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The Effect of *T. harzianum*isolates and AVG on growth of fenugreek Seeds Under Laboratory Conditions

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بسم الله الرحمن الرحيم

يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا يَذَّكَّرُ إِلَّا أُولُو الْأَلْبَابِ

صدق الله العلي العظيم

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1. Introduction

Fenugreek (*Trigonellafoenumgraecum L.*) is an annual plantthat belongs to the family fabeacese. It is the famous spices inhuman food. The seeds and green leaves of fenugreek are usedin food as well as in medicinal application that is the old practiceof human history. It has been used to increase the flavoring andcolor, and also modifies the texture of food materials. Fenugreek is known for its pleasantly bitter, slightly sweet seeds. The seeds are available in any form whether whole or ground form is used to flavour

many foods mostly curry powders, teas and spice blend. Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Betty, 2008).

The chemical constituents of fenugreek include fibers, saponins, flavonoids, fixed oils, and alkaloids, namely, trigonelline and choline (Shailajan*et al*., 2011). Additionally, the steroidal sapogenindiosgenin is a secondary metabolite produced by fenugreek and other plants that are widely studied due to its bioactive properties in the medical field (Jesus *et al*., 2016). The nutritional benefits and curative applications of fenugreek as an effective therapeutic agent against inflammation and diseases, such as cancer and diabetes, have been reported (Syed *et al*., 2020).

Trichoderma species are widely used in agriculture to stimulate plant growth and to control different pathogens affecting crops, representing useful tools for sustainable food production (Ferreira and Musumeci, 2021.).

*Trichoderma*has an enormous capacity to produce secondary metabolites (Zeilinger*et al.*, 2016); thus, playing an important role in ecosystem health (Rajput *et al*., 2014). The efficacy of *Trichoderma* spp. has been identified as effective plant-beneficial microbial inoculants, these fungi act as bio-stimulants that promote plant growth (Lombardi et al., 2020). Plant associations with *Trichoderma* strains have been shown to enhance plant growth by improving root development, water-holding capacity (Harman *et al*., 2004), and nutrient uptake (Yildirim*et al*., 2006). Also, (Hosseini*et al*., 2018) , reported that the Fenugreek growth factors and Trigonelline biosynthesis can be affected by Trichoderma strains. Moreover, it was recently reported that fenugreek inoculation with Trichoderma treatment

increased trigonelline accumulation, which acts as a strong inducing factor for secondary metabolite production (Hosseini*et al*., 2018).

Aloe leaf extract (ALE) has been used to improve the vegetative growth of *Abelmoschusesculentus, Oenotherabiennis, and Majoranahortensis* (Padmaja*et al*., 2007). Fayez and Bazaid, (2014)suggested that ALE is an efficient alternative source to improve the growth of Populus trees grown under in vitro conditionsParameshaet *al*.,(2021) showed that the early staged fenugreek leaves have the best metabolite and antioxidant capacity. The seeds had higher antioxidant and metabolite activity, but the tender leaves of the Kasurimethi (KS) variety of the plant had the strongest activity for secondary metabolite accumulation.

The aim of this research is Investigate the effect of *Trichodermaharzianum,* and Aloe veraand there interaction on the growth of Fenugreek plants.

2 .**Methods**

2.1 Source of Fenugreek seeds

Seeds of *T. foenum-graecum L*. were purchased from the local market from Al-Hilla City, Babylon Province, Iraq.

2.2Preparation Culture media

2.2.1 Potato Dextrose Agar (PDA)

PDA medium was prepared according to the manufacturer's instructions, then sterilized by autoclaved at 121 ° C for 15min. at 15psi and poured in sterilized plates. This medium was used for the growth and maintenance of *T. harzianum* isolates (Colleeet *al*., 2015).

2.2.2 Potato Dextrose Broth (PDB)

PDB medium was prepared by dissolving 24 gm of this medium in 1L of distilled water, mixed thoroughly then serialized and autoclaved.

2.3Fungal Growth

T. harzianum isolates were first cultured on Petri plates containing sterilized PDA, incubated at 26˚C for 7 days, in the dark and after that placed under continuous light to promote sporulation

2.4Sterilization and selection of the fenugreek seeds

Before starting the experiment, all the glassware that we need to prepare treatment formulations were sterilized in an oven for 24 hours at 110 ^{o}C. Homogeneous fenugreek seeds, one-year-old with no cracks or other visible deformations were selected, their viability was checked by suspending them in distilled water, then the seeds that settled to the bottom were chosen. Seeds were surface sterilized using 2% NaOCl for 5 min. Seeds were rinsed thoroughly several times with sterile distilled water, the seeds were dried on sterile filter paper under a laminar flow hood. The standard germination of the seeds was 98 %

2.5 **Experimental treatment and design**

Ten treatments were prepared, in which two isolates of T. harzianum: Th-1 and Th-2 (1×10^{8} spores/ml), and Aloe Vera Gel (AVG) at 50% concentration were conducted. However, ten treatments were applied as the following (the concentration of combination treatments was 50% each):

1. Control, seeds treated with distilled water

2. Seeds treated with (Th-1)
3. Seeds treated with (Th-2)
4. Seeds treated with AVG (100% concentration)
5. Seeds treated with (Th-1) + AVG
6. Seeds treated with (Th-2) + AVG

All ttreatments were planted in pots after completing the experiment

2.6Growth parameters

Fenugreek plants were harvested soon after flowering 45 days old, the plant was removed from the pots and the shoots and roots of plants were separated and washed with distilled water three times, and then dried and weighed. and the roots gently washed off from the soil using running tap water. Several leaves, chlorophyll content, shoot length, root length, fresh weight of shoot was evaluated. Shoot length was calculated from the soil line to shoot top with slight modification and then weighed with sensitive balance.

3. Results

The effect of *T. harzianum*isolates and AVG on growth of fenugreek seeds under laboratory conditions

Growth parameters of fenugreek plants were varied according to the type of treatment. The results (Table 1) of this experiment revealed that the Th-1treatment and AVG and their combinations were enhanced the growth capacity of fenugreek plants which summarized as the following:

Root length increased significantly ($P<0.001$) in plants treatedShoot height, similar tendency was observed with shoot height. Both parts of plant were taller when seeds were treated with Th-1 in lonely.

Fresh weight of shoots was significantly (P<0.001) increased due to seed treatment with Th-1, and AVG and their combinations.

Significant variation was observed in number of leaves in different treatments.

Significant difference was observed in chlorophyll percentage with Trichoderma treatments.

Table (1). Effects of treatments with *T. harzianum* isolates (Th-1 and Th-2) in combination with AVG on root length, shoot height, shoot fresh weight, number of true leaves, and chlorophyll content. of fenugreek (*Trigonellafoenum-graecum*) seed treatment Data are means ± standard errors of four measurements.

Treatments	Root length	Shoot height	FW of of shoots	No. of true leaves	Chlorophyll content
Control	9.12±0.59	23.30±1.09	2.10±0.27	9.20±0.47	49.07±0.59
Th-1	14.87±0.65	32.00±0.70	3.50±0.29	12.00±0.40	56.12±0.62
Th-2	8.35±0.69	19.57±0.33	1.10±0.29	6.50±0.86	40.94±0.33
AVG	14.95±0.68	31.92±0.07	7.90±0.49	23.50±0.64	59.25±0.61
Th-1+AVG	14.00±1.35	33.75±0.47	6.52±0.57	28.50±0.75	61.45±0.86
Th-2+AVG	10.67±0.41	20.25±0.47	2.11±0.12	8.25±0.47	45.32±1.07
$L.S.D_{005}$	1.3	1.52	0.90	2.38	1.83

4. Discussion

Trichoderma is one of the most economically important microorganisms in the agro-ecosystem and industry that influence soil health and crop productivity, anti-pathogenic activity, bio-fertilizer, promoter of plant growth, bioremediation and increase in crop yield, both biological and economic output (Rai*et al.*, 2020).

The present experiment demonstrated the substantial effect of *T. harzianum* and its combinations on the growth of fenugreek plants. Since the results of this experiment revealed an increase in root length, shoot height, fresh weight of the shots, number of true leaves, in addition to the chlorophyll

content, these parameters reflecting growth improvement (Ezzi& Lynch, 2002).

Some Trichoderma species may release soluble compounds and that may exerted beneficial effects not only for plants but also for those microbial groups cohabiting the rhizosphere (Herrera-Jiménez, *et al.*, 2018).

Current experiment revealed that the application of Th-1, and AVG. showed a significant impact on the number of true leaves, shoot length, root length, fresh weight and dry weight of fenugreek plants compared to control. The rapid growth of the plant, number of leaves, plant height, fresh weight and dry weight of plant are the important criterion of growth parameter.

our findings indicated that the effects of Th-1and AVG alone or in combination with each other on growth mainly depend on the type of *T. harziamun* isolate/strain applied. In some cases, Trichoderma has a stimulatory effect on plant growth by modifying the soil conditions, besides, the increased growth response of the plant, caused by Trichoderma strains, mainly depends on the ability to survive and establishment in the rhizosphere (Harman *et al*. 2004). These studies have been confirmed in the case of T. harzianum enhancing seed germination, root and shoot length (Dubey*et al*. 2007) as well as increasing the frequency of healthy plants. Ozbay and Newman (2004) also reported that T. harzianum strains have significantly ($P<0.05$) increased the germination, height, shoot and root dry weight in fenugreek plants transplanted into pots in a greenhouse.).

On other hand, the increasing of plant height in plants that treated with *T. harzianum* may due to produceauxins that can stimulate plant growth and root development (Contreras-Cornejo et al., 2009). This result was similar to that obtained by Nagata *et al.*, (2005) who stated that chlorophyll percentage also increased using Trichoderma in tomato. For this case, the range of

survival percentage was found by Trichoderma to improve plant performance under different biotic and abiotic stress (Mastouri 2010).

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