

Evaluation of Certain Biocides and Chemicals Inducing Resistance in Management of Cumin Wilt (*Fusarium oxysporum* f. sp. *cumini*)

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F*usarium* wilt of cumin (*Cuminum cyminum* L.) caused by *Fusarium oxysporum* f. sp. *cumini* is a destructive disease, responsible for pre-mature plant death leading to serious economic losses in all cumin-growing areas in Middle and Upper Egypt. The fungus was isolated from the four surveyed governorates (Beni-Sueif, Minia, Assiut and Fayoum). Pathogenicity tests showed that the isolates obtained from Matai County followed by those from Beni Mazar, Minia governorate were the most aggressive and showed the highest frequencies (%) compared to other isolates. Efficiency of some biocides namely Bio-Cure-F (*Trichoderma viride*, 2×10^6 spore/cm³), Bio-Cure-B (*Pseudomonas fluorescens*, 1×10^8 spore/cm³) and inducer resistance chemicals (chitosan, kinetin and salicylic acid) either individually or in combinations was evaluated in controlling the disease in the greenhouse. Combined treatments between bioagents and the tested elicitors were more effective in reducing the disease incidence than using each treatment alone. *P. fluorescens* combined with chitosan was the superior treatment in this respect.

Keywords: Biocides, chemical control, chitosan, elicitors, kinetin, *Pseudomonas fluorescens*, salicylic acid, seed treatment and *Trichoderma viride*.

Cumin (*Cuminum cyminum* L.) belonging to family Apiaceae occupies an important position among condiments, as its dry seed(s) is one of the most important condiments consumed in Egypt. Essential oil of seeds was reported to have antimicrobial activity (Begum *et al.*, 2008). The oil has a great importance in pharmaceutical industry and as food additives as well. Fusarium wilt is known to be favored by warm climates in winter, especially under dry conditions. The fungus can attack the crop up to maturity phase, being more destructive at the seedling stage. Infected plants display dropping leaves, yellowing, damage of vascular elements that lead to plant death. The disease is one of the most important cumin diseases (Nawade *et al.*, 2014). The causal fungus is a soil and seed borne pathogen. Also, it is widely prevalent in almost all cumin growing countries (Agnihotri, 1991 and Hilal *et al.*, 2009). In Egypt, cumin is seriously affected by the Fusarium wilt that causes devastating damage to cumin in southern regions of Egypt (Hilal *et al.*, 2009). The long-term cumin cultivation at the same place and the probable use of contaminated seeds are among the reasons contributing to increasing the pathogen population in the soil, making the continuous planting of this crop undesirable.

Losses due to cumin wilt have been estimated according to conditions to range from 5-60% depending on the conditions. In India, Champawat and Pathak (1991) tested seven fungicides in controlling wilt and found that mixed carbendazim and benomyl treatment gave the best disease control and increased yield, though chemical control alone was not sufficient to manage the disease.

Enhanced disease resistance in plants was found to occur in response to biotic and abiotic inducing agents with special concern of salicylic acid, oxalic acid, hydrogen peroxide, 2,6 dichloroisonicotinic acid, kinetin, chitosan and chitine and others (Bashir *et al.*, 1997).

The present study was undertaken to determine the effect of some biocides and elicitors on Fusarium wilt of the highly susceptible cumin cultivar (cv. Balady), through seed treatment.

Materials and Methods

I- Isolation from infected plants:

Diseased plants at various growth stages were collected from cumin fields distributed in Middle and Upper Egypt, basically from Beni-Sueif, Minia, Assiut and Fayoum governorates, where cumin is intensively cultivated. Plants showing typical wilt symptoms were cleaned from adhering soil and washed several times in running tap water. The entire stems and the main roots of plant samples were cut into small pieces (5 mm segments), surface disinfected with 3% sodium hypochlorite solution for 2 min, rinsed three times in sterilized water, then dried between folds of sterilized filter paper and the segments were placed in sterilized Petri-dishes containing sterilized potato dextrose agar medium (PDA). Seven days after incubation at 25°C in the dark, the emerged fungi from each segment were purified and examined macroscopically and microscopically. Identification of the isolated fungi was carried out using single spore isolates according to the descriptions of Booth (1971) and Domsch *et al.* (1980). Frequency percentage of each fungus was also determined using the following formula:

$$\text{Frequency (\%)} = \frac{\text{No. of fungal colonies of each fungus}}{\text{Total No. of fungal colonies of all fungi}} \times 100$$

II- Pathogenicity tests:

The experiment was carried out at the Plant Pathol. Res. Inst., ARC, Giza, Egypt during November 2015. Six single-spore isolates were randomly chosen from a collection of Egyptian native *F. oxysporum* recovered from wilted cumin plants according to location and morphological characteristics. Inocula were prepared by growing the fungal isolates on PDA medium for 10 days at 30±1°C. Discs (5mm), taken from the margins of the fungal colonies were used to inoculate 100 ml potato dextrose broth (PDB) in 250ml flasks and incubated at 30±1°C for 15 days. Fresh mycelial mat was blended for 1 min in sterilized distilled water. A sufficient volume of suspension was used to give a concentration of 10⁶ spores/ml using a hemocytometer slide. Clay soil was sterilized with 5% formalin solution, covered with polyethylene sheets for 7 days, and left uncovered for 10 days. Sterilized pots (30-cm-diam.) were filled with the sterilized soil at the rate of 4 kg soil/pot. Soil

infestation was carried out by adding 90 ml of 10^6 spore/ml suspension of any of the tested isolates / pot .The infested soil was kept moist for 7 days in order to stimulate fungal growth and ensure homogeneous distribution of the fungus. Control treatment was prepared by adding the same amount of the sterilized potato dextrose broth (PDB) to the sterilized soil of each pot. Apparently healthy seeds of cumin (cv. Balady) obtained from growers, were disinfected with 1% sodium hypochlorite for 3 min and left to dry air for 6 hours. These seeds were planted in the infested soil at the rate of 25 seeds /pot. Randomized complete block design with 4 replicates was followed. The percentages of disease incidence as dead plants (pre- and post-emergence damping-off and wilt) as well as healthy survived plants, were determined after 21, 45 and 60 days after sowing, respectively. Percentages of damping-off incidence, wilt and survived plants were estimated using the following formula:

$$\text{Pre-emergence (\%)} = \frac{\text{No. of non-emerged seedlings}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{No. of dead seedlings}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Survived plants (\%)} = \frac{\text{No. of survived plants}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Wilt (\%)} = \frac{\text{No. of wilted plants}}{\text{Total No. of sown seeds}} \times 100$$

III- Identification of *F. oxysporum forma specialis*:

F. oxysporum isolates were selected for use in this experiment based on consistent infection and re-isolation in the pathogenicity experiments. Eight herbs belonging to the family Apiaceae namely: parsley (*Petroselinum crispum* L.), coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* L.), caraway (*Carum carvi* L.), cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisum* L.) and celery (*Apium graveolens* L.) were tested for their reactions to *F. oxysporum* isolated from cumin plants.

Pots (30-cm-diam.), filled with infested soil as described before, and were sown with each plant species. Pots containing non-infested soil were sown with seeds of the tested plant species to serve as healthy control. Pots were distributed out in randomized block design with four replication pots, consisting of 25 seed/pot. Three weeks after inoculation, plants were checked for symptoms of Fusarium wilt. The percentages of disease incidence as dead plants (Pre- and Post-emergence damping-off and wilt), as well as healthy survivals were determined at 21, 45 and 60 days after sowing, respectively. Roots were cut and 1-cm sections from the lower portions of the stem surface, disinfected in 3% NaOCl for 2 min. and placed onto PDA medium in petri dishes for recovering *F. oxysporum*. Plates were incubated at $30 \pm 1^\circ\text{C}$ for 5 days. Colonies from tested plant tissues were identified as mentioned before.

IV- Greenhouse experiments:

A- Induction of resistance:

Salicylic acid and Kinetin as growth regulators, as well as chitosan as chemical resistance inducer were evaluated for their effect on defense responses against Fusarium wilt under greenhouse conditions. Three concentrations per each compound were used as follows:

- Salicylic acid (0.5, 2.0 and 4.0 mM^l).
- Kinetin (0.5, 15.0 and 25.0 µg ml⁻¹).
- Chitosan (0.5, 2.0 and 4.0 g l⁻¹).

Pot experiments were carried out using *F. oxysporum* isolate No. 4 (a highly virulent isolate). Seeds of the highly susceptible cumin cv. Balady were dipped in each tested elicitor concentration for 20 min before sowing in Fusarium-infested soil. Moreover, cumin seeds were treated with the fungicide Topsin M 70% WP to serve as additional control.

Four replicates were used for each individual treatment and 25 seeds were sown in each pot and the treatments were as follows:

- 1- Disinfected untreated – seeds planted in artificially Fusarium – infested soil (infested control).
- 2- Seeds treated with Topsin M 70% WP at the rate of 1g /kg seeds (second control).
- 3- Seeds treated individually with salicylic acid at three concentrations (0.5, 2.0 or 4.0 mM^l).
- 4- Seeds treated individually with Kinetin at three concentrations (0.5, 15.0 and 25.0 µg ml⁻¹).
- 5- Seeds treated with chitosan at concentrations (0.5, 2.0 and 4.0 g l⁻¹).
- 6- Disinfected seeds soaked in sterilized distilled water were planted in Fusarium- infested soil (by adding 90 ml of 10⁶ spore/ml suspension/pot) to serve as third control.

B- Biological control:

The biocides, Bio-Cure –F (*Trichoderma viride*, 2x10⁶/cm³) and Bio-Cure-B (*Pseudomonas fluorescens*, 1x10⁸/cm³), T. Stanes & Company limited, India, were used as biocontrol agents. Seeds of the highly susceptible cumin cultivar (cv. Balady) were immersed with gentle stirring in Bio-Cure –F or Bio-Cure –B for 20 min before sowing. Topsin M 70% WP treatment was used as additional control at the rate of 1g/kg seeds; Arabic gum solution (5%) was used as a sticker. Seeding was made at the rate of 25 seeds/pot. Check treatments were inoculated with the pathogen alone. The pots were arranged in a complete randomized block design with four replicates in the greenhouse.

Disease incidence was determined as pre- and post-emergence damping-off, wilt (number of dead plants) as well as survived plants for each treatment 21, 45 and 60 days after sowing, respectively, and the efficiency of each treatment was calculated according to the following formula:

$$\text{Efficiency \%} = \frac{\text{No. of dead plants in the control} - \text{No. of dead plants in the treatment}}{\text{No. of dead plants in the control}} \times 100$$

C- Application of the bioagents combined with the different IRCs:

The experiment was conducted to determine whether incorporation of an inducer tested with the bioagents, *T. viride* or *P. fluorescens* would improve the efficacy of Fusarium wilt control. The combination between bioagents and IRCs was prepared with dissolving chemical inducers in the suspensions of the bioagents (Abdel-Monaim, 2013). Apparently healthy cumin cv. Balady seeds were soaked for 20 min in each of the following treatments. (1) *P. fluorescens* + chitosan, (2) *P. fluorescens* + kinetin, (3) *P. fluorescens* + salicylic acid, (4) *T. viride* + chitosan, (5) *T. viride* + kinetin, (6) *T. viride* + salicylic acid, (7) Topsin M70, (8) Untreated seeds (control). Plastic pots were filled with sterilized soil and mixed with the tested fungal pathogen, by adding 90 ml of 10^6 spore/ml suspension/pot, seven days before planting as previously mentioned. In control treatment, cumin seeds were soaked in water only for 20 min. Extra 10 ml of each treatment were added as soil drench immediately after sowing. All pots were examined, 21, 45 and 60 days after sowing. Treatments were arranged in a complete randomized block design with four replicate pots and 25 seeds per pot. Disease incidence (%) as pre- and post-emergence damping-off, wilt (number of dead plants) as well as survived plants were recorded as previously mentioned.

Statistical analysis:

The data were statistically analyzed for computing L.S.D. according to the procedure outlined by Snedecor and Cochran (1989).

Results and Discussion

Relative distribution of the disease:

Fusarium wilt was found in all the major cumin growing areas in the governorates of Middle and Upper Egypt, where losses due to the disease are rated high. The pathogen was recovered from roots and stems of diseased cumin plants collected from the different growing areas in Beni-Sueif, Minia, Assiut and Fayoum governorates. The fungus was consistently isolated from the diseased organs on potato dextrose agar medium (PDA). Data in Table 1 show that 115 isolates belonging to 3 genera, 103 fungal isolates were found to belong to the genus *Fusarium*, 81 isolates were identified as *Fusarium oxysporum* that was most frequently isolated from diseased plants where the frequency of this fungus was 70.4% whereas, the other species were infrequently associating fungi to wilt disease. Data in Table 2 show that successful isolation was obtained with higher frequency from samples of Matai County followed by Beni Mazar County, Minia governorate, being 23.4 and 18.5%, respectively.

Table 1. Frequency percentages of the associated fungi isolated from wilted cumin plants

Isolated fungi	No. of isolates	Frequency (%)
<i>Fusarium moniliforme</i>	9	7.83
<i>Fusarium oxysporum</i>	81	70.43
<i>Fusarium semitectum</i>	10	8.69
<i>Fusarium solani</i>	3	2.61
<i>Alternaria alternata</i>	7	6.09
<i>Aspergillus niger</i>	5	4.35
Total	115	-

Table 2. Relative occurrence of Fusarium damping-off in samples from different cumin plantations

Location	No. of plants	No. of positive isolations	% Isolates to the No. of plants	Frequency (%)
Beba, Beni-Sueif	30	13	43.3	16.1
Beni Mazar, Minia	33	15	45.5	18.5
Samalut, Minia	30	14	46.7	17.3
Matai, Minia	35	19	54.3	23.4
Abanob, Assiut	26	11	42.3	13.6
Fayoum	25	9	36.0	11.1
Total	179	81	45.3	-

Pathogenicity of F. oxysporum isolates:

Results in Table 3 indicate that all the tested isolates obtained from naturally infected cumin plants showing wilt symptoms were able to attack cumin plants causing wilt symptoms on cv. Balady cumin plants grown in infested soil, as expressed by counting pre-and post-emergence damping-off, wilt as well as reduction in the survived plants. Diseased plants showed red-brown shriveling of the foliage and most of them died within 60 days after inoculation. The tested isolates varied significantly in their ability to cause damping-off symptoms (Table 3). However, no significant differences were found among the isolates in case of pre-emergence damping-off. Isolate of Matai (isolate 4) caused the highest percentage of post-emergence damping-off (24%), followed by Samalut isolate, (isolate 3), being 20%.

Identification of F. oxysporum forma specialis:

Specificity of the pathogen to cumin was determined in the greenhouse by means of artificial inoculation of eight plant species with each of the six isolates of *F. oxysporum* tested. Results in Table 4 indicate that among the plant species tested, cumin plants (cv. Balady) only showed the typical Fusarium wilt symptoms by any of the six tested Fusarium isolates. No symptoms were visible on the other inoculated plant species or non-inoculated control plants. All these tested plant

species stayed healthy with no observed differences with their respective non-inoculated control plants until the harvest. Therefore, it could be concluded that the tested isolates were *Fusarium oxysporum* f. sp. *cumini*.

Table 3. Pathogenicity of *F. oxysporum* isolates obtained from naturally infected cumin plants collected from different locations, greenhouse experiment

Isolates	% Damping - off		% Wilted plants***	% Dead plants	% Survived Plants	% Decrease****
	Pre-emerg.*	Post-emerg.**				
Isolate (1) Beba, Bani-Sueif	12	15	30	57	43	57
Isolate (2) Beni Mazar, Minia	14	16	34	64	36	64
Isolate (3) Samalut, Minia	18	20	28	66	34	66
Isolate (4) Matai, Minia	16	24	36	76	24	76
Isolate (5) Abanob, Assiut	10	18	25	53	47	53
Isolate (6) Fayoum	7	11	18	36	64	36
Control (without fungus)	0	0	0	0	100	0.0
L.S.D. at 5%	5.0	2.0	1.0	4.0	3.0	-

*Pre-emergence = assessed at 21 days after sowing

***Wilt=assessed at 60 days after sowing

**Post-emergence = assessed at 45 days after sowing

****Decrease in healthy survivals relative to the control

Table 4. Pathogenicity of six isolates of *F. oxysporum* on eight different plant species belonging to family Apiaceae

English name	Scientific name	Reaction to the six isolates
Parsley	<i>Petroselinum crispum</i> L.	-
Coriander	<i>Coriandrum sativum</i> L.	-
Dill	<i>Anethum graveolens</i> L.	-
Fennel	<i>Foeniculum vulgare</i> L.	-
Caraway	<i>Carum carvi</i> L.	-
Cumin	<i>Cuminum cyminum</i> L.	+
Anise	<i>Pimpinella anisum</i> L.	-
Celery	<i>Apium graveolens</i> L.	-

- Non-infected + Infected

Efficacy of three resistance inducers on disease infection:

Data in Table 5 show that the tested chemicals were significantly effective in decreasing the infection compared with untreated control. Chitosan, as seed dressing treatment caused the lowest values of post-emergence damping-off, being 8-12% when the soil was artificially infested with the highly virulent isolate (No.4). Seed dressing with the tested elicitors (IRCs) at higher concentrations resulted in a significant increases in healthy unaffected cumin plants. In addition, chitosan

treatment at concentrations 0.5, 2.0 and 4.0 g caused the lowest incidence of total infection with wilt, pre- and post-emergence damping-off, being 25-35% on the average.

The response of cumin plants to elicitor's can be attributed to the direct antimicrobial activity of these substances and their ability to induce plant defense response against pathogens without undergoing any deterioration due to plant infection (Burrows *et al.*, 2007). Chitosan, which is chemically amino substances, B-D-glucosamine, may has assisted in stimulating the synthesis of protective agents and contributed to plant growth by acting as natural elicitors or catalysts inducing pathogenesis-related proteins such as chitinase enzymes for young seedlings as indicated by Ohta *et al.* (2001). Also, chitosan provides protection against environment stress such as drought and may assist in water balance in plants by closing the stomata, decreasing transpiration, and maintaining production of plants (Bittelli *et al.*, 2001). Induced resistance against many fungal diseases using chitosan was also reported by Abd-El-Kareem (2002). The results reported herein indicate a negative relationship between increasing the concentrations of elicitors tested and disease progress. Increasing the concentration decreased the percentage of *Fusarium* wilt incidence. This result is in agreement with that mentioned by El-Mougy *et al.* (2006).

Table 5. Effect of three IRCs on the incidence of pre- and post-emergence damping-off and wilt diseases in cumin (cv. Balady), grown in soil infested with *F. oxysporum* f. sp. *cumini* (isolate 4), under greenhouse conditions

Treatment	Conc./ L or/ kg	% Damping - off		*** % Wilted plants	% Dead plants	Efficiency (%)	% Survived plants
		*Pre-emerg.	**Post-emerg.				
Chitosan	0.5 g	8	12	15	35	53.9	65
	2.0g	7	10	13	30	60.5	70
	4.0g	5	8	12	25	67.1	75
Kinetin	5.0 ppm	7	18	30	55	27.6	45
	15.0 ppm	6	15	26	47	38.2	53
	25.0 ppm	6	13	25	44	42.1	56
Salicylic Acid	0.5 mM	7	19	36	62	18.4	38
	2.0 mM	6	18	25	49	35.5	51
	4.0 mM	5	16	22	43	43.4	57
Topsin M70	1g/kg seeds	5	10	25	40	47.4	60
Control		16	24	36	76	-----	24
L.S.D. at 5%		2.0	1.0	4.0	-----	-----	3.0

* Pre-emergence = assessed at 21 days after sowing.

** Post-emergence = assessed at 45 days after sowing.

*** Wilt=assessed at 60 days after sowing.

Effect of two biocides on infection:

The effect of Bio-Cure-B and Bio-Cure-F on controlling Fusarium wilt of cumin (cv. Balady) is shown in Table 6, where a noticeable decrease in the infection by wilt was noticed, being 26 and 30%, respectively while plants dressed with Topsin -M showed 25% infection. Moreover, cumin seed dressed with Topsin -M resulted the lowest values of both pre- emergence and post- emergence damping-off incidence, where percentage of infection, expressed as pre-, post-emergence damping-off and wilted plants, recorded 40% compared to the control (76%). In general, data showed significant differences between the treatments and the control. However, the differences among the treatments were in most cases, not significant.

Several strains of *P. fluorescens* were reported to control various Fusarium wilt and root rot pathogens including *F. oxysporum* f. sp. *dianthi* in carnation (Van Peer *et al.*, 1991). Seed treatment with *P. fluorescens* indicated certain biochemical changes in the plant leading to induce disease resistance against many pathogens (Sivakumar and Sharma, 2003). On the other hand, the antagonistic activities of *T. viride*, which are found in Bio-Cure-F, may be attributed to antibiosis (Ghisalberti *et al.*, 1993); competition for nutrients and/or space (Inbar *et al.*, 1994). Higher efficacy of the bioagent *Trichoderma* sp. over fungicidal seed treatment for cumin wilt suppression has also been reported in Iran (Aghnoom *et al.*, 1999). Vyas and Mathur (2002) recorded *T. harzianum* as a potential bioagent against Fusarium wilt of cumin through antibiosis and hyper-parasitism. Tawfik and Allam (2004a) reported that *T. harzianum*, *T. hamatum* and *T. viride* exhibited a mycoparasitism associated with high level of growth reduction induced by their filtrate on *F. oxysporum* f. sp. *cumini*. Antagonists *T. harzianum*, *T. viride* and *B. subtilis* and the biocide (Plant Guard) showed significantly lower percentages of infection compared to control. The least infection was found in pre-sowing treatment with *T. harzianum* (Tawfik and Allam, 2004b).

Effect of seed dressing with biocides and IRCs as soil treatment on infection:

Data presented in Table 7 show that cumin plants can be efficiently protected against wilt using seed dressing by biocides and drenching soil with the tested chemicals just before sowing. *P. fluorescens* plus any elicitor was the most effective treatment in decreasing wilt infection than the combined treatment with *T. viride*. Generally, combined treatments between bioagents (*P. fluorescens* or *T. viride*) as seed dressing and each of the tested elicitors as soil amendments effectively improved the level of disease reduction compared to each treatment alone. Combination between *P. fluorescens* and chitosan was superior to the other treatments in minimizing disease amount and maximizing plant survivals, respectively. The survived seedlings were apparently healthy and free from noticeable wilt symptoms (71.0% efficiency) and showed the highest plant survivals. Moreover, wilt infection reached 76.0% in the untreated control compared to 40% in fungicide-treated plants. The proposed mechanisms of these combinations which provide an increase in plant protection may be attributed to the synergistic action of the antifungal metabolites such as antibiotics and hydrolytic enzymes that restrict fungal growth in the root tissues, decrease pathogen viability (Duffy and Weller

1996) and associated with marked accumulation of newly formed products in the host cells (Benhamou *et al.*, 1998).

Table 6. Effect of two biocides as seed treatment on the incidence of pre- and post-emergence damping-off and wilt diseases on highly susceptible cumin (cv. Balady) grown in soil infested with *F. oxysporum* f. sp. *cumini*, under greenhouse conditions

Biocide	% Damping - off		***% Wilted plants	% Dead plants	Efficiency (%)	% Survived plants
	*Pre-emergence	**Post-emergence				
Bio-cure-B	5	12	26	43	43.4	57
Bio-cure-F	5	10	30	45	40.8	55
Topsin M70	5	10	25	40	47.4	60
Control	16	24	36	76	-----	24
L.S.D. at 5%	2.0	1.0	2.0	-----	-----	3.0

* Pre-emergence = assessed at 21 days after sowing

** Post-emergence = assessed at 45 days after sowing

*** Wilt = assessed at 60 days after sowing

Table 7. Effect of the combination of the IRCs with two bioagents on the incidence of damping -off and wilt on cumin (cv. Balady), grown in soil infested with the highly virulent isolate of *F. oxysporum* f. sp. *cumini*, under greenhouse conditions

Treatment	Bioagents											
	Bio-Cure-B					Bio-Cure-F						
	Pre-emerg [*]	Post-emerg ^{**}	Wilted Plants ^{***}	% Dead plants	Efficiency %	% Survived plants	Pre-emerg [*]	Post-emerg ^{**}	% Wilted plants ^{***}	% Dead plants	% Efficiency	% Survived plants
Chitosan	3	7	12	22	71.0	78	3	8	13	24	68.4	76
Kinetin	6	13	23	42	44.7	58	5	13	26	44	42.1	56
Salicylic acid	5	11	24	40	47.4	60	5	10	30	45	40.8	55
Topsin M70	5	10	25	40	47.4	60	5	10	25	40	47.4	60
Control	16	24	36	76	-----	24	16	24	36	76	-----	24
L.S.D. at 5%	2.0	1.0	2.0	--	-----	4.0	n.s.	2.0	2.0	-----	-----	2.0

* Pre-emergence = assessed at 21 days after sowing

** Post-emergence = assessed at 45 days after sowing

*** Wilt = assessed at 60 days after sowing

References

- Abd-El-Kareem, F. 2002. Integrated treatments between bioagents and chitosan on root rot diseases of pea plants under field conditions. *Egypt. J. Appl. Sci.*, **17**:257-279.
- Abdel-Monaim, M.F. 2013. Improvement of biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. *Mycobiol.*, **4**(1): 47-55.
- Aghnoom, R.; Falahati-Rastegar, M. and Jafarpour, B. 1999. Comparison of chemical and biological control of cumin wilt (*Fusarium oxysporum* f. sp. *cumini*) in laboratory and greenhouse conditions. *Iran. J. Agric. Sci.*, **30**(3):619-630.
- Agnihotri, J. P. 1991. Diseases of arid zone seed species: present status and future strategies for their management. In: Dry Land Resources and Technology, (ed: L. L. Somania) Scientific Publishers, Jodhpur, **6**:1-110.
- Bashir, N.; Hashmi, M.I. and Jamil, F.F. 1997. Induction of systemic acquired resistance by oxalic acid in chickpea (*Cicer arietinum*) against *Ascochyta rabiei*. *Pak. J. Phytopathol.*, **9**:18-20.
- Begum, J.; Nazrul, M.; Chowdhury, J.U.; Hoque, M.N. and Anwar, M.N. 2008. Antimicrobial activity of essential oil from seeds of *Carum carvi* and its composition. *Bangl. J. Microbiol.*, **25**(2):85-89.
- Benhamou, N.; Klopper, J.W. and Tuzun, S. 1998. Induction of resistance against Fusarium wilt of tomato by combination of chitosan with endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta*, **209**: 153-168.
- Bittelli, M.; Flury, M.; Campbell, G. and Nichils, E.J. 2001. Reduction of transpiration through foliar application of chitosan. *Agric. Forest Meteorol.*, **107**: 167-175.
- Burrows, F.; Louime, C.; Abazinge, M. and Onokpise, O. 2007. Extraction and evaluation of chitosan from crop exoskeleton as a seed fungicide and plant growth enhancer. *American-Eurasian J. Agric. & Environ. Sci.*, **2**(2):103-111.
- Champawat, R.S. and Pathak, V.N. 1991. Effect of fungicidal seed treatment on wilt disease of cumin. *Turkish Phytopathol.*, **20**(1): 23-26.
- Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK. 237p.
- Domsch, K.H.; Gams, W. and Anderson, T.H. 1980. Compendium of Soil Fungi. Academic Press, New York, USA, 1156p.
- Duffy, B.K. and Weller, D.M. 1996. Combination of *Trichoderma koningii* with fluorescent *Pseudomonas* for control of take-all on wheat. *Phytopathology*, **86**: 188-194.

- El-Mougy, Nehal S.; El-Gamal, N.G.; Fotouh, Y.O. and Abd-El-Kareem, F. 2006. Evaluation of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse and field conditions. *Res. J. Agric. Boil. Sci.*, **2**(5):190-195.
- Ghisalberti, E.L.; Rowland, C.Y. and Sivasitamparam, K. 1993. Metabolites of *Trichoderma harzianum*. 6th ICPP Montreal, Canada Abstract pp. 292 (c. after Vyas and Mathur, 2002).
- Hilal, A.A.; Soliman, G.L.; El-Shaer, A.H. and El-Zefzaf, H.M. 2009. Bio-and chemical controls of soil-borne fungal diseases of the medicinal and aromatic plants: Cumin (*Cuminum cyminum* L.) and pelargonium (*Pelargonium graveolense* L.). *Egypt. J. Appl. Sci.*, **24**(1):90-112.
- Inbar, J.; Abramshy, D.; Cohn, D.S. and Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Eur. J. Plant Pathol.*, **100**: 337-346.
- Nawade, B.D.; Jadeja, K.B.; Talaviya, J.R. and Vyas, U.M. 2014. Comparative analysis of *Fusarium oxysporum* f. sp. *cumini* isolates using RAPD marker and cultural characteristics. *Trends Biosci.*, **7**(17): 2475-2478.
- Ohta, K.; Asao, T. and Hosoki, T. 2001. Effects of chitosan treatments on seedling growth, chitinase activity and flower quality in *Eustoma grandiflorum* (Raf.) Shinn 'Kairyoku Wakamurasaki. *J. Hort. & Biotechnol.*, **76**:612-614.
- Sivakumar, G. and Sharma, R.C. 2003. Induced biochemical changes due to seed bacterization by *Pseudomonas fluorescens* in maize plants. *Indian Phytopathol.*, **56**(2):134-137.
- Snedecor, G.W. and Cochran, W.G. 1989. *Statistical Methods*. 8th ed. Iowa State Univ. Press, Ames, Iowa, USA.

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تقييم فعالية بعض المركبات الحيوية والمواد المستحثة للمقاومة في مكافحة مرض ذبول الكمون

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يُعتبر مرض ذبول الفيوزاريوم على الكمون والذي يسببه فطر فيوزاريوم اوكسيسبورام كيوميني من الأمراض المدمرة والتي تسبب موت النباتات قبل مرحلة النضج مما يؤدي إلى خسائر اقتصادية جسيمة في جميع مناطق زراعته في صعيد مصر. تم عزل الفطر من ٤ محافظات (بني سويف، المنيا، اسيوط والفيوم) وكانت أعلى نسبة عزل وكذلك قدرة مرضية تحت ظروف العدوى الصناعية بالصوبة هي عزلة مركز مطاي محافظة المنيا. تم دراسة تأثير اثنان من الكائنات المضادة (ترايكودرما فيردي وسيدومونس فلوريسنس) في صورة مركبات تجارية وثلاثة من المواد المستحثة للمقاومة (شيتوزان، كينينين و حمض سلسيليك) سواء بصورة منفردة أو مشتركة. ووجد أن المعاملة المشتركة بين الكائنات المضادة والمواد المستحثة كانت أكثر فعالية في مقاومة المرض عنها في حالة الاستخدام المنفرد. كما اتضح أن استخدام سيدومونس فلوريسنس كمعاملة بذرة مع إضافة الشيتوزان للتربة كانت أكثر المعاملات تأثيراً في هذا الشأن.