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Biocontrol Agent Talaromyces flavus Stimulates the Growth of Cotton and Potato

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Abstract Beneficial plant-microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility. Some antagonistic fungi have shown great effects toward the growth of plant crops. In this study, two major crops, cotton and potato, were selected to evaluate their growth promotion by the antagonistic fungus Talaromyces flavus. For each plant, five T . flavus isolates were selected from our fungal collection which had shown the highest antagonistic activities against the causal agent of wilt diseases on these plants. In the next step, for every crop, five isolates were used under greenhouse conditions. For evaluation of the plant growth promotion ability of T . flavus isolates, a split-plot trial was arranged in a randomized complete block design with four replications. The main factor was the method of application of T. *flavus* as a soil treatment, a seed treatment, and a combination of both methods. The subfactor was the use of different fungal isolates. Measured parameters were root length, crown length, plant height, plant fresh weight, and plant dry weight. Results showed that the maximum increase in the above parameters was mediated by the seed treatment method. The most

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M. Razavi e-mail: mor845@mail.usask.ca effective isolate for cotton plants was TF-Co-M-23, which increased root length, plant height, plant fresh weight, and plant dry weight by 1.80-, 2.26-, 1.23-, and 1.19-fold, respectively. There were no significant differences among the various treatments affected by T . flavus in terms of crown length. The most effective isolate for potato plants was TF-Po-V-50, which increased root length, crown length, plant height, and plant dry weight by 1.71-, 1.09-, 1.45-, and 3.75fold, respectively. The overall results of this study suggest that it may be possible to promote cotton and potato growth characteristics by using the antagonistic fungus T. flavus.

Keywords Plant growth promotion · Talaromyces flavus · Cotton - Potato

Introduction

Environmental concerns have led to the need for sustainable use of natural resources. Conventional agriculture has had a considerable negative impact on soil and water. It is important to change certain management practices to environmentally cleaner techniques (Sudha and others [2011](#page-6-0)). Sustainable agriculture employs many approaches and techniques to reduce the negative effects of conventional agricultural practices on the environment. One of these strategies is the utilization of soil microorganisms for the promotion of plant growth and control of plant diseases (Botelho and Hagler [2006\)](#page-5-0).

The widespread use of chemical pesticides and fertilizers has been a concern of the public and environmental protection agencies. The harmful chemicals may affect human health, contaminate the environment, and negatively affect biological resources. In addition, their high production cost and the appearance of resistant pests

should also be considered (Zaki and others [1998;](#page-6-0) Heydari and Misaghi [1998;](#page-6-0) [2003\)](#page-6-0).

Some antagonistic fungi play direct and indirect roles in promoting plant growth characteristics. In a direct role, metabolites of antagonistic fungi promote plant growth by providing soluble elements that are necessary for plant nutrition. Indirectly, some microorganisms can act as biological control agents and affect plant growth promotion indirectly through decreasing plant diseases (Le Floch and others [2003\)](#page-6-0).

Talaromyces flavus (Klocker) Stolk and Samson is one of the most important species of antagonistic fungi. This ascomycete is frequently isolated from soil, although it may also occur on organic materials undergoing decomposition (Domsch and others [1980](#page-5-0)). The organic soluble metabolites of this fungus include D-glucono-1,4-lacton; 5-hydroxymethylfurfural; 4,6-dihydroxy-5-methylphthalimide; methyl 4-carboxy-5-hydroxyphethalal dehydrate; hexaketide; 7 hydroxy-2,5-dimethylchromone; 3-hydroxymethyl-6,8-dimethoxycoumarin; altenusin, desmethyldehydroaltenusin, talaroflavone, deoxytalaroflavone, 2-methylsorbic acid, sorbic acid, bromomethylsorbic acid, and bromosorbic acid (Ayer and Racok [1990;](#page-5-0) Wakelin and others [2004](#page-6-0)).

Some of above-mentioned metabolites (2-methylsorbic acid, sorbic acid, bromomethylsorbic acid, and bromosorbic acid) play a fundamental role in the biogeochemical cycling of phosphorus (P) in natural and agricultural ecosystems (Wakelin and others [2004\)](#page-6-0). Harvey and others [\(2009](#page-6-0)) demonstrated that microbiological activity in the rhizosphere could dissolve sparingly soluble inorganic phosphorus and increase plant growth. On the other hand, T. flavus is a biological control agent that has been used in biological control of important soil-borne pathogens such as Verticillium dahliae, V. albo-atrum, Rhizoctonia solani, and Sclerotinia sclerotiorum (Marois and others [1984](#page-6-0)). Naraghi and others [\(2007](#page-6-0); [2010a,](#page-6-0) [b,](#page-6-0) [c\)](#page-6-0) showed that this fungus is a very effective biocontrol agent in controlling Verticillium wilt disease on different crops, including cotton, greenhouse cucumber, potato, and tomato.

Talaromyces flavus is a common mycoflora member of cotton and potato, which are two major crops in the world including Iran. In this study we investigated the possible growth promotion effects of different T. flavus isolates on some characteristics of these crops, including plant height, root length, crown length, plant fresh weight, and plant dry weight.

Materials and Methods

The following experiments were conducted and executed in 2010 in the greenhouses of the Iranian Research Institute of Plant Protection (IRIPP).

Preparation of T. flavus Isolates

For each plant (cotton and potato), five T . flavus isolates were selected from our fungal collection. They had shown the highest antagonistic activities against the causal agent of wilt diseases of these plants in previous studies (Naraghi and others [2007](#page-6-0); [2010b\)](#page-6-0). The isolates for cotton (TF-Co-G-1, TF-Co-N-15, TF-Co-N-20, TF-Co-N-21, and TF-Co-M-23) were obtained from cotton fields in the Razavi Khorasan (Neishaboor) and Ardebil (Moghan) provinces of Iran, and the isolates for potato (TF-Po-V-48, TF-Po-V-49, TF-Po-V-50, TF-Po-V-51, and TF-Po-V-52) were obtained from potato fields in Tehran (Varamin) province (Naraghi and others [2010b](#page-6-0)).

Preparation of T. flavus Inoculum

Preparation of inoculum for every T. flavus isolate was carried out separately as described below.

Talaromyces flavus isolated from soil was cultured in 1.6 cm-wide \times 15 cm-high test tubes containing TF medium [1 l distilled water, 39 g potato dextrose agar (PDA), 2.0 ml of a 50% solution of lactic acid, 100 mg streptomycin sulfate, 50 mg chloramphenicol, 50 mg chlortetracycline, 30 mg nystatine (Mycostatin, 4,960 U mg⁻¹), 4 mg pimaricin, and 0.5 g oxgall (Bile, bovine)]. Five days after culturing, conidial suspension of T. flavus was prepared and 20 ml of this suspension was poured into a sterile plastic bag containing 250 g rice bran. In next step, these bags were incubated in 30 \degree C for germination of *T. flavus* conidia on the rice bran. The content of each plastic bag was emptied after the surface of the rice bran was completely covered with T. flavus hyphae. The number of T. *flavus* ascospores in each gram of rice bran was determined using a hemocytometer. In this procedure, 1 g of rice bran was suspended in 10 ml SDW and the number of ascospores was calculated by counting ascospores in 1 ml of this prepared suspension. T. flavus inoculum was added to the soil in different pots at the rate of $10⁷$ ascospores per g soil (Chet and Baker [1981\)](#page-5-0). For seed treatment preparation, seed potato tubers were floated in this inoculum.

For cotton seed treatment, seeds were coated with antagonistic inoculum (10^7 CFU g^{-1}) at the ratio of 1:3 (v/v). In this step, no adhesive compound was used for seed coating.

Study of the Promotion Effects of T. flavus Isolates on Cotton and Potato Growth Characteristics

The experiment for studying the effect of T . flavus isolates on the growth characteristics of cotton and potato was carried out as described below.

Soil samples (56% sand, 30% silt, 14% clay, 0.25% N, 0.01% P, and 0.11% K) were collected from potato fields in the Karaj area, in the Tehran Province of Iran, and placed in experimental pots after autoclaving. For every plant, the experiment was performed as a split plot arranged in a randomized complete block design with four replications. The main factor was different types of T. flavus treatments at three levels $(1 = soil, 2 = seed, 3 = soil$ and seed) and the subfactor was different fungal isolates at six levels (5 T. flavus isolates and without fungal inoculum).

Inoculated plants were kept at 22° C in the greenhouse under a 12-h light period. Plants were harvested 90 days after sowing for growth evaluation. Measured parameters included root length, crown length (the lower swollen section of the stem), plant height, plant fresh weight, and plant dry weight. Data were analyzed by analysis of variance using MSTAT C statistical software (Michigan State University, 1988), and means were separated by Duncan's multiple-range test.

Results

Study of the Promotion Effects of T. flavus Isolates on Cotton and Potato Growth Characteristics

The effects of the main factor (methods of application of T. flavus), subfactor $(T.$ flavus isolates), and both factors on the growth characteristics of cotton and potato, which were determined separately, are presented below.

The Effects of the Main Factor on Cotton Growth Characteristics

The results of this experiment showed that the effects of the main factor on all growth parameters (root length, plant height, plant fresh weight, and plant dry weight) except crown length were significant (Table 1). According to the results, there were no significant differences among the various methods of application of T. flavus in the effect on root length, plant fresh weight, and plant dry weight. However, the maximum significant increase of plant height was obtained when the method of seed coating was applied (Table 1).

The Effects of the Subfactor on Cotton Growth **Characteristics**

The results of this experiment showed that the effects of subfactor on all parameters (root length, crown length, plant height, plant fresh weight, and plant dry weight) were also significant (Table 2). In this study, most treatments by different isolates of T. flavus mediated significant increases in growth characteristics when compared with control. Among these treatments, maximum and minimum means related to growth characteristics, except root length and crown length, were observed in treatments with TF-Co-M-23 and TF-Co-N-15, respectively (Table 2).The maximum root length mean belonged to treatment with TF-Co-N-21. However, there were no significant differences among T. flavus isolates in the effect on crown length (Table 2).

The Effects of Both Factors on Cotton Growth **Characteristics**

The effects of both factors on growth parameters (root length, plant height, plant fresh weight, and plant dry weight) except crown length of cotton were significant (Table [3\)](#page-3-0). In this study, all treatments except TF-Co-G-1,

Table 1 The effects of the main factor (method of application of T. flavus) on plant height, root length, plant fresh weight, and plant dry weight of cotton plants

Variable source	Treatment	Root length (cm)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)
Main factor	Soil	6.78a	25.25b	13.71a	4.54a
	Seed	6.34a	29.13a	14.05a	4.88a
	Soil and seed	5.94a	22.44c	13.37a	4.20a

In each column values marked with the same letter are not significantly different ($P < 0.01$)

Table 2 The effects of subfactor (different T. flavus isolates) on root length, crown length, plant height, plant fresh weight, and plant dry weight of cotton plants

Variable source	Treatment	Root length (cm)	Crown length (cm)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)
Subfactor	TF -Co-G-1	7.00ab	2.00a	28.00b	13.24ab	4.07bcd
	TF -Co-N-15	4.00c	2.00a	19.00d	12.72b	3.98cd
	TF -Co-N-20	6.33 _b	2.50a	26.33c	13.94a	4.69abc
	$TF-Co-N-21$	7.67a	2.33a	27.83b	13.40ab	4.42bc
	$TF-Co-M-23$	7.50ab	2.50a	29.33a	14.08a	5.05ab
	Control	5.00c	1.50a	15.00e	12.83b	3.23d

In each column values marked by the same letter(s) are not significantly different ($P \lt 0.01$)

Variable source	Treatment	Root length (cm)	Height plant (cm)	Plant fresh weight (g)	Plant dry weight (g)
Both factors	TF-Co-G-1, Soil	8.00b	24.00I	13.55cde	4.56cd
	TF-Co-N-15, Soil	6.50 _{de}	29.00f	13.59cde	4.63cd
	TF-Co-N-20, Soil	6.00e	21.00k	13.80bcd	4.49cd
	TF-Co-N-21, Soil	9.00a	29.00f	13.30def	4.53cd
	TF-Co-M-23, Soil	7.50 _{bc}	29.00f	13.71bcd	4.33ab
	TF-Co-G-1, Seed	6.50de	37.00a	13.55cde	4.47cd
	TF-Co-N-15, Seed	3.50 _g	18.001	12.41gh	4.10de
	TF-Co-N-20, Seed	6.50 _{de}	32.00d	14.30b	5.00 _{bc}
	TF-Co-N-21, Seed	6.00e	36.00b	14.05bc	4.63cd
	TF-Co-M-23, Seed	9.00a	34.00c	15.86a	4.62a
	TF-Co-G-1, Soil and seed	6.50 _{de}	23.00 _i	12.63gh	3.48fg
	TF-Co-N-15, Soil and seed	2.00 _h	10.00n	12.17h	3.21 _g
	TF-Co-N-20, Soil and seed	6.50 _{de}	26.00 _g	13.72bcd	4.58cd
	TF-Co-N-21, Soil and seed	8.00b	18.001	12.83fg	4.13 _{de}
	TF-Co-M-23, Soil and seed	6.00e	25.00h	12.67 fgh	4.19 _{de}
	Control	5.00f	15.00 m	12.83fg	3.23 _g

Table 3 The effects of both factors (method of application of T. flavus and different T. flavus isolates) on root length, plant height, plant fresh weight, and plant dry weight of cotton plants

In each column values marked by the same letter(s) are not significantly different ($P < 0.01$)

Table 4 The effects of the main factor (method of application of T. flavus) on root length, crown length, plant fresh weight, and plant dry weight of potato plants

Variable source	Treatment	Root length (cm)	Crown length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Main factor	Soil	3.31ab	5.22a	85.70a	16.04a
	Seed	3.77a	5.46a	87.00a	17.67a
	Soil and seed	3.24 _b	5.56a	91.11a	17.20a

In each column, values marked with the same letter(s) are not significantly different ($P < 0.01$)

soil and seed; TF-Co-N-15, seed; TF-Co-N-15, soil and seed mediated a significant increase in growth characteristics when compared with control. The maximum increase in root length and plant fresh weight was observed when TF-Co-M-23 was applied as a seed coating (Table 3).

The Effects of the Main Factor on Potato Growth Characteristics

The results of this experiment showed that the effects of the main factor on all parameters (crown length, root length, plant fresh weight, and plant dry weight) except plant height of potato were significant (Table 4). Results also showed that no significant differences were observed among the various methods of application of T. flavus in crown length, plant fresh weight, and plant dry weight. However, the maximum significant increase in root length was obtained when the method of seed coating was applied (Table 4).

The Effects of Subfactor on Potato Growth Characteristics

Results of this experiment showed that the effects of subfactor on all growth parameters of potato were significant (Table [5\)](#page-4-0). Plant height did increase significantly in some cases. The maximum means related to growth characteristics were observed in treatment by TF-Po-V-51 (Table [5](#page-4-0)).

The Effects of Both Factors on Potato Growth Characteristics

The effects of both factors on all growth parameters of potato were significant (Table [6\)](#page-4-0). This experiment showed that TF-Po-V-49, seed; TF-Po-V-50, seed; and TF-Po-V-51, seed mediated a significant increase in growth characteristics when compared with control (Table [6](#page-4-0)). The maximum increase in root length was mediated by TF-Po-V-51 as seed coating (Table [6](#page-4-0)).

Table 5 The effects of subfactor (different T. flavus isolates) on root length, crown length, plant height, plant fresh weight, and plant dry weight of potato plants

Variable source	Treatment	Root length (cm)	Crown length (cm)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)
Subfactor	$TF-Po-V-48$	4.16 _{bcd}	5.08b	63.92c	49.00d	20.40b
	$TF-Po-V-49$	4.35b	6.00a	79.33ab	91.70b	19.27bc
	$TF-Po-V-50$	4.28 _{bc}	6.00a	82.25ab	70.21c	19.71bc
	$TF-Po-V-51$	4.75a	3.60c	89.17a	100.61a	23.18a
	$TF-Po-V-52$	3.13e	5.12b	74.67b	86.85b	16.35d
	Control	2.50f	5.50ab	55.50c	69.42c	7.49e

In each column values marked by the same letter(s) are not significantly different ($P \lt 0.01$)

Table 6 The effects of both factors (method of application of T. flavus and different T. flavus isolates) on root length, crown length, plant height, plant fresh weight, and plant dry weight of potato plants

Variable source	Treatment	Root length (cm)	Crown length (cm)	Height plant (cm)	Plant fresh weight (g)	Plant dry weight (g)
Both factors	TF-Po-V-48, Soil	3.01 g	4.75de	59.00hi	47.76j	26.00b
	TF-Po-V-49, Soil	3.88def	6.00a	71.25ef	84.15e	17.70e
	TF-Po-V-50, Soil	3.83ef	6.00a	93.25a	105.00b	14.58hi
	$TF-Po-V-51$, Soil	3.30 g	3.36 g	90.75ab	104.37b	16.55f
	TF-Po-V-52, Soil	2.30 _{hi}	5.19bc	81.50 cd	76.55f	15.33gh
	TF-Po-V-48, Seed	4.16bcd	4.500e	71.00ef	54.28i	15.80 fg
	TF-Po-V-49, Seed	4.35b	6.00a	85.50bc	62.68 h	24.60c
	TF-Po-V-50, Seed	4.28bc	6.00a	81.00 cd	48.07j	28.10a
	TF-Po-V-51, Seed	4.75a	3.42 g	83.50c	89.09d	24.15c
	TF-Po-V-52, Seed	3.13 g	5.23bc	72.50ef	97.69c	15.30gh
	TF-Po-V-48, Soil and seed	4.25bc	6.00a	61.75 fg	44.97 _i	19.40d
	TF-Po-V-49, Soil and seed	3.72ef	6.00a	81.25 cd	128.25a	15.50 fgh
	TF-Po-V-50, Soil and seed	3.00 g	6.00a	72.50ef	57.57i	16.44 fg
	TF-Po-V-51, Soil and seed	2.15i	4.04f	93.25a	108.38b	28.85a
	TF-Po-V-52, Soil and seed	4.00cde	4.96 cd	70.00ef	86.32de	18.43de
	Control	2.50h	5.50b	55.50i	69.42 g	7.49j

In each column values marked by the same letter (s) are not significantly different ($P \lt 0.01$)

Discussion

The overall results of this study show that it may be possible to promote growth in some plant characteristics by different T. flavus isolates. The results of this study reveal the potential of T. flavus for increasing plant growth in cotton and potato. The type of growth promotion may be similar to that produced by the addition of Trichoderma spp. which has been found to enhance the growth of various plants (Chang and others [1986;](#page-5-0) Paulitz and others [1986](#page-6-0); Windham and others [1986](#page-6-0); Baker [1988](#page-5-0); Kleifeld and Chet [1992;](#page-6-0) Ousley and others [1994a,](#page-6-0) [b](#page-6-0); Phuwiwat and Soytong [1999;](#page-6-0) Harman [2000](#page-6-0); Contreras-Cornejo and others [2009;](#page-5-0) Hajieghrari [2010;](#page-5-0) Masunaka and others [2011\)](#page-6-0).

The increased plant growth induced by Trichoderma spp. has been shown to be dependent on many factors such as plant species, the strain of Trichoderma, the form of inoculum, the concentration of inoculum, and the soil environmental conditions (Paulitz and others [1986](#page-6-0); Baker [1988;](#page-5-0) Kleifeld and Chet [1992](#page-6-0); Ousley and others [1994a](#page-6-0), [b](#page-6-0)).

In this study, it was shown that T . flavus had a significant effect on the growth of cotton and potato by increasing root

length, crown length, plant height, plant fresh weight, and plant dry weight. In this regard, there are previous reports that T. flavus and Penicillium notatum may enhance plant growth directly (Phuwiwat and Soytong [2001;](#page-6-0) Bewley and others 2006). Phuwiwat and Soytong [\(2001](#page-6-0)) showed that P. notatum isolated from rhizospheric soil gave higher plant yields of treated Chinese mustard compared to nontreated plants. The Chinese mustard grown in sterilized planting medium mixed with P. notatum yielded the highest plant growth. Plant height, root length, root diameter, fresh and dry weights of shoot and root, and the total plant dry weight increased gradually as the inoculum of P. notatum was applied.

Penicillium species (including the teleomorphic states of Talaromyces and Eupenicillium) are considered a key group of soil microflora involved in P cycling (Whitelaw [2000\)](#page-6-0). This activity is generally attributable to the production of organic acids that can directly dissolve P precipitates, or chelate P-precipitating cations with the concomitant release of P into solution (Kucey and others [1989;](#page-6-0) Gadd 1999). This could be an important reason for the increase in growth in cotton and potato observed in the present study.

However, many studies have shown that T . flavus is capable of controlling some important soil-borne pathogens such as Verticillium dahliae, Rhizoctonia solani, Sclerotinia sclerotiorum, and S. rolfsii in several crops, including cotton, potato, tomato, eggplant, and bean (Dutta 1981; Madi and others [1997;](#page-6-0) Tjamos and Fravel [1997](#page-6-0); Menendez and Godeas [1998;](#page-6-0) Naraghi and others [2006](#page-6-0)). Naraghi and others $(2010a, b, c)$ reported that T. flavus could reduce Verticillium wilt disease caused by V. alboatrum in potato, tomato, and greenhouse cucumber. Despite the presence of T. flavus in cotton and potato fields of Iran, its population density is not enough to mediate soil nutritional processing. It should be added to the soil as inoculum until it gets active for decomposition, nutrient mobilization, mineralization, and storage and release of nutrients and water (Pandya and Saraf [2010\)](#page-6-0).

There is overwhelming evidence in the literature indicating that plant growth-promoting fungi (PGPF) can be a true success story in sustainable agriculture (Sudha and others [2011\)](#page-6-0). In fact, through their numerous direct and indirect modes of action, PGPF can allow a significant reduction in the use of pesticides and chemical fertilizers. The beneficial effects of PGPF, that is, biological control of diseases and pests, promotion of plant growth, increase in crop yield, and improvement in crop quality, can take place simultaneously or sequentially. Plant age and the soil's chemical, physical, and biological properties will greatly influence the outcome of PGPF inoculation (Hyakumachi and Kubota [2004](#page-6-0); Siddiqui and others [2008](#page-6-0)). Presently, the absence of a universal magic PGPF bioinoculant

formulation for each important field crop simply reflects the complexity of the interactions and of the molecular signal exchanges taking place in the soil-plant-organisms ecosystems (Arora 2004).

It would be interesting to test T . flavus for biological control of important plant pathogens. Further investigations are needed to determine the potential of this fungus in promoting the growth of different crop plants. The effective strains would need to be selected and the suitable concentrations for plant growth promotion must be investigated. Moreover, the soil environmental conditions that are suitable for promoting plant growth also have to be studied.

The overall results of the present study are promising and may be used in the biological fertilization of cotton and potato in the field. Because the tested fungal isolates have shown both antagonistic and plant-growth-promoting properties, their successful application in the field may result in the reduction in the use of chemicals in agriculture and protection of the agricultural environment and biological resources which are very important factors for a sustainable agricultural system.

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