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ACTIVITY OF *TRICHODERMA* SPP. AGAINST *ERWINIA CAROTOVORA* CAUSAL AGENT OF POTATO TUBER SOFT ROT

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Abstract

This study was conducted to evaluate the efficacy of *Trichoderma* spp. in restriction *Erwinia carotovera*, subsp *carotovera*, causative agent of potato tuber soft rot. Erwinia growth was highly inhibited by Trichoderma isolates showing high inhibition halo attained to 1.45 cm width *Trichoderma viride* isolate 3 (TV3), and 1.82 cm width with *T. harzianum* isolate 2 (TH2). High significant reduction in disease incidence on potato slices treated with TV3 and TH2 isolates was manifested, 26.25 % and 20.00 % respectively compared with 94.25% in control. Significant reduction in disease incidence in tubers produced from tubers treated with TV3 and TH2 was manifested, 20.27% and 16.47% respectively compared with 90.42% in control. The restriction of Erwinia growth was found associated with increases in plant length, fresh and dry weights in the plant emerged from tubers treated with Trichoderma isolates, 17.58 cm, 23.06 g, 4.60 g for shoot compared with 9.63 cm, 6.95 g, 1.40 g in control respectively, 6.0 cm, 6.43 g, 1.20 g for roots compared with 3.98 cm, 5.15 g, 0.96 g in control respectively in the plant treated with TV3. The three parameters were found to be 16.11 cm, 21.30 g, 4.36 g compared with 9.65 cm, 6.95 g, 1.40 g in control for shoots respectively, 5.66 cm, 5.93 g, 1.2g compared with 3.98 cm, 5.15 g. 0.15 g, 0.96 g in control for root respectively in the plant treated with TH2. Significant increase in leaves chlorophyll content that attaine to 74.16, 59.96, 49.8 in plants treated with TV3, 66.68, 51.44, 39.12 in plant treated with TH3 after 30, 60, 90 days respectively compared with 44.08, 30.52, 28.16 in infected non-treated plants respectively.

Keywords: *Trichoderma spp*, *Erwinia carotovora*, potato tuber soft rot

Introduction

Potato, *Solanum tuberosum*, family Solancum, is considered as one of the most nutritive vegetable crops in the word and main nutritive crop in Europe countries. Potato tubers were reported to contain 17.8% starch, 6.8% sugars, 7.8% protein, 0.39% fats, 3.9% cellulose as well as vitamins and amino acids (Salman and Alaa, 2011).

Potato tuber are subjected to infection with several pathogens including *Erwinia carotovera*, causative agent of soft rot disease in the field and during storage causing high losses in yield (Up to 60%) (Abo-Elyouser *et al.,* 2010; Toth *et al.,* 2011; Ngadze *et al.,* 2012; Montsebo *et al.,* 2014).

Soft rot bacteria survive in soil, volunteer potato, and in plant debris. The bacterial infection occur through wounds and natural openings in the tuber producing a wide range of cell wok degrading enzymes including proteases, celluloses, pectinases, and xylenes responsible of tissue maceration and symptoms development at (Peronbelon, 2002; Prichard *et al.*, 2012). The symptoms of soft rot are manifested as water soaked lesions on tuber, gradually become soft, (mushy, disintegrated and discolored). The tissue within the infected lesions become black, creamy and slimy, and finally, disintegrated (Rahman *et al.*, 2012). The disease caused reduction in plant emergence, weak plants, curling and leaves drooping resembling wilt disease or water deficiency and finally yellowing symptoms may develop (Rosenzweig *et al.,* 2016).

The management of the disease was restricted on the application of copper based compounds to reduce the spread of bacteria to healthy plant. The treatment with copper docs not remedy infected plants and has shown limited effectiveness (Stevenson *et al,* 2001). Therefore the research was oriented toward biological control using non-pathogenic microorganism isolated from plant rhizosphere (bacteria and fungi) for control the disease. It was reported that rhizosphere microorganisms are present in large numbers in plant rhizosphere. Among the microorganisms isolated from plant rhizosphere *Trichoderma* spp. showed high antagonistic activity against many soil borne pathogens (Yedidia *et al.*, 2001; Viterbo *et al.*, 2004).

The study was conducted to evaluate the activity of Trichoderma spp. to control potato soft rot disease.

Material and Methods

Erwinia isolation

Sample of potato tubers showing symptoms of soft rot where collected from the market. The tubers were wounded and placed in plastic container with 15 ml of sterilized water and maintained at 28±2 ºC for 15 days. The tuber showing soft with undesirable odor were placed on nutrient glucose agar (NGA) and maintained at 27±2 ºC for 72 hrs. The growing bacterial colonies were purified and identified as described (Lelliett and Stead, 1987).

Tricoderma isolation

The fungus was isolated from the soil through making serial dilutions 10^{-1}-10^{-5}. One ml of each dilution was spread on potato dextrose agar (PDA) in 9 cm dim petri plates and maintained as 25±2 ºC. The growing fungus colonies were purified by successive transfer on PDA. The fungal isolated were identified based on morphological and microscopical characteristics as described by (Jassim and Abdullah, 2016).

Antagonistic Activates of Trichoderma against Erwinia on Culture Medium

A suspension of each isolated of Trichoderma was prepared by addition a 1 cm dim disc of fungal colony on PDA into 250 ml of potato dextrose broth (PDB) in 500 ml

flask and maintained at 25±2ºC for 3 days. A liquid culture of Erwinia was obtained by transfer a small part of bacterial colony, on Nutrient agar, (NA) into 250 ml of Nutrient broth in 500 ml flask and maintained at 28±2ºC for 48 hrs.

One ml of the bacterial suspension on NB was uniformly spread on the NA medium in each of 9 cm dim petriplate. Disc of filter paper, 0.5 cm dim, dipped in fungal suspension for 10 min were distributed in the plates, 4 discs/plate. Bacterial suspension was added to other plates containing NA for control. Four plates for each isolate were used. The plates were maintained at 25±2ºC for 72 hrs. and the inhibition zones around the discs were measured. The more effective isolate for each Trichoderma species was selected for the next experiments.

Antagonistic activity of Trichoderma isolates TV3,TH2, against Erwinia on potato slices

Healthy potato tubers were service sterilized with 2% sodium hypochlorite for three min, rinsed with sterile distilled water and cut into 1cm thick slices. The slices were placed in sterile petriplates containing sterilized filter paper soaked with sterile distilled water. The slices were inoculated with Erwinia by spreading the bacterial suspension on the surface by sterile inoculation loop. Trichoderma isolated suspension were separately added to each slices with suitable humidity. Potato slices contaminated with Erwinia only were used as control. Four plates for each isolated were used. The plates were maintained at 25±2°C for 7 days and the percentage of infected tissue was estimated by the equation:

$$\%\,\text{Infected tissue} = \frac{\text{Infected tissue}}{\text{Total tissue}} \times 100$$

(Naggash *et al.,* 2016)

Activity of Trichoderma against Erwinia under field conditions

The experiment was carried out in the fields of Suez Canal University/ faculty of Agriculture/ Agriculture plant Department, to control potato soft rot in pots 30x30cm. Potato tubers were soaked in Erwinia suspension for 20min, let to dry in isolation room, and soaked in Trichoderma suspension for 20 min for each isolate separately. Tuber treated with Erwinia suspension and tubers treated with Trichoderma only were used as control. The tubers were cultivated in pots (one tuber/pot). Disease incidence, length of plants, fresh and dry weights, were determined in plants after 120 days of cultivation. The chlorophyll content in leaves were determined in plants after 30, 60 and 90 days of cultivation. The disease incidence was estimated by the equation:

$$\%\,\text{Disease incidence} = \frac{\text{No. of inf ected tubers}}{\text{Total tubers}} \times 100$$

(Masum *et al.,* 2011)

Result

The isolation and identification

The pathogen

The observation of morphological and biochemical tests of the bacteria isolated from potato tubers showing soft rot symptoms indicated that the bacteria is *Erwinia carotovora* supsp *carotovora*. It was reported that *Erwinia carotovora* infect potato tubers causing soft rot disease in the field and during storage (Abo-Elyouser *et al.*, 2010; Toth *et al.*, 2011; Ngadze *et al.,* 2012; Mantsebo *et al.,* 2014).

The biological agent

Two species of Trichoderma isolated from the soil were identified as *T. viride* and *T. horzianum*. Three isolates of *T. viride,* Tv1, Tv2, Tv3, and four isolates of *T. harzianum,* TH1, TH2, TH3, TH4 were obtained.

Antagonistic Activity of *Trichoderma* spp. against *Erwinia carotovora* growth in culture medium

Result Table (1), showed that all Trichoderma isolates caused reduction in Erwinia growth on Nutrient Agar (NA). The width of the inhibition zones around the site of Trichoderma inoculum were 0.60, 0.97, 1.45cm for the isolates, TV1, TV2,TV3 of *T. viride*, 0.80, 1.82, 0.70, 0.37cm for the isolates TH1, TH2,TH3, TH4 of *T. harziznum* respectively compared with 0.00 cm in control. The more effective of the isolates were TV3 and TH2 that used in the next experiments.

Table 1 : Antagonistic activity of *Trichoderma* spp. against *Erwinia carotovora* growth in culture medium

(A) Antagonistic activity of *Trichoderma viride*. against *Erwinia carotovora* growth in culture medium

Treatment ***T. viride***	**Inhibition zone/cm**
TV1	0.6 b
TV2	0.97 b
TV3	1.45 a
Control	0 c
LSD	0.426

(B) Antagonistic activity of *Trichoderma harzianum*. against *Erwinia carotovora* growth in culture medium

Treatment ***T. harzianum***	**Inhibition zone/cm**
TH1	0.8 b
TH2	1.82 a
TH3	0.7 b
TH4	0.37 c
Control	0 d
LSD	0.310

Effective of Trichoderma on *Erwinia carotovora* growth on potato slices

High significant reduction in Erwinia growth on potato slices treated with Trichoderma suspension was manifested. The disease incidences were found, 26.25 and 20.00% for the tow isolates, TV3 and TH2 respectively compared with 94.25% in control Table (2).

Table 2 : Effect of *Trichoderma* spp. on *Erwinia carotovora* growth on potato slices

Treatment	**Infected tissue %**
TV3	26.25 b
TH2	20 c
Control	94.25 a
LSD	4.813

Activity of Trichoderma spp. in restriction of Erwinia growth under field conditions

The treatment of Erwinia contaminated potato tubers with Trichoderma induced high reduction in disease incidence in the tubers obtained. The disease incidence were found to be 20.27% and 16.74% in the tubers produced from tubers treated with TV3 and TH2 isolates respectively compared with 90.42% in control Table (3).

Table 3 : Effect of *Trichoderma* spp. on disease incidence in tubers under field condition

Treatment	Infected tuber %
TV3	20.27 b
TH2	16.47 c
Control	90.42 a
LSD	2.880

Effect of treatment with Trichoderma on plant growth parameters

High increase in plant lengths, fresh and dry weights of shoot and roots, in the plants emerged from tubers treated with Trichoderma, were manifested Table (4). The three parameters were, 17.58cm, 23.06g, 4.6g for the shoot respectively compared with 9.65cm, 6.95g, 1.4g in control (infected) respectively the lengths, fresh and dry weights of root were, 6.0 cm, 6.58g, 1.26g respectively compared with 3.98cm, 5.15g, 0.96g in control respectively in the plants emerged from tubers treated with TV3. The three parameters were found to be, 16.11cm, 21.30g, 4.36g for shoots, 5.66cm, 5.93g, 1.2g for root respectively in plants emerged from tubers treated with TH2 compared with 9.65cm, 6.95g, 1.4g for shoots, 3.98cm, 5.15g, 0.96g for root respectively in control.

Table 4 : Effect of treatment with Trichoderma on plant growth parameters

Treatment	Shoot			Root		
	Weight (g)		Length (cm)	Weight (g)		Length (cm)
	Fresh	Dry		Fresh	Dry	
TV3	24.81 a	5.11 a	19.53 a	7.33 a	1.5 a	6.63 a
TH2	23.66 ab	4.73 ab	18.31 ab	6.58 ab	1.6 ab	6.18 ab
TV3 + E.c	30.06 b	4.6 ab	17.58 bc	6.43 ab	1.26 ab	6 ab
TH2 + E.c	21.3 c	4.36 b	16.11 e	5.93 be	1.2 ab	5.66 be
Control	17.65 d	3.48 c	13.96 d	5.63 be	1.01 b	5.01 c
E.c	6.95 e	1.3 d	9.65 e	5.15 e	0.96 b	3.98 d
LSD	1.635	0.701	1.552	1.166	0.319	0.957

The infection with Erwinia caused high reduction in leaves content of chlorophyll compared with control

The chlorophyll contents were found 44.08, 30.52, 28.18 in infected plants compared with 56.36, 42.48, 38.62 in control after 30, 60, 90 days respectively. The treatment of Erwinia inoculated plants with Trichoderma induced high significant increase in chlorophyll contents that attained to 74.16, 59.96, 49.80 in plants emerged from tubers treated with TV3, 66.68, 51.44, 39.12 in plants emerged from tubers treated With TH2 after 30,60,90 days respectively Table(5).

Table 5 : Effect of treatment with Trichoderma on chlorophyll content

Chlorophyll content			
Treatment	30 Days	60 Days	90 Days
TV3	98.14 a	80.38 a	70.54 a
TH2	91.74 b	70.9 b	56.72 b
TV3+ Ec	74.16 c	59.96 c	49.8 c
TH2 + Ec	66.68 d	51.84 d	39.12 d
Control	56.36 e	42.48 e	38.62 d
E.c	44.08 f	30.52 f	28.18 e
LSD	3.868	5.994	5.156

Discussion

The results of this study demonstrate that potato tubers are highly infected with *Erwinia carotovora* subsp carotovora causing soft rot disease leading to significant losses in potato production. It was reported that potato soft rot caused by *E. carotovora* subsp carotovora is one of important diseases on potato causing great reduction in yield resulting in economic losses in the field, during transit, and in storage (Abo-Elyouserv *et al.,* 2010; Toth *et al.,* 2011; Ngadze *et al.,* 2012; Mautsebo *et al.,* 2014). *E. carotovora* subsp carotovora is listed among the top ten planted pathogenic bacteria (Mansfield *et al.,* 2012).

Erwinia growth was highly inhibited by Trichoderma isolates tested showing inhibition halo on nutrient at different extent. The more effective isolates, TV3 and TH2 caused high reduction in disease incidence on potato slices compared with control.

The activity of *Trichoderma* is restriction Erwinia growth may be through producing substances possessing on agonistic effects against Erwinia. Several studies reported to the activity of *T. virde* in restriction different plant pathogens growth (Bhattocharrya and Purohit 2008, Harman, 2006). The effected of Trichoderma can be resulted from direct parasitism on Erwinia cells followed by penetration the cells and producing enzymes that degrade host cells wall leading to cells death. It was reported that some fungi penetrate host cells through excursion certain enzyme including, chitinase, B-1,3-glucanase and protease (McQuilken and Gemmell, 2004; Al-Jarah *et al.,* 2013).

The treatment of Erwinia contaminated potato tubers with Trichoderma induced high reduction in diseases incidence in tubers produced, associated with promotion of plant growth. The activity of Trichoderma can be the result of direct suppression of Erwinia through antibiosis or secretion lytic enzymes, or indirectly through activation of defense mechanisms in the plant. It has been reported that treatment of points with a variety of agents, biotics or non-biotics can lead to induction of resistance in the plants characterized by restriction of pathogen growth and suppression of disease symptoms development (Pieterse and Van Loon, 2007; Walters, 2010).

Results of this study suggest it may be possible to use Trichoderma spp. as a biological agent to control soft rot caused by *Erwinia carotovora* subsp carotovora in potato.

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