33	15.00
Catechol (1 mM)	13.33	26.67	20.00	10.00	16.67	13.33
Catechol (2 mM)	33.33	26.67	30.00	16.67	6.67	11.67
Control	36.67	36.67	36.67	23.33	23.33	23.33
Mean	22.86	21.91	22.38	14.29	12.38	13.33

L. S. D. at 5 % for:

Inducers (A) = 9.29	Inducers (A) = 6.24
Isolates (B) = 4.96	Isolates (B) = 3.34
Interaction (AxB) = 13.13	Interaction (AxB) = 8.83

In season 2005/06, data indicate that salicylic acid at concentration 1 mM gave the highest reduction of pre-emergence damping-off in cumin compared to other treatments. Salicylic acid and ascorbic acid at both concentrations gave the highest reduction of post-emergence damping. On the other hand, ascorbic acid at concentration 1 mM and catechol at 2 mM was the lowest effect in reduction pre-emergence damping-off percentage in cumin. Also, catechol at 1 and 2 mM concentrations gave the lowest effect.

In season 2006/07, salicylic acid and ascorbic acid at 1 mM concentrations gave the highest reduction of percentage of pre-emergence damping-off compared to other treatments. Salicylic acid at 1 and 2 mM concentrations gave the highest reduction of percentage of post-emergence damping-off. While, catechol and ascorbic acid at 2 mM concentration gave the lowest reduction of damping-off.

4- Effect of seeds soaking in different concentrations of resistance inducers on controlling cumin wilt disease under greenhouse conditions:

Data presented in Table (5) showed that all tested treatments with resistance inducers significantly reduced the percentage of wilted plants compared to the control. In general, all tested resistance inducers at concentration 2 mM reduced the percentage of wilted plants in both tested seasons.

In season 2005/06, salicylic acid and ascorbic acid at concentration 2 mM gave the highest reduction of wilt percentage. While, ascorbic acid at concentration 1 mM gave the lowest reduction on controlling cumin wilt with isolate No. 1.

In season 2006/07, salicylic acid and catechol at both concentrations were more effective on the disease reduction than the other treatments. In addition, ascorbic acid and catechol at concentration 1 mM caused the lowest reduction in cumin wilt disease.

Table (5): Effect of soaking cumin seeds in different concentrations of resistance inducers on controlling cumin wilt disease in 2005/06 and 2006/07 seasons.

Isolates No. / Inducers	Wilt %					
	Season 2005/06			Season 2006/07		
	Iso. No.1	Iso. No.19	Mean	Iso. No.1	Iso. No.19	Mean
Salicylic acid (1mM)	13.33	10.00	11.67	10.00	6.67	8.33
Salicylic acid (2mM)	10.00	10.00	10.00	6.67	10.00	8.33
Ascorbic acid (1 mM)	16.67	13.33	15.00	20.00	6.67	13.33
Ascorbic acid (2 mM)	13.33	6.67	10.00	6.67	13.33	10.00
Catechol (1 mM)	6.67	16.67	11.67	10.00	6.67	8.33
Catechol (2 mM)	16.67	16.67	16.67	10.00	6.67	8.33
Control	50.00	30.00	40.00	23.33	20.00	21.67
Mean	18.10	14.76	16.43	12.38	10.00	11.19

L. S. D. at 5 % for:

Inducers (A) = 7.67	Inducers (A) = 7.72
Isolates (B) = 4.10	Isolates (B) = 4.13
Interaction (AxB) = 10.85	Interaction (AxB) = 10.92

Discussion

Fusarium cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini.* is one of the most important cumin diseases in Egypt and world-wide (Arafa, 1985; Champawat, 1986; Champawat and Pathak, 1991 and Moustafa, 2006). Two isolates of the pathogen were obtained from wilted cumin plants collected from different localities of Assiut Governorate. They proved to be pathogenic to Baladi cumin variety (Kasha-Chand *et al.*, 1999 and Ghasolia and Jain, 2004).The effectiveness of certain resistance inducers (salicylic acid, ascorbic acid and catechol) against *F. oxysporum* f. sp. *cumini* were studied. Data indicated that addition of inducers to Czapek's medium revealed that all tested inducers were able to inhibit the mycelial growth of the pathogen. Salicylic acid caused the highest reduction in growth of the pathogen followed by the other tested inducers. Such finding are in agreement with those found by (Mandavia *et al.*, 2000 and El-Ganaieny *et al.*, 2002) they reported that theses compounds significantly inhibited fungal spore germination and mycelial growth of different *Fusarium species*, in *vitro*.

Under greenhouse conditions, cumin seeds treatment by the three tested resistance inducers for 3 minutes before sowing was very effective against the pathogen in both seasons. Data revealed that salicylic acid and ascorbic acid gave the highest reduction of percentage of pre and post-emergence damping-off. While, catechol gave the least reduction in damping-off. The results are in agreement with those reported by many workers (Rasmussen *et al.*, 1991; Farmer and Ryan, 1992; Raskin, 1992; Pena-Cortes *et al.*, 1993; Attitalla *et al.*, 1998; Ghonim, 1999; Abdou *et al.*, 2001 and Mandavia *et al.*, 2003), they reported that SA may have different actions at the local and at the systemic levels. Other known actions of SA include a direct antimicrobial effect. He and Wolyn (2005) reported that SA activated peroxidase and phenylalanine ammonialyase, as well as lignifications, upon *F. oxysporum* f. sp. *asparaggi* attack. In conclusion, soaking cumin seeds in SA before sowing reduced the damping-off and wilted plants, and has not any harmful effect against human and plants.

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فاعلية بعض مستحثات المقاومة في مقاومة مرض الذبول الفيوزاريومى في الكمون.

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أجريت هذه الدراسة بهدف معرفة تأثير بعض مستحثات المقاومة وهى حمض السالسليك، وحمض الأسكوربيك والكاتيكول في مقاومة مرض الذبول في الكمون. وتحت ظروف المعمل تم دراسة تأثير هذه المستحثات للمقاومة بعشر تركيزات مختلفة وهى (1،2،.....،10 ملليمول) على النمو الميسيليومى لعزلتين من الفطر فيوزاريوم أكسيسبورم كيومينای المسبب للمرض على البيئات المغذية فى المعمل، وأظهرت النتائج أن كل هذه المواد لها تأثيرا معنويا في تثبيط نمو الفطر المسبب للمرض، وعموما وجد أن حمض السالساليك كان الأكثر تثبيطا للنمو الميسيليومى للفطر المختبر، بينما كان الأسكوربيك الأقل تأثيرا على نمو الفطر.

* وتحت ظروف الصوبة وجد أن نقع البذور لمدة ثلاث دقائق قبل الزراعة في هذه المستحثات للمقاومة بتركيز 1، 2 ملليمول أدى إلى تقليل نسبة الإصابة بالمرض. حيث أدى نقع البذور في محلول من السالساليك أو الأسكوربيك بتركيز 2 ملليمول إلى تقليل نسبة الذبول و سقوط البادرات في الكمون. وعموما كان حمض السالساليك الأكثر تأثيرا في تقليل نسبة المرض، علاوة على أنها آمنة على الإنسان والنبات والبيئة.