

Role of some bioagents and inducer resistance chemicals in management of potato dry-rot caused by *Fusarium* species

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ABSTRACT—Dry-rot of potato caused by *Fusarium* species is a serious disease, which causes significant losses in potato tubers all over the world. A survey of diseased potato tubers in markets and seed production storage facilities was carried out in four Egyptian Governorates during 2012 and 2013. Twenty-eight fungal isolates were collected from diseased potato tubers showing typical dry rot symptoms. The isolated fungi were purified and identified as *F. culmorum*, *F. oxysporum*, *F. sambucinum*, *F. semitectum* and *F. solani*. Pathogenicity tests showed clear variations among the 28 *Fusarium* isolates on potato tubers (cv. Spunta), eighteen isolates were pathogenic to potato tubers and ten isolates were non-pathogenic. In vitro tests were conducted in Petri-dishes to evaluate the antagonistic effect of twelve bioagents against *F. culmorum* growth. Obtained results revealed that *T. viride*-1 and *T. harzianum* caused the highest reduction to the mycelial growth of the tested *F. culmorum* isolate. Moreover, in vitro tests were conducted to determine the inhibitory effect of three inducer resistance chemicals (IRCs), i.e. catechol, chitosan and salicylic acid, against *F. culmorum* mycelial growth. In this concern, results cleared that chitosan and salicylic acid were more effective more than catechol, which had no effect against the mycelial growth of tested fungus. On the base of the aforementioned results, in vivo experiments were conducted to evaluate the antagonistic effect of six bioagents and three IRCs against dry-rot disease on potato tubers (cv. Spunta). Results showed that the tested IRCs have high ability to reduce dry-rot disease compared with the tested bioagents, which show a moderate ability.

Keywords: Bioagents, catechol, chitosan, disease management, dry-rot, *Fusarium* spp., potatoes and salicylic acid.

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is known as one of the most important crops overall the world. It is ranked as the fourth main food crop after wheat, maize and rice (Hawkes, 1994). The world production of potato reached about 368 million ton, of which 4.8 million tons are produced in Egypt (Anonymous, 2013).

Potato dry-rot disease leads to significant losses in both quality and quantity of potato tubers. The causal pathogen kills potato sprouts and reduces crop establishment, when the crop losses reached up to 25% in the field and may be reached more than 60% of tubers during storage (Ghadiri *et al.*, 2013).

Up to date, thirteen species of genus *Fusarium* were found to be the causal of potato dry-rot around the world. In this concern, the most important *Fusarium* spp. are *F. solani*, *F. sambucinum* and *F. avenaceum*. In North America and parts of Europe, *F. sambucinum* and *F. coeruleum* are considered to be the most significant causal agent of potato tuber dry-rot. In Britain, *F. coeruleum* is more prevalent, meanwhile in Iran and South Africa, *F. solani* is the main causal species of potato dry-rot (Chehri *et al.*, 2011).

Concerning the disease control, application of thiabendazole (TBZ) is well known as a primary control of potato dry-rot disease. However, due to the

issues raised by the development of the pathogens resistance to the fungicide and potential harmful effects on the environment and human health, new strategies for controlling postharvest-diseases have been proposed. Therefore, resistance in plant tissue through elicitor to protect against infection of pathogens is considered as a promising approach for disease management. Moreover, chitosan and sodium silicate were reported to induce resistance in potato tuber against dry-rot caused by *F. sulphureum* (Yan *et al.*, 2010). Recently, intense research efforts have been devoted to the development of antagonistic microorganisms to control postharvest disease. So far, biological control of dry and soft-rots with different bioagents, i.e. fungi, bacteria, and yeasts, have been reported as effective under experimental conditions.

Several microbial antagonists have been identified and shown to reduce *Fusarium* dry-rot on potatoes (Sadfi *et al.*, 2002).

This research was carried out to evaluate some abiotic agents (IRCs) which induce plant resistance in comparison with bioagents to manage potato dry-rot under *in vitro* and *in vivo* conditions.

2 MATERIALS AND METHODS

1. Source of infected potato tubers:

Diseased samples of potato tubers, showing typical symptoms of dry-rot, were collected from several store-houses and markets in Giza, Behera (Nobaria), Qualiobia and Fayoum Governorates.

2. Isolation and identification of the causal organism and associated fungi:

Collected potato tuber samples were washed under tap water, surface sterilized with sodium hypochlorite (0.5%) for 5 min. before washing 3 times in sterilized water, then cut into small pieces and transferred into Petri-dishes containing PDA medium and incubated at 26±2°C for 3-5 days. The isolated fungi were purified using single spore technique and/ or hyphal tip methods and identified according to their morphological characteristics of colony and conidia as described by Gilman (1957) and Booth (1971). Identification of the isolated fungi was confirmed at the Mycol. Res. and Dis. Survey Dept., Plant Pathol. Res. Inst., ARC, Giza.

3. Pathogenicity test:

Apparently healthy potato tubers (cv. Spunta), uniform in size and weight (100-120g), were selected and surface sterilized by 1% sodium hypochlorite and washed with sterilized water three times, then inoculated by removing a plug of tissues (5-mm-diam. by approximately 7- mm- deep) using a sterilized cork borer, and replacing it with a 5-mm-diam. mycelial growth plug taken from a 6-day-old actively growing *Fusarium* culture. Inoculated tubers were wrapped in paper bags and incubated in the dark at 25±1°C for 4 weeks and then rotted areas measurements were recorded. Non-inoculated PDA plugs inserted into wounded tubers were kept as check treatments (Estrada *et al.*, 2010). On the base of obtained results, the most pathogenic isolate was chosen for further studies.

4. Sources of the tested bioagents:

Twelve bioagents, belonging to eight bacterial isolates, *i.e.* (4) *Bacillus subtilis*; (1) *B. thuringiensis* and (3) *Pseudomonas fluorescens*, as well as four isolates belonging to two *Trichoderma* spp., *i.e.* (1) *T. harizianum* and (3) *T. viridi*, were evaluated for their antifungal activity against the most virulent *Fusarium culmorum* isolate. Tested *Bacillus* and *Trichoderma* spp. were isolated from the rhizospheric soil of potato plants grown in different fields located at Giza, Behera (Nobaria), Qualiobia and Fayoum Governorates.

Serial dilution plate technique was used to isolate native antagonistic *Trichoderma* spp. on PDA medium and *Bacillus* spp. on nutrient agar medium (Oedjijono and Dragar, 1993).

The isolated *Bacillus* spp. were purified and identified using the description of Parry *et al.* (1983) and Holt and Krieg (1984).

Also, the fungal cultures of *Trichoderma* spp. were selected and purified by hyphal tip method and then identified on the basis of cultural and microscopic morphological characters (Rifia, 1969 and Bissett, 1991).

Meanwhile, the three *Pseudomonas fluorescens* isolates were kindly provided by Dr. El-Said Mohamed El-Shabrawy, Plant Pathol. Res. Inst., ARC, Giza.

4.1. In vitro preparation of the bioagents suspension:

Tested bacterial antagonists, taken from 24 hr grown cultures, were individually streaked onto nutrient agar Petri-plates (9-cm-diam.). Plates were then incubated for 4 days at 25±2°C. Bacterial suspension was prepared and adjusted to 10⁸ CFU/ml. *Trichoderma viride* and *T. harizianum* were grown on PDA for 7 days at 25±2°C then the conidial suspension was prepared and adjusted to 10⁵ spores/ml using a hemocytometer slide.

4.2. Effect of the bioagents:

Bacterial and fungal isolates were cultured in order to evaluate their antifungal activity against the most virulent *F. culmorum* isolate, the causal of potato dry-rot. They were cultured separately for 7 days on PDA medium, then 0.5-cm plugs from the edge of the colonies of *Trichoderma* spp. and *F. culmorum* were placed in pairs 4 cm apart from each other into PDA plates (9-cm-diam.). In addition to, the antifungal activity of bacterial isolates was evaluated on PDA medium by using the dual culture technique described by Dennis and Webster (1971). Bacterial isolates were streaked at one side of the Petri-dishes (9-cm-diam), 2 cm away from the edge. Disks (5-mm-diam.), taken from the margin of 7-day-old *F. culmorum*, were placed at the opposite side of the Petri-dishes perpendicular to the bacterial streak and incubated at 25±2°C for 7 days. Three replicate plates were prepared for each isolate.

The check treatments consisted of pure *F. culmorum* cultures. The plates were incubated at 25±2°C. and the development of the colonies was observed until the controls covered the whole PDA medium. Growth inhibition (I %) was calculated using the following equation:

$$I (\%) = (D_0 - D_r / D_0) \times 100$$

Where:

D₀= Average diameter of fungal colony in check treatment and

D_r= Average diameter of fungal colonies in the presence of tested bioagent.

4.3. Effect of inducer resistance chemicals (IRCs):

Three IRCs, *i.e.* catechol, chitosan and salicylic acid were evaluated for their activity against *F. culmorum* growth. Catechol and chitosan were dissolved in 0.04 N HCl, and the pH was adjusted to 5.6 using 2N KOH. Chitosan was tested at four concentrations, *i.e.* 50, 250, 500 and 1000 mg/l. Whereas, catechol was tested at concentrations of 5, 25, 50, and 100 mg/ml. Salicylic acid dissolved in sterile distilled water to obtain the concentrations of 10, 30, 60 and 90 mg/l.

The aim of this experiment was designed to determine whether the effect of these inducers is related to their direct effect on the fungus or indirect way by stimulate the induce resistance of the plant.

The effect of catechol, chitosan and salicylic acid on the mycelial growth of *F. culmorum* were assessed according to Yao and Tian (2005). Mycelial disks (5-mm-diam.), taken from one-week-old culture of the fungus were placed in the centre of 90-mm Petri-dishes containing PDA medium with one of the different concentrations of the tested salts and then incubated at 25°C in the incubator. Mycelial growth was determined by measuring the colony diameter after 7 d of inoculation. Mean diameter of colony was measured and inhibition of mycelial growth was calculated as the following formula:

$$\text{inhibition of mycelial growth} = \frac{(\text{control radial growth} - \text{salt-amended radial growth})}{\text{control radial growth}} \times 100.$$

5. In vivo assays under storage conditions:

5.1. Effect of the bioagents:

Based on the antifungal activity on Petri-plates, selected bioagents, i.e. *B. Subtilis*-1,2; *T. viridi*-1,2 and *T. harizianum*. The preparations of the of selected bioagents were tested for their activity against *F. culmorum* in potato tubers under storage conditions. Bacterial strains were grown on NA for 48 h at 25°C and the bacterial cell suspension was adjusted to 10⁸ cfu/ml water. Meanwhile, the spore suspension of *Trichoderma* spp. was prepared at 10⁵ conidia/ ml. water. The pathogenic fungal isolates of *F.culmorum* were obtained from the pure cultures of 7-days-old PDA agar cultures incubated at 25°C.

Table 1. The tested inducer resistance chemicals , their formula and molecular weights.

| IRCs | Molecular formula | MW (g/mol) |
|----------|---|------------|
| Catechol | 1,2-dihydroxbenzene (C ₆ H ₆ O ₂) | 110.1 |

4 RESULTS

1. Isolation, purification and identification of *Fusarium* spp.

Twenty-eight isolates of *Fusarium* spp. (Table,2) were isolated , purified and identified depending on the basis of their morphological characteristics .

Table 2. Frequency (%) of *Fusarium* spp. isolated from

different governorates of diseased potato tubers.

| No. | <i>Fusarium</i> spp. | Frequency (%) |
|-----|------------------------|---------------|
| 1 | <i>F. sambucinum</i> | 10.7 |
| 2 | <i>Fusarium solani</i> | 35.7 |
| 3 | <i>F. oxysporum</i> | 21.4 |
| 4 | <i>F. culmorum</i> | 28.5 |
| 5 | <i>F. semitectum</i> | 3.5 |

| | | |
|----------------|---|--------|
| Chitosan | C ₅₆ H ₁₀₃ N ₉ O ₃₉ | 1526.5 |
| Salicylic acid | C ₇ H ₆ O ₃ | 138.12 |

The selected potato tubers of cv. Spunta were washed and sterilized then air-dried. Potato tubers were injured (5mm) in diameter by cork borer, then soaked in the preparation of the suspension of bacterial bioagents and *Trichoderma* spp. individually for 30 min . then air dried, inoculated with a disk from *F. culmorum* and placed in paper bags.

Sterile water was used as negative control and tubers which just inoculated with pathogen were used as positive control. Four weeks after incubation at 25°C under storage

conditions, the diameters of decayed area on potato tubers were measured. All treatments consisted of four replicates and the averages of the lesions size were recorded (Recep *et al.*, 2009).

5.2. Effect of IRCs :

The same technique followed to assay the effect of the tested bioagents against *F. culmorum* was followed to assay the effect of three salts of IRCs to decrease the development of potato dry-rot caused by the causal pathogen.

The tested IRCs were applied 24 h before inoculation with the pathogen at three concentrations for 30 min , air dried and inoculated with active disk of the margin 7-day old colony of the causal pathogen.

6. Statistical analysis:

The experimental design of all experiments was randomized complete block design . Analysis of variance (ANOVA) of the data were performed with Assist of Lest Significant Different was used to compare treatment means.

The isolates of genus *Fusarium* were belonged to five species, i.e. *F. sambucinum*, *F. solani*, *F. oxysporum*, *F. culmorum* and *F. semitectum*. In addition to, both *Rhizoctonia solani* and *Alternaria solani* were , also, isolated.

2. Pathogenicity test:

Data in Table (3) indicate that there was an obvious variation in the pathogenicity of 28 species of *Fusarium* on potato tubers (cv. Spunta). Ten species of *Fusarium*, i.e. FCU4, FSA2, FSO3, FSO4, FSO8, FSO9, FOX2, FOX3, FOX6 and FSEM were non-pathogenic to potato tubers and the eighteen species were pathogenic to potato tubers. The most pathogenic species was FCU8 caused lesions more than double of that of the lowest species.

Based on Duncan's test, species lies in one of the two groups, i.e. pathogenic and non-pathogenic. The most pathogenic isolate was *F. culmorum* (FCU8) from Giza, which showed 1.96 cm lesions followed by isolate (FCU1) of *F. culmorum* from Giza, FCU7 from

Behera and FSO1 from Fayoum without significant differences. While the less pathogenic isolate was *F. oxysporum* (FOX1) from Fayoum governorate which showed 0.98 cm lesions followed by *F. culmorum* (FCU3) from Fayoum governorate and *F. solani*

(FSO5) from Fayoum governorate without significant differences. On the other hand, results showed ten isolates were non-pathogenic, and the other of isolates were differed in their pathogenicity.

Table 3. Average lesion size on tubers of potato artificially inoculated with different *Fusarium* species

| <i>Fusarium</i> spp. | Strain No. | Governorate | Rotted area (cm) | | Average lesion area (cm) |
|----------------------|------------|-------------|------------------|-------|--------------------------|
| | | | Depth | Width | |
| <i>F.culmorum</i> | Fcu1 | Giza | 1.40 | 1.90 | 1.65 ^{abc} |
| <i>F.culmorum</i> | Fcu2 | Behera | 1.23 | 1.40 | 1.32 ^{bcde} |
| <i>F.culmorum</i> | Fcu3 | Fayoum | 0.75 | 1.25 | 1.00 ^e |
| <i>F.culmorum</i> | Fcu4 | Behera | 0.00 | 0.00 | 0.00 ^f |
| <i>F.culmorum</i> | Fcu5 | Giza | 1.93 | 1.63 | 1.78 ^{ab} |
| <i>F.culmorum</i> | Fcu6 | Fayoum | 1.03 | 1.53 | 1.28 ^{bcde} |
| <i>F.culmorum</i> | Fcu7 | Behera | 1.40 | 1.84 | 1.62 ^{abcd} |
| <i>F.culmorum</i> | Fcu8 | Giza | 1.80 | 2.13 | 1.96 ^a |
| <i>F.sambucinum</i> | Fsa1 | Giza | 0.73 | 1.35 | 1.04 ^{de} |
| <i>F.sambucinum</i> | Fsa2 | Behera | 0.00 | 0.00 | 0.00 ^f |
| <i>F.sambucinum</i> | Fsa3 | Fayoum | 1.32 | 1.28 | 1.30 ^{bcde} |
| <i>F.solani</i> | Fso1 | Fayoum | 1.73 | 1.38 | 1.55 ^{abcde} |
| <i>F.solani</i> | Fso2 | Giza | 1.52 | 0.92 | 1.22 ^{bcde} |
| <i>F.solani</i> | Fso3 | Behera | 0.00 | 0.00 | 0.00 ^f |
| <i>F.solani</i> | Fso4 | Giza | 0.00 | 0.00 | 0.00 ^f |
| <i>F.solani</i> | Fso5 | Fayoum | 0.73 | 1.30 | 1.01 ^e |
| <i>F.solani</i> | Fso6 | Behera | 1.60 | 0.90 | 1.25 ^{bcde} |
| <i>F.solani</i> | Fso7 | Giza | 1.30 | 1.10 | 1.20 ^{bcde} |
| <i>F.solani</i> | Fso8 | Behera | 0.00 | 0.00 | 0.00 ^f |
| <i>F. solani</i> | Fso9 | Qalyubia | 0.00 | 0.00 | 0.00 ^f |
| <i>F. solani</i> | Fso10 | Qalyubia | 0.80 | 1.45 | 1.13 ^{cde} |
| <i>F.oxysporum</i> | Fox1 | Fayoum | 0.67 | 1.30 | 0.98 ^e |
| <i>F.oxysporum</i> | Fox2 | Giza | 0.00 | 0.00 | 0.00 ^f |
| <i>F. oxysporum</i> | Fox3 | Qalyubia | 0.00 | 0.00 | 0.00 ^f |
| <i>F. oxysporum</i> | Fox4 | Qalyubia | 0.77 | 1.40 | 1.08 ^{cde} |
| <i>F. oxysporum</i> | Fox5 | Qalyubia | 0.63 | 1.50 | 1.06 ^{cde} |
| <i>F. oxysporum</i> | Fox6 | Qalyubia | 0.00 | 0.00 | 0.00 ^f |
| <i>F.semitectum</i> | Fsem | Qalyubia | 0.00 | 0.00 | 0.00 ^f |

The same letter means no significant differences at level $\alpha=0.05$, according to Duncan's multiple range tests.

3. In vitro assay:

3.1. Effect of the bioagents:

The interaction between *F. culmorum*, the causal of potato dry- rot, and different fungal and bacterial bioagents was assessed seven days after incubation 25

°C. The obtained results showed significant reduction to the growth of the causal fungus due to the effect of the tested bacterial and fungal bioagents .

Results shown in Table (4) show that *T. viride*-1 and *T. harzianum* antagonists were the best treatments, which made the high reduction of mycelial growth of *F.*

culmrum without significant differences, the inhibition percentage were 64.4 and 61.9% respectively followed

by *T.viride*-3, *B. subtilis*-3, *T. viride*-2 making medium inhibition of fungal mycelial of the tested fungus. On the other hand, *B. thuringiensis* was the lowest antagonist one, being 31.1% reduction .While the

3.2. Effect of IRCs:

Data in Table (5) indicate that using of chitosan in four concentrations have a little effect on inhibition of mycelial growth of *F. culmorum*, being 6.67% at 50 mg/L and increased steadily to reach 26.11% at 1000mg/l.

The same trend was noticed by using different concentrations of salicylic acid, being 0% at 10 mg/L and increased steadily to reach 21.11% at 90mg/l. On the other hand, using different concentrations of catechol were have no effect or very weak effect on mycelial growth of tested pathogen. i.e., about 3,3-0% inhibition of the tested pathogen.

lowest pathogenic isolate was *F. oxysporum* (FOX1) from Fayoum governorate , which showed 0.98 cm lesions followed by *F. culmorum* (FCU3) from Fayoum governorate and *F. solani* (FSO5) from Fayoum governorate without significant differences. On the other hand, results showed ten isolates were non-pathogenic, and the other of isolates were differed in their pathogenicity.

4. In vivo assay :

4.1. Effect of bioagents:

The tubers which inoculated and treated individually with five biological agents and stored for 28 days, there was significant differences among treatments as shown in (Fig.1).

Treating tubers with *T. harizianum* and *T. viridi*-2 were the less disease incidence without significant differences in comparing with control, followed by treatment with *T. viride*-1 with significant differences. Whereas, treatments with *B. subtilis* 1,2 have a weak effect on disease reduction.



Fig.1. Dry rot incidence after storage tubers 28-days and treating them with different biological agents.

The treatment with *P. Fluorescens*-1was the more disease incidence with significant differences compared with other treatments.

Table 4. Reduction percentage of mycelial growth of *F. culmorum* due to different bacterial and fungal antagonistic

| No. | Tested bioagent | Reduction (%) |
|-----|--------------------------|--------------------|
| 1 | <i>P. fluorescens</i> -1 | 50.3 ^{cd} |
| 2 | <i>P. fluorescens</i> -2 | 50.0 ^{cd} |
| 3 | <i>P. fluorecens</i> -3 | 48.9 ^{de} |
| 4 | <i>B. thuringiensis</i> | 31.1 ^f |
| 5 | <i>B. subtilis</i> -1 | 51.1 ^{cd} |
| 6 | <i>B. subtilis</i> -2 | 44.4 ^{de} |
| 7 | <i>B. subtilis</i> -3 | 52.0 ^{cd} |
| 8 | <i>B. subtilis</i> -4 | 43.3 ^e |
| 9 | <i>T. viride</i> -1 | 64.4 ^a |
| 10 | <i>T. viride</i> -2 | 51.9 ^{cd} |
| 11 | <i>T. viride</i> -3 | 56.7 ^{bc} |
| 12 | <i>T. harzianum</i> | 61.9 ^{ab} |

The same letter means no significant at level $\alpha=0.05$, according to Duncan’s multiple range tests.

Table 5. Reduction percentage of mycelial growth of *F. culmorum* due to different concentrations of IRCs.

| No. | IRCs | Conc. (mg/L) | Colony diameter | (%) Inhibition |
|-----|----------|--------------|-----------------|----------------|
| 1 | Chitosan | 50 | 8.40 | 6.67 |
| 2 | Chitosan | 250 | 7.55 | 16.11 |
| 3 | Chitosan | 500 | 7.15 | 20.55 |
| 4 | Chitosan | 1000 | 6.65 | 26.11 |

| | | | | |
|----|----------------|-----|------|-------|
| 5 | Salicylic acid | 10 | 9.0 | 0.00 |
| 6 | Salicylic acid | 30 | 8.2 | 8.88 |
| 7 | Salicylic acid | 60 | 7.43 | 17.44 |
| 8 | Salicylic acid | 90 | 7.1 | 21.11 |
| 9 | Catechol | 5 | 9.0 | 0.00 |
| 10 | Catechol | 25 | 9.0 | 0.00 |
| 11 | Catechol | 50 | 8.8 | 2.22 |
| 12 | Catechol | 100 | 8.7 | 3.33 |
| 13 | Control | - | 9 | 0.00 |

4.2. Effect of IRCs:

At the end incubation period of the treated tubers, tubers were cut at the location of inoculation and measure the diameter of lesions.

The obtained results showed high difference between treatments and within same treatments with different concentrations.

Using of four concentrations of chitosan showed steady increased with increase of concentration, and it ranged between 13-87% disease reduction.

With regard to, use different concentrations of salicylic acid displayed little reduction to the disease in first two concentrations (5-7%) compared with second two concentrations which showed high reduction of the disease (62 and 84%). The same trend was noticed by applying four concentration of catechol, where first two concentration have a weak effect (1.3-1.7%) comparing with two second concentration 37-96%.

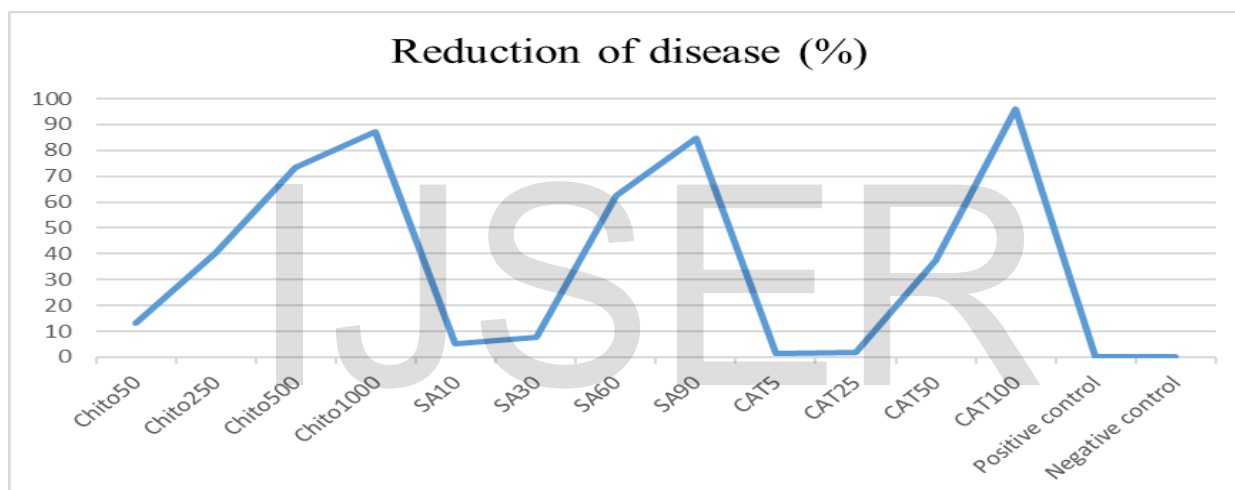


Fig.2. Dry rot incidence after storage the tubers for four weeks and treating them with different IRCs.

Table 6. Effect of three IRCs on development of potato dry-rot.

| No. | IRC | Conc. (mg/L) | Diam. Of lesion (cm) | % Reduction |
|-----|------------------|--------------|----------------------|-------------|
| 1 | Chitosan | 50 | 1.98 a | 13.16 |
| 2 | Chitosan | 250 | 1.36 b | 40.35 |
| 3 | Chitosan | 500 | 0.61 cd | 73.25 |
| 4 | Chitosan | 1000 | 0.29 ef | 87.28 |
| 5 | Salicylic acid | 10 | 2.16 a | 5.260 |
| 6 | Salicylic acid | 30 | 2.10 a | 7.900 |
| 7 | Salicylic acid | 60 | 0.85 c | 62.72 |
| 8 | Salicylic acid | 90 | 0.35 de | 84.65 |
| 9 | Catechol | 5 | 2.25 a | 1.320 |
| 10 | Catechol | 25 | 2.24 a | 1.750 |
| 11 | Catechol | 50 | 1.43 b | 37.28 |
| 12 | Catechol | 100 | 0.09 ef | 96.05 |
| 13 | Positive control | - | 2.28 a | 0.00 |
| 14 | Positive control | - | 0.00 f | 0.00 |

The same letter means no significant at level $\alpha=0.05$, according to Duncan's multiple range tests.

5 DISCUSSION

Potato dry-rot caused by *Fusarium* species is an important disease worldwide which causes post-harvest rotting and seed piece decay after planting (Du *et al.*, 2012).

Isolation trials from potato tubers samples showing dry-rot symptoms collected from four Governorates yielded many fungal isolates. The isolated fungi were purified and identified as *F. solani*, *F. oxysporum*, *F. culmorum*, *F. semitectum* and *F. sambucinum*, which proved their pathogenic capability to potato

tubers. In addition, *F. culmorum* was the most virulent one.

At least, thirteen *Fusarium* species were considered as causal agents of dry rot of potato worldwide (Cullen *et al.* 2005). Five *Fusarium* species, *i.e.* *F. sambucinum*, *F. culmorum*, *F. oxysporum*, *F. semitectum* and *F. solani*, were found during the progress of the present study covering four areas in Egypt. One additional species, *i.e.* *F. equiseti* was previously reported to be associated with potato dry-rot in Egypt with *F. sambucinum* and being the most frequently reported species (Elhassan, 2008). On the other hand, Du *et al.* (2012) identified five different *Fusarium* spp., *i.e.* *F. sambucinum*, *F. avenaceum*, *F. oxysporum*, *F. equiseti*, and *F. acuminatum* out of 260 *Fusarium* isolates were collected in six important potato production regions of northern China.

In vitro twelve isolates of *Bacillus* spp., *P. fluorescens*, and *Trichoderma* spp. were tested for their inhibitory effect against *F. culmorum* and the highest five antagonistic isolates were used to evaluate their ability to reduce potato dry-rot disease.

All the tested bioagents have inhibitory effect to the mycelial growth of the tested fungus on Petri-dishes. The evaluation of different bioagents to inhibit *Fusarium* spp. were tested by many researchers, *i.e.* Sadfi *et al.* (2002); Chérif *et al.*(2002); Recep *et al.* (2009) and Rojo *et al.*(2007).

Five isolates, *i.e.* three isolates of *T. viride*, one isolate of *T. harizianum* and one isolate of *B. subtilis* were the most antagonists bioagents, which caused the highest inhibition to *F. culmorum*. These results are in agreement with those obtained by El-Kot (2008) who reported that *T. harizianum* was the most effective bioagents in suppressing the radial growth of the black scurf and potato dry-rot pathogens. Foroutan (2013) mentioned that inhibition zone could be due to the effect of diffusible inhibitory substances produced by the *Trichoderma* strains, which suppressed the growth of *F. graminearum*. The presence and size of the zone of inhibition have been used as evidence of the production of antibiotics by the *Trichoderma* strains.

There are a lot of studies in the literature indicating that strains of PGPR species (*Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp.) may be used as potential bioagents against potato dry-rot (Schisler *et al.*, 1997 and Sadfi *et al.*, 2002).

The use of bacterial and fungal bioagents to control potato dry-rot has received a great deal of attention, because postharvest conditions provide an ideal role for

bioagents. *Trichoderma* spp. have been developed into several commercial biological control products and are used in field crops and greenhouse systems. These products are known to control numerous soil-borne diseases (Rojo *et al.*, 2007). These findings can be agreed with the obtained findings of Foroutan (2013) who documented the high ability of *Trichoderma* spp. to suppress soil plant diseases.

The capability of *Trichoderma* strains can be related to antibiotic substances such as tubercidin, candicidin, phospholactomycin, phenasin and 4-diacetylphloroglucinol or 2,4-diacetylphloroglucinol, produced by some antagonists like *Pseudomonas fluorescens*, *Streptomyces* spp. and *Trichoderma* spp. (Mazzolla *et al.* 1992).

In this present study, we observed that *Trichoderma* spp. were more effective than *Bacillus* spp. as bioagents to reduce potato dry-rot disease. The different efficacy of the bioagents to the same pathogen could be due to the influence of several factors. These factors include the efficiency of the type or strain of bioagent, the type or aggressiveness of pathogens, the susceptibility of the host to the pathogen(s) (Frances *et al.*, 2006).

In the second part of this study was tested three IRCs, *i.e.* catechol, chitosan, and salicylic acid in four concentrations. Obtained results exhibited that each of catechol (50 and 100 mg/L), chitosan (500 and 1000 mg/L) and salicylic acid (60 and 90 mg/L) were effective to suppress dry-rot development in potato tubers. This may be agreement with the obtained data by Trabelsi *et al.* (2009) who found that chitin and chitosan at 400 µg/ml and BABA and salicylic acid at 30 µg/ml effectively inhibited the development of *Fusarium* dry-rot on potato tubers. Different studies such as those of Fritig *et al.* (1998) showed that concentrations of salicylic acid were ranging from some ten to hundred nanograms per gram of fresh tissue in healthy plants. These levels need equilibrium between the role of salicylic acid as a signal to systemic acquired resistance and its phytotoxic effects of its accumulation. In addition to, chitosan has been proven to control effectively postharvest diseases on various horticultural commodities such as apple, kiwifruit, pear, tomato, table grape, strawberries, raspberries and others. Recent studies have shown that chitosan treatment not only is effective in halting pathogen growth, but also results in marked morphological changes, structural alterations, and molecular disorganization of the fungal cells (Ait Barka *et al.* 2004). Also, it was documented that treatment of susceptible tomato plants with catechol prevented disease symptom expression after infection by *Fusarium oxysporum* f. sp. *lycopersici*. A marked accumulation of total phenols was observed in the catechol-treated plants (Retig and Chet, 1974).

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