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Bioactive Compounds Produced by *Trichoderma harzianum* 1-SSR for controlling *Fusarium verticillioides* (Sacc.) Nirenberg and Growth Promotion of *Sorghum vulgare*

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ABSTRACT

Twenty sorghum seed samples were collected in 2015 from sorghum fields in southern region of Saudi Arabia and investigated for seed-borne fungi. Thirteen fungal genera and twenty-one fungal species were recorded on sorghum seeds using agar plate technique. *Fusarium verticillioides* was the most common pathogenic fungus in the present survey. In a trial to find out dual-purpose microorganisms, which able to stimulate sorghum growth and control its pathogens with friendly environmental impact 6 species of *Trichoderma* were evaluated. One of them was selected and identified as *T. harzianum* 1-SSR. The high production of indole-3-acetic acid (IAA, 52.57 µg/ml), and total phenols (53.30 µg/ml) as well as the reasonable amount of gibberellic acid (AG3, 34.80 µg/ml) during fermentation process and the reduction of the final culture pH (4.6) compared to other *Trichoderma* isolates introduced explanation for such choice. Under laboratory, *Trichoderma* bioactive compounds (TBA) reduced total infection on sorghum seeds, especially *F. verticillioides* that was completely inhibited and colonized by *T. harzianum*. Inoculation with *T. harzianum* and/or its TBA reduced the pre- and post-emergence damping-off, increased growth and phenol content of sorghum seedling. This in turn may encourage the practical application of such dual-purpose fungus on large scale.

Key words: *Trichoderma harzianum, Fusarium verticillioides*, sorghum, Indole acetic acid, Gibberellic acid.

INTRODUCTION

Sorghum (*Sorghum vulgare*) is an important crop used for human food and animal feeding. Grains are principal sources of several nutritional elements, especially at semi-arid tropics, representing about 30 countries (FAOSTAT, 2016). Industrially, various components and edible oils are manufactured from sorghum grains. Saudi Arabia imports additional varying quantities of sorghum annually (FAOSTAT, 2016).

Several fungi have been reported causing sever diseases for sorghum that affect plant growth, most of them are transported by grains, affecting negatively quantity and quality of the crop yield (Yassin *et al*., 2010). Seed-borne fungi associated externally or internally with the grains lead to deterioration of seeds and reduction of germination, resulting systemic seedling diseases (Rodriguez *et al*. 2006 and Yassin *et al*., 2010).

The phytopathogen; *Fusarium verticillioides* (Sacc.) Nirenberg, is the major worldwide causative agent of stalk rot that can be seed transmitted and causes severe infection to sorghum, leading to complete disintegration of the root and shoot systems (Zummo, 1984), another more serious problem, is the secretion of mycotoxins such as fumonisins, vomitoxin and zearalenone on sorghum grains (Isakeit *et al.,* 2008 and Yassin *et al*., 2010).

Searching for microbes with significant antagonistic impact as well as growth promoting activity has increased. Various microorganisms are able to synthesis numerous valuable bioactive substances, *e.g*. *Trichoderma* species are free-living and/or endophytic fungi that grow vigorously in soil and plant root ecosystems, representing 90% of fungi utilized as biocontrol agents; therefore, they have received much concern as economic and safe biocontrol agents for different pathogens and enhancers of plant defense mechanisms. Additionally, because of their capacity to produce plant growth promoters, they are widely applied in stimulation of plant growth (Saber *et al.,* 2009; Hassan *et al.,* 2013; Vinale *et al*., 2014 and Ezzat *et al.,* 2015).

Plant growth regulators (PGR) have critical role during the life cycle of plants. They are produced within the plant in very low levels and involved in all the stages of plant life from seeds to production of fruits (Bhalla *et al*., 2010 and Boopathi *et al.,* 2013). Phytohormones control several processes such as specific division, elongation and cellular differentiation; moreover, they are necessary in the primary and secondary metabolism processes (Vinale *et al*., 2014). IAA (indoleacetic acid) and GA3 (gibberellic acid) are high-valued well-studied PGR.

Phenolic compounds are another fungal secondary metabolites that known to be participated in a number of biochemical processes, such as redox reactions and stimulation, they include simple phenols, coumarins, most flavenoids, certain amino acids, prosthetic groups of some enzymes, plant pigments and complex derivatives such as lignins (Saber *et al*., 2009 and Ezzat *et al*., 2015). Phenolic compounds are principal protection agents for the growing plants. They are frequently reported as significant antioxidant, anti-tumoral and antibiotics, importantly, therefore, they provide protection from microbial invasion (Apak *et al.,* 2007).

A trial to screen active *Trichoderma* isolates for their potency in biosynthesis of the bioactive metabolites; IAA, GA3 and phenolic compounds was carried out. Also, the most active isolate was applied in greenhouse experiment to evaluate its efficiency on both growth and biological control of *F. verticillioides* on *Sorghum vulgare* plants.

MATERIALS AND METHODS

Trichoderma isolates

Trichoderma sp.1 and sp.2 were obtained from Prof. W. E. A. Saber, Microbial Activity Unit, Microbiology Department, Research Institute of Soils and Water and Environment, Agricultural Research Center, Egypt, and *Trichoderma* sp. 3, sp. 4, sp. 5 and sp. 6) were obtained from Prof. K. M. Ghoneem, Department of Seed Pathology, Research Institute of Plant Pathology, Agricultural Research Center, Egypt. The fungi were preserved (at 4°C) on slants of potato dextrose agar (PDA) after growing at 28±2°C for one week. These *Trichoderma* isolates were inoculated in 50 ml Czapek broth supplemented with 1% tryptophan. The medium was inoculated with one disk (0.5 cm) from the edge of 5 days old culture grown on Czapek agar medium. Incubation (30°C) lasted for seven days under shaking at 150 rpm. The filtrate was separated from fungal mycelia at 5000 rpm for 15 min. The filtrates containing the *Trichoderma* bioactive compounds (TBA) of the 6 *Trichoderma* isolates and/or their spore suspension (10^8spore/ml) were used during the subsequent investigations.

Detection of TBA of the tested isolate

Filtrates of the six *Trichoderma* spp. were evaluated for the biosynthesis of IAA according to the procedure of Glickmann and Dessaux (1995), GA3 by the procedure of (Holbrook *et al.*, 1961) and total phenols by the procedure reported by (Malik and Singh, 1980**)**. Final culture pH was measured in the fermented broth using pH-meter with glass electrode (HI 9321 microprocessor pH-meter).

Detection of sorghum seed-borne fungi

Sorghum seed (20 samples, 1 kg each) were collected from sorghum various fields of different districts of Southern region of Saudi Arabia, including Jizan, Abha and Najran, during the harvesting season of 2015. Seeds were brought in sterile plastic bags, and kept at 4°C. All the samples were mycologically analyzed. Mycological analysis of sorghum seeds was performed as follows; the surface of sorghum seeds was sterilized by sodium-hypochlorite (2%) for 3 min two times with sterile distilled water, then 10 seeds were placed on Potato dextrose agar plates. Incubation (20±2°C) was carried out with alternating cycles light (12h) and darkness (12h), using white fluorescent light. After 7 days, stereoscopic and compound microscopes were utilized to examine and identify the developed fungi on plates (ISTA, 2007). Isolation of the pure fungal cultures was performed by hyphal-tip and/or single-spore techniques. Obtained fungi were grown and saved on potato carrot agar slants. Identification was done according to (Raper and Fennel, 1965; Ellis, 1971; Booth, 1977 and Domsch *et al.*, 1980). Average intensity percentage of infected samples by a fungus (I%) and frequency occurrence percentage of a fungus on the samples (F%) were calculated using the following formulas:

$$I\% = \frac{\Sigma \text{ fungus incidence in examined samples}}{\text{Number of investigated samples}} \times 100$$

$$F\% = \frac{\text{Number of infected samples with the fungus}}{\text{Number of investgated samples}} \times 100$$

Germination and pathogens of sorghum seeds as affected by TBA

Influence of TBA of *Trichoderma* spp., on germination and fungal infections of sorghum seeds was investigated. Out of the previously investigated twenty-seed samples of sorghum, 3 samples had lowest germination (~60%) and highest infections with fungal pathogens were selected. The seeds were soaked in each of the prepared *Trichoderma* filtrate for 24 hrs. Another sample was soaked in Rhizolex-T (2.5 g kg^{-1} seeds) for 1 hour, in addition to the untreated seeds that served as controls. The seeds were drained out in a Laminar flow chamber for 1 hour before plating on a wet blotter. Seeds were grown and the recovered fungi were detecting, following the International Seed Testing Association **(**ISTA, 2007**)**. In this respect, 25 seeds were placed per Petri dish containing moist filter paper. Four hundred seeds of a 100 seeds per replicate were used for each treatment. The Petri dishes containing seeds were kept at 22±2°C for one week under cycles of darkness (12 h) and light (12 h). Observation for the incidence of fungal pathogens was made under stereomicroscopes. In some cases, fruiting bodies and spores were examined using the compound microscope to achieve accurate identification.

Antifungal activity

Antagonistic activity of *Trichoderma* sp.1 was carried out against *F. verticillioides,* using the dual culture test on PDA (Difco, USA). A 5 mm diameter mycelial disc of the bioagent and pathogen, taken from 5-days old culture, were paired at opposite ends on 9 cm diameter PDA plates. Both fungal discs were place at the same distances from the edge of the plate. Individually inoculated plates by the antagonist fungus or the pathogen was used as controls, and then incubated at 25±2 ºC.

Identification of *Trichoderma* sp.1

Trichoderma sp. 1 was selected as the best bioagent for both TBA producer and antagonist fungus for the phytopathogen *F. verticillioides*. Identification was carried based on properties and morphology of fungal culture, and microscopic investigation (Domsch *et al.*, 1980 and Gams and Bissett, 2002).

Greenhouse experiment

The pathogen (*F. verticillioides*) and the antagonistic fungus (*T. harzianum*) were individually cultured on PDA plate and incubated at 25±2°C. Three days later, plugs of mycelia of each fungus were separately inoculated sterilized medium of sorghum seeds: coarse sand: water (2:1:2, v/v), incubation lasted for 10 days, at room temperature; this was the standard inoculum. Plastic pots of 20-cm diameter containing 2 kg sterilized soil (2 clay: 1 sand. v/v) with 1% NaOCl. Sorghum seeds were prepared by sterilization in 1% sodium hypochlorite, followed by 2-time washing with sterilized water. Some pots were infected singly with the previously prepared pathogen (P) inoculum (0.4%, w/w). The soil was mixed well with *F. verticillioides* and regularly irrigated with sterilized water and left for one week to ensure even distribution of the pathogen. Pots inoculated with *T. harzianum* (T) received 0.4% (w/w) of the inoculum. The chemical treatment was carried out by soaking sorghum seeds in Rizolex-T (50%) (tolclofos methyl-thiram, Sumitomo Chemical Co., Japan) at 2.5 g. kg^{-1} seed for 1 h and then dried using sterilized paper. Another set of pots of disinfected soil with the pathogen were either infected by the antagonistic fungus (P+T) or the seeds were soaked in *Tichoderma* bioactive compounds (TBA) for 24 h, that previously prepared as mentioned above. Finally, a combination of P+T+TBA was applied in another set of treatment. Pots of disinfected soil and untreated seeds were used as a control. Accordingly, the treatments applied in this study were; 1) non-treated control; (2) infection with *F. verticillioides* alone (P); (3) inoculation with *T. harzianum* only (T); (4) treatment with Rizolex-T and infection with *F. verticillioides* (Rhizolex + P); (5) T+P; (6) soaking in and infection with the *F. verticillioides* (TBA+P) and (7) T+TBA+ P. 10 seeds for each pot were grown in a greenhouse for 40 days at temperature ranged between 20–30°C. Ten replicates were used for each treatment.

Assessments of disease, growth and total phenol content

Under greenhouse conditions, the daily data of damping off were recorded on the base of the percentage of rotted seeds (pre-emergence), and percentage of infected seedlings (post-emergence) as well as the survival plants was recorded. After 40 days from sowing, the lengths of root and shoot and fresh weight were measured. The total phenols content in sorghum plant was determined according to (Malik and Singh,1980).

Statistical analysis

Analysis of the data, including ANOVA test of the completely randomized block design, and post comparison, as well as the simple correlation coefficient, were performed using CoStat program (CoHort Software, U.S.A) version 6.4 at level of probability (P)≤ 0.05.

RESULTS AND DISCUSSION

Six *Trichoderma* spp. were screened for the secretion of various plant hormones as well as the total phenol production and the final culture pH (Table 1). Obviously, *Trichoderma* sp. 1 recorded significant superior in IAA and total phenols production, compared to the other tested *Trichoderma* spp. Conversely, *Trichoderma* sp.5 was the highest in GA3 production with significant variance, in this respect. An important non-direct indication for the production of phytohormones was the final culture pH, in this respect *Trichoderma* sp.1 and 2 showed lowest values, indicating the presence of substances with acidic nature, *e.g.* IAA and/or GA3 in the filtrate of both fungi.

Trichoderma spp. are known as plant growth promoting fungi, they are able to produce various bioactive secondary metabolites, which stimulate plant growth and protect it from being infected with various phytopathogens (Martínez-Medina *et al.*, 2014). Similarly, various fungal species have been reported to secrete GAs in their culture medium, *e.g. Gibberella fujikuroi*, *Sphacelomam anihoticola* (Bomke *et al.*, 2008), *Scolecobasidium tshawytschae* (Hamayun *et al.*, 2009) and *Paecilomyces formosus* LHL10 (Khan *et al.*, 2012). Moreover, other *Trichoderma* spp. were detected to produce total phenol in their culture supernatant, *e.g. T. koningi* MTCC 796,*T. hamatum* NBAII Tha 1 and *T. harzianum* NBAII Th 1, however, positive

Table (1): The potentiality screening of the different *Trichoderma* spp. for the production of bioactive compounds *in vitro*

Isolates	IAA (µg/ml)	GA3 (µg/ml)	Total phenol (µg/ml)	Final culture pH
Trichoderma sp. 1	52.57[a]	34.80[b]	53.30[a]	4.6[c]
Trichoderma sp. 2	35.33[b]	0.00[c]	43.13[b]	4.6[c]
Trichoderma sp. 3	0.00[c]	0.00[c]	40.97[b]	5.0[b]
Trichoderma sp. 4	0.00[c]	0.00[c]	36.07[c]	5.8[a]
Trichoderma sp. 5	0.00[c]	37.41[a]	12.63[d]	5.7[a]
Trichoderma sp. 6	0.00[c]	0.00[c]	12.67[d]	5.8[a]

Same letter within a column, indicate non-significant difference

correlations among pathogen inhibition, coiling pattern of antagonist, and total phenol content were recorded (Gajera *et al.*, (2012).

Regarding to the reduction in the surrounding environmental pH as a result of *Trichoderma* spp., Martínez-Medina *et al.* (2014) stated an explanation on the base of the secretion of organic acids that resulted from the ability of *Trichoderma* to metabolize various carbon sources, mainly glucose, which reduces the pH of the surrounding environment, this have additional benefits, *i.e.* solubilization of various elements to available form. The relationships among the previously tested variables were measured in termed of simple correlation coefficient (analysis not shown). All possible relationships were calculated. IAA was significantly correlated with total phenol and final culture pH, the relation was positive with total phenol (0.720) and negative with final culture pH (-0.836). The total phenol and the final culture pH recorded negative significant correlation (-0.821). On the other hand, the other relations showed non-significant correlation.

Seed-borne fungi of sorghum

Mycological analysis of sorghum seeds was performed to explore the intensity percentage of every isolated fungus on the sorghum samples (I%) as well as the frequency percentage of each fungus on the tested samples (F%). A total of 21 species, representing 13 genera of fungi, was recorded from the collected sorghum seed samples by the agar plate technique (Table 2). *F. verticillioides*, *Penicillium* spp., *Aspergillus flavus*, *Curvularia lunata* and *Rhizopus stolonifer* were the most common fungi. *F. verticillioides* was the most common among all *Fusarium* species (80%), followed by *F. incarnatum* (20%) and *F. chlamydospoum* (10%). Moreover, high incidence of pathogenic fungus, *C. lunata* (70%) on sorghum seeds, while low incidence of *Bipolaris sorgicola* (20%) was recorded. The presence of pathogenic fungi in sorghum seeds, even in low levels, could be harmful to whole seed lot. Beyond seed rots and seedling blights caused by these fungi, their presence at high levels in seeds is an indication for several problems (*i.e.* the secretion of mycotoxins, for instance, *F. incarnatum* and *F. verticillioides* have been known to produce deoxyninalenol, zearalenone, fusaric acid and trichothecene. *Aspergillus flavus* is known to secret aflotoxin (B1, B2, G1 and G2) but *Alternaria alternata* produces alternariols). These toxins are very carcinogenic and may cause liver cancer in human and livestock animals, as well as weight loss in cattle and poultry, leading to economic casualties for the producers. However, *F. verticillioides* was the most frequent in the present study, and is known pathogenic fungus on sorghum seeds worldwide (Isakeit *et al.*, 2008 and Yassin *et al.*, 2010).

Laboratory evaluation of TBA on sorghum seeds

For evaluation of *Trichoderma* bioactive compounds (TBA) as a biocontrol agent for seed-borne pathogenic fungi as well as their impact on seed germination of sorghum, 3 seed samples having low germination and high infection were soaked in TBA and then examined on wet blotters under laboratory conditions.

Effect of TBA on sorghum seed-borne pathogens

Data of Table (3) indicate that all tested TBA of the 6 isolates significantly reduced the total infection by the seed-borne mycoflora on sorghum seeds as compared to control. TBA obtained from *Trichoderma* sp. 1 was superior, recording (58.1%) reduction in total infection, in comparison to the control treatment. TBA obtained from *Trichoderma* sp. 1 was also superior, without significant differences between the fungicide, in reducing the incidence of *F. verticillioides* pathogen, down to (7.3%), as compared to control (23.7%). Moreover, the incidence of *B. sorgicola* was not affected by any of the tested TBA as well as the fungicide treatments. While insignificant variation was detected between TBA treatments in reducing the incidence of *A. niger* and *A. alternata*. Additionally, TBA treatments came next to fungicide, in reducing the incidence of *C. lunata*. The tested fungicide gave highest reductions in the incidence of most of saprophytic fungi *e.g.*, *A. flavus* and *R. stolonifer* (0 %). Several reports indicated that treatment of seeds of various crops

Table (2): Mycological evaluation of the occurrence of sorghum seed-borne fungi using agar plate method

Fungus	Frequency on the samples (F%)	Intensity of a fungus (I%)
Alternaria alternate	50	2.0
Aspergillus albicans	10	0.2
A. flavus	70	8.3
A. glaucus	30	1.3
A. nidulans	10	0.4
A. niger	40	5.0
A. ochraceus	20	0.2
A. terreus	20	0.1
Bipolaris sorgicola	20	0.6
Cephalosporium acremonium	10	0.1
Chaetomium sp.	40	0.8
Cladosporium cladosporioides	50	3.6
Curvularia lunata	70	20.1
Fusarium chlamydosprium	10	0.1
F. incarnatum	20	0.6
F. verticillioides	80	3.0
Nigrospora oryzae	20	1.0
Penicillium spp.	80	1.1
Rhizopus stolonifer	70	1.4
Trichoderma harzianum	50	1.1
Trichothecium roseum	20	0.3

Table (3): Effect of seed treatment with TBA on prevalence of seed-borne fungal infections of sorghum

Treatments	*F. verticillioides*	*Curvularia lunata*	*Rhizopus stolonifera*	*Bipolaris sorgicola*	*A. flavus*	*A. niger*	*Penicillium* sp.	*Alternaria alternata*	Total infection	Reduction (%)
Control	23.7 a	47.0 a	2.7 bc	1.5 a	9.3 a	1.5 a	1.5 a	3.0 a	90.2	0.0
Trichoderma sp. 1	7.3 bc	17.3 b	3.0 b	0.0 a	4.7 a-c	1.7 a	0.5 a	3.3 a	37.8	58.1
Trichoderma sp. 2	8.3 b	32.0 b	5.7 a	0.0 a	5.7 a-c	2.0 a	0.3 a	0.0 a	54.0	40.1
Trichoderma sp. 3	8.0 b	26.7 b	2.7 bc	0.0 a	2.7 bc	0.7 a	1.7 a	2.3 a	44.8	50.3
Trichoderma sp. 4	11.7 b	23.0 b	6.0 a	0.7 a	2.0 bc	0.0 a	0.3 a	0.7 a	44.4	50.8
Trichoderma sp. 5	9.3 b	29.0 b	5.3 ab	1.0 a	8.3 ab	0.7 a	4.3 a	0.3 a	58.2	35.5
Trichoderma sp. 6	12.0 b	26.0 b	2.7 bc	0.3 a	2.3 bc	0.3 a	0.3 a	1.3 a	45.2	49.9
Rhizolex	1.7 c	2.3 c	0.0 c	0.0 a	0.0 c	0.0 a	0.0 a	0.0 a	4.0	95.6

Same letter(s) within a column, indicate non-significant difference

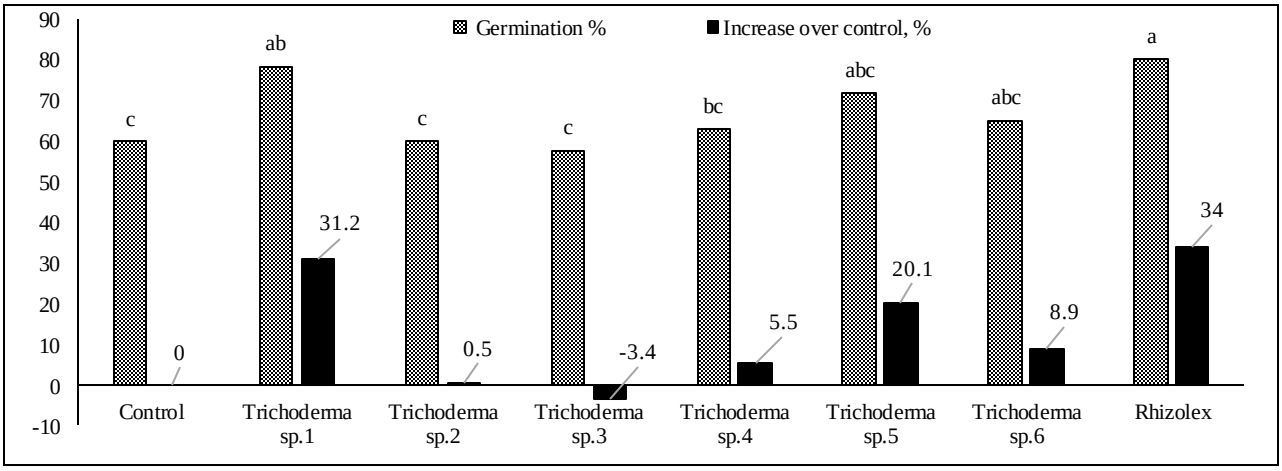

Fig. (1): Influence of TBA on germination of sorghum seed.

Columns designated by similar letter(s) are not significantly different.

with *Trichoderma* spores or its filtrate effectively suppressed several plant pathogens (Asaduzzaman*et al*., 2010). This suppression action of *Trichoderma* may be back to the several bioactive compounds that are involved in *Trichoderma*-pathogen interaction, for example, β-glucanase, chitinase and proteinases were reported to degrade the host cell wall (Munir *et al.*, 2013).

Effect of TBA on germination of sorghum seed

Results of Fig. (1) show that most of TBA treatments effectively enhanced the germination percentage, compared to control. Among all TBA treatments, *Trichoderma* sp.1 as well as the fungicide exhibited the highest significant enhancement of germination percentages; being 78.3 and 80.0%, recording 31.2 and 34%, increase over the untreated control, respectively**.** *Trichoderma* sp. 5 came next in this respect. TBA of *Trichoderma* sp. 3 was the only exception, where it recorded lower germination (57.7%) that was reduced by 3.4%, compared to the control.

Treatment of seeds with *Trichoderma* spores or its filtrate effectively increased seed germination percentages, vigor index, growth and yield by suppressing the plant mycoflora associated with the seeds that could kill the embryo of the seeds (Asaduzzaman *et al.*, 2010). However, separation of seeds from *Trichoderma* using a cellophane layer led to the same increment in seed germination that leads to the conclusion that seed germination is greatly mediated by the growth factors secreted by *Trichoderma* (Benítez *et al.* 2004). Recently, *Trichoderma* spp. have been detected to produce various plant hormones, *e.g.* GA3 and IAA, which are reported to maximize the germination of seeds (Bhalla *et al.*, 2010; Boopathi *et al.*, 2013 and Martínez-Medina *et al.*, 2014). Additionally, *Trichoderma* fungi can secret several organic acids, which acidify their medium, leading to increase the solubilization of the complicated minerals (Martínez-Medina *et al.*, 2014).

Trichoderma sp. 1 was selected for further application. There are several evidences for the selection of such fungal isolate. As it could be seen, *Trichoderma* sp.1 was found to be the active strain in the various tested phytohormones. The values of IAA, GA3 and total phenols were 52.57, 34.80 and 53.30, respectively. These were accompanied with significant reduction in the final culture pH (4.6). Additionally, induced seed germination and significantly reduced the overall infection on seed sorghum by the various mycoflora, especially *F. verticillioides*, which is highly occurred on sorghum seeds.

In vitro antagonism between *Trichoderma* sp. 1 and *F. verticillioides*

Antagonistic properties of *Trichoderma* sp.1 was tested against *F. verticillioides* through dual Petri plate method. The assay showed a pronounced reduction of the mycelial growth of *F. verticillioides* by (74.8%) after 5 days of incubation. However, several days later *Trichoderma* mycelia completely grow and colonize over *F. verticillioides* (Fig. 2). In this respect, Saber *et al.* (2009) reported that once the pathogen and *Trichoderma* contact, the later attaches and parasites the pathogen, by the secretion of enzymes that degrade the pathogen cell wall and other antifungal agents, leading to the decomposition of the cell walls of the pathogen.

Trichoderma sp. 1 was identified as *T. harzianum* 1-SSR. It shows potential activity as TBA producer and antagonist fungus for the phytopathogen *F. verticillioides*.

Greenhouse evaluation of *T. harzianum* 1-SSR

For practical confirmation, the application of the selected *T. harzianum* 1-SSR was carried out in greenhouse trial, to investigate its effect on the pathogenic parameters caused by *F. verticillioides*, in addition to its effect on growth and physiological activity of sorghum plants.

In-vivo control of *F. verticillioides*

Controlling of the most common and aggressive seed-borne pathogen *e.g.*, *F. verticillioides* of sorghum was carried out under greenhouse conditions. *T. harizanum* 1-SSR was chosen according to *in vitro* experiments. Obtained data (Table 4) on disease parameters indicated that all tested biological treatments showed significantly improvements in reducing plant disease severity compared to infected control. However, under infection, seed soaking in TBA prior to sowing and *Trichoderma* added to soil or their combination came next to fungicide, in significantly reducing rotted seeds syndrome as compared to infected control. Soil application of *T. harzianum*1-SSR even in the presence of infection showed the highest reduction of post-emergence damping-off syndrome as compared to infected control. On the other side, maximum significant increase was observed in seedlings survivals due to soil application by *T. harizanum* 1-SSR (P + T) or its combined application with seed treatment by TBA. *Trichoderma* spp. can induce plant resistance locally or systemically, moreover, many *Trichoderma* spp. were found to induce the emergence of seedlings (Benítez *et al.*, 2004 and Hassan *et al.*, 2013) by several secondary metabolites, like hydrophobins, koninginins and viridians that were also reported to enhance root development and/ or resistance of plant (Munir *et al.*, 2013 and Vinale *et al.*, 2014).

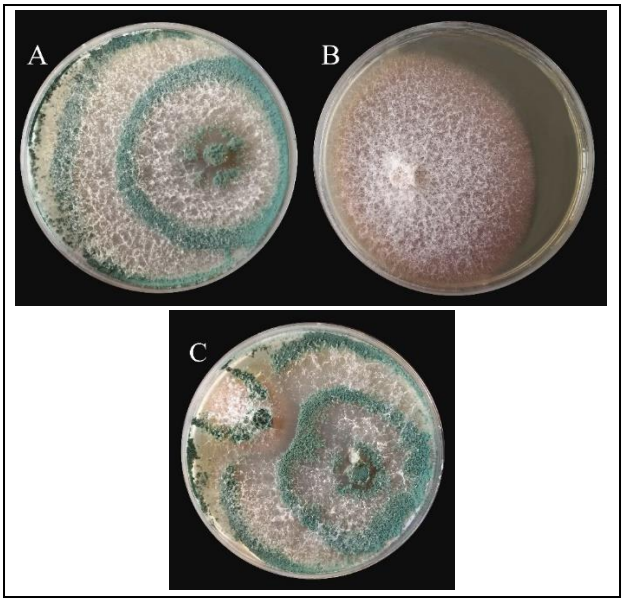

Fig. (2): Single growth of *Trichoderma* sp. 1 (A) and *F. verticillioides* (B) on Petri-plates, as well as the dual culture of both fungi (C) showing the over growth of *Trichoderma* sp. 1 on *F. verticillioides*

Table (4): Pathogenicity and control of *F. verticillioides* by *T. harzianum* and its bioactive compounds under greenhouse

Treatment	Survival seedlings (%)	Emergence damping off, %	
		Rotted seeds (%)	Infected seedlings (%)
Control	90^{a}	10^{d}	0c
F. verticillioides (P)	46^{e}	41^{a}	13^{ab}
T. harzianum (T)	88^{a}	12^{d}	0^{c}
Rhizolex + P	58^{d}	31^{b}	11^{ab}
P + T	73^{b}	20^{cd}	7^{bc}
TBA + P	62^{cd}	23^{c}	15^{a}
P + T + TBA	70^{bc}	19^{cd}	11^{ab}

Same letter(s) within a column, indicate non-significant difference ($P \leq 0.05$)

Table (5): Greenhouse evaluation of *T. harzianum* and its bioactive compounds on sorghum seedlings growth parameters and phenol content under infection by *F. verticillioides*

Treatments	Vegetative growth			Total phenol (mg 100 g^{-1} fresh wt.)
	Length of root (cm)	Length of shoot (cm)	Shoot fresh weight (gm)	
Control	24.7^{ab}	15.7^{c}	0.80^{b}	205.18^{b}
F. verticillioides (P)	6.40^{e}	9.1^{d}	0.28^{cd}	59.47^{f}
T. harzianum (T)	26.5^{a}	25.5^{a}	1.20^{a}	247.35^{a}
Rhizolex + P	13.6^{d}	9.8^{d}	0.14^{d}	95.28^{e}
P + T	18.3^{cd}	15.0^{c}	0.54^{bc}	148.91^{c}
TBA + P	17.4^{cd}	14.9^{c}	0.63^{b}	121.36^{d}
P + T + TBA	20.5^{bc}	18.9^{b}	0.70^{b}	192.92^{b}

Same letter(s) within a column, indicatenon-significant difference ($P \leq 0.05$)

Morphological characters and total phenol contents

Significant differences were recorded on growth parameters (root and shoot length, and fresh weight of shoot) of sorghum seedlings (Table 5) when treated with *T. harzianum*. The single application of *Trichoderma* came in the first order in increasing seedlings growth parameters. However, under infection with the pathogen, combined soil applications of *T. harizanum* with soaking seeds in its TBA, resulted in the highest increase of seedlings growth parameters. Regarding to total phenol content, the obtained results indicated that under infection with the pathogen, combined *Tichoderma* treatments (P + T + TBA) led to a significant increase in total phenol contents in shoots (192.92mg 100 g^{-1}), followed by soil application by *T. harizanum* 1-SSR treatment (148.91 mg 100 g^{-1}), when compared to untreated-infected control (59.47). Moreover, the single application of *T. harzianum* (T) were found to be superior in all tested criteria, indicating the validity of such isolated for improving growth and physiological activity of sorghum plants. The infection with *F. verticillioides* alone reduced vigorously the survival seedlings, this was expected, since the pathogen was previously reported to destroy the root system and eventually infect stalks, later, the root and stalk tissue completely disintegrated (Zummo, 1984). On the other hand, presence of *T. harizanum* prevented the previous symptoms to develop on the sorghum seedlings. *Trichoderma* spp. have been reported to increase the various growth parameters as well as production of toxic metabolites that act as inhibitors to wide range of phytopathogenic microorganisms (Hassan *et al.*, 2013 and Vinale *et al.*, 2014). In this respect various workers (Apak *et al.*, 2007; Saber *et al.*, 2009 and Ezzat *et al.*, 2015) reported that infection in certain diseases is characterized by increased synthesis of certain precursors of phenolic compounds and oxidation products of phenolics, such as quinines, which exhibit more toxicity to the pathogens invasion. In many instances, positive correlation was found between the amount of phenol content and degree of resistance to plant disease. Moreover, *Trichoderma* spp. are widely known as endophytic fungi that could inhabit inside plant tissues, implying a symbiotic interaction without any damage to the host plant (Hassan *et al.*, 2013 and Nicoletti and Fiorentino, 2015). This symbiosis can stimulate plant growth and its physiological status and protect plants against biological (pathogen) and non-biological stresses, because of its ability to produce several kinds of bioactive metabolites (Khan *et al.*, 2012). Of these bioactive compounds, IAA and GA3 that play critical roles in initiation of lateral and adventitious root and emergence, as well as in development of shoot by activating an auxin-dependent mechanism and/or producing IAA, which subsequently, induce plant growth and crop production (Bhalla *et al.*, 2010; Boopathi *et al.*, 2013 and Vinale *et al.*, 2014).

Inoculation of the rhizosphere area with beneficial microorganisms, may supply plants with a continual source of biologically active metabolites, through the production of auxins in the area of plant roots, as a result, improving the plant development during all growth stages. Oppositely, one-time utilization of these compounds has limited advantages, suggesting that microbial application in the area of plant roots may guarantee the presence and raise the advantages of the secreted auxins, hence maximize the response in the host plant (Vinale *et al.*, 2014). The presence of in situ source of such TBA is favorable, as this source is acting as continuous one.

Evidently, the pollution and economic problems of condensed application of fungicides and plant growth promoters could be easily managed by single application of the proposed dual-purpose bioagent; *T. harzianum*. The single application is useful from different points of view: economically one-time application will reduce the production cost compared to two or more applications, environmentally, minimal application of fungicide and importantly, the presence of the bioagent *T. harzianum* in the rhizosphere of sorghum will guarantee continuous supplementation of TBA during the growth of the plants as well reduce or prevent the harmful effect of the various pathogens in soil.

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