

EFFECT OF WHEAT SEED TREATMENT WITH SPORULATION SUSPENSION OF THREE ISOLATES OF *TRICHODERMA HARZIANUM* AND ITS INTERACTION WITH HALF OF NPK FERTILIZER RECOMMENDATION ON SOME GROWTH CHARACTERISTICS OF WHEAT CROP

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Abstract

The study aimed at the use of three isolates of *Trichoderma harzianum* fungi with the addition of half a recommendation of NPK fertilizer to the wheat crop (variety IBAA 99) and its effect on some growth and yield characteristics. Three isolates of *T. harzianum* (T.h D, T.h A and T.h G) were used and NPK fertilizer levels were (0 and a half-fertilizer recommendation), *T. harzinaum* D isolate without the addition of NPK was the highest height of wheat plants (80.58 cm). T.h G isolate treated with half recommended NPK had the highest chlorophyll content of wheat leaves were 37.12 SPAD. The isolates of *T. harzinaum* (A, D and G) treated with half recommended of NPK fertilizer achieved the highest significant values for the study of wet and dry weight, 1000 grain weight and bio-yield weight.

Key words: wheat crop , Trichoderma harzianum , NPK

Introduction

Wheat Triticum aestivum L. is the most important staple crop in temperate zones and is in increasing demand in countries undergoing urbanization and industrialization, In addition to being a major source of starch and energy, wheat also provides substantial amounts of a number of components which are essential or beneficial for health, notably protein, vitamins (notably B vitamins), dietary fiber, and phytochemicals. (Shewry and Hey, 2015). World demand for wheat is rising rapidly as the global population continues to increase. World Bank estimates the demand for wheat in developing countries will increase 60% by 2050 (Scott, 2018). Wheat growers around the world need to increase their productivity, while the prices of wheat and other cereal grains decrease. In addition, with the expectation that prices for fertilizers and chemicals will continue to rise in the future, wheat producers must substantially improve their production efficiency to stay competitive. Grain yield (GY) and quality are the most important parameters affecting gross returns for wheat producers. In addition, wheat needs to reach specific quality criteria to be eligible for export (Taulemesse et

al., 2015). Trichoderma spp. are most popular research bioagent as microbial inoculants that have been largely used against several fungal plant diseases causing soil borne, air borne and post-harvest diseases of plant through their high antagonistic and mycoparasitic potential in lab conditions. In recent years, they have become popular as plant growth promoter (Hermosa et al., 2012). Some Trichoderma rhizosphere-competent isolates have been shown to have direct effects on plant crops, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defenses against many biotic and abiotic diseases (Shoresh et al., 2010). Beneficial effects of Trichoderma as plant growth stimulator and promoter are attributed to several discovered mechanisms which include root colonization as endophyte symbiont, solubilization of the minerals and increasing availability of insoluble nutrients and therefore plant nutrient uptake, plant growth regulatory materials (phytohormons), secretion of siderophores, enzymes and vitamins (Gravel et al., 2007, de Santiago et al., 2011). Hajieghrari and Mohammadi (2016) explain the role Growth-promoting

activity of indigenous Trichoderma isolates on wheat seed germination, seedling growth and yield, and Trichoderma rhizosphere-competent strains stimulate plants growth via several direct mechanisms that affect various growth parameters. Hermosa et al., (2012) found that it is possible to increase plant growth and nutrient uptake by the direct effect of some strains of Trichodermarhizosphere as well as increasing the efficiency of fertilizer use. Singh and Sharma (2002) noted the interaction between biofertilizers and chemical fertilizers that also led to an increase in potassium uptake and increased concentration in the vegetative part of the full and half fertilizer recommendation. The study aimed to use three isolates of Trichoderma harzianum with the addition of half the recommendation of NPK fertilizer to the wheat crop and its effect on some growth and yield characteristics.

Material and Methods

Three isolates of *T. harzianum* were diagnosed according to the table below :

A spore stuck was prepared for each of *T. harzianum* isolates separately for use in study treatments. A set of petri dishes containing PDA medium was prepared and inoculated with three isolates. After 7 days, each petri dish containing pure growth of isolates was placed 10 mL of sterile distilled water and shaken for several seconds to obtain the sporadic suspension of the fungus. The suspension was collected in 250 ml sterile flasks. Then, gum Arabic was brought and grinded and then mixed with sporophyte to isolate *T. harzianum* and added to the seeds of wheat intended for planting category Iba 99 in order to adhere to the spores of mushrooms with grain and sure. The cultivation was done in the season 2017-2018 wheat crop category parents 99 where the following characteristics were measured:-

Plant height: The height of the plant was measured at 50% of syphilis growth by randomly measuring the height of 10 plants per experimental unit. Thus, the height of the plant was calculated for the rest of the other experimental units.

Chlorophyll content: The chlorophyll content of the plant was calculated by taking 5 plants per experimental unit and then we measured the chlorophyll

Table 1: Sources of T. harzianum isolates used in the study.

Isolation source	Isolation	Isolation
	symbol	number
Directorate of Agriculture in Najaf	T.h A	1
Karbala University / College of Agriculture	T.h D	2
University of Basra / College of Agriculture	T.h G	3

content by the Opti-Sciences device. One plant rate, and so we find the rest of the measurements for the other four plants within the same experimental unit, and then we enter the value of the chlorophyll content of each experimental unit in a special formula to measure the content of chlorophyll are as follows

Chlorophyll content = $A \times 10.40 - 80.05$

Since A represents the value of the chlorophyll content calculated for each experimental unit, so we can find the full value of the chlorophyll content for each experimental unit.

Wet weight: After calculating the characteristics of the number of seeds in one spike and the number of seeds in 10 spikes as well as the number of fertile spikes, bio and economic yield, the weight of the total weight of the vegetative total was calculated by taking a sample of 10 plants in all parts except the roots and from each experimental unit after harvest. Shortly to avoid stiffness of these parts before the calculation of wet weight, where it is calculated wet weight by a sensitive balance and thus calculates the wet weight for the rest of the experimental units.

Dry weight: Calculate the dry weight after leaving those samples taken from wheat plants consisting of 10 plants per experimental unit exposed to air for 7 days in order to dry, then the dry weight was calculated by a sensitive balance and as in the case of calculating the wet weight of the vegetative total.

1000 grain weight: The weight of 1000 grains was calculated by counting one thousand seeds of wheat seeds per experimental unit and then calculating the weight of these 1000 seeds by using an electronic scale of 2 kg in order to obtain the weight of one thousand grains per experimental unit.

Weight of the biological product: Wheat plant biomarker was measured using an electronic weighing scale of 5 kg, by measuring the biomarker of each experimental unit by placing the plant package for each experimental unit on that scale in order to obtain its own weight and then record these values in a table Vital to those experimental units. This vital weight for each experimental unit is 0.4 m of total experimental unit area. The experimental data were analyzed using global experiments (factor I: three isolates of *T. harzianum* and factor II: half of the fertilizer recommendation without adding fertilizer) using the RCBD design and three replicates.). And using GenStat version 12.

Results and Discussion

Plant height: When examining the effect of wheat

seed treatment with *T. harzianum* isolates, the results of Table 2 showed that the high wheat yield in Th A and T.h D isolates had the highest height of 73.89 and 76.65 cm with a significant difference over the treatment of Th G which was 69.61. The height of the plant was not significantly affected when treated with half fertilizer recommendation or without adding NPK fertilizer. In the study of the interaction between *T. harzinaum* isolates and NPK fertilizer in this study, the treatment of Th D and without the addition of fertilizer achieved the highest height of wheat plants was 80.58 cm and the same effect was the treatment of Th A with the addition of half of the fertilizer recommendation as 77.16 cm and differences Significant for all other transactions.

The effect of *T. harzinaum* isolates and NPK fertilizer doses on the plant height of the wheat crop is consistent with the results of Suhail *et al.*, 2010. Biofertilizers, if added to the soil, will significantly increase plant height and dry weight of plants compared to not adding soil fertilizer. Regardless of the addition of chemical fertilizer, the addition of the double biofertilizer (*T. harzianum* + *Azotobacter chroococcum*) recorded higher values in plant height and dry weight compared to the absence of biofertilizer with individual additives of biofertilizers and significant increase (57.37 and 120.83%). Also the results of Mohammed and Kassem, 2005 that the height of the plant and some of the other yield characteristics of the study were superior when using fertilization treatment.

Table 2: Effect of wheat seed treatment with *T. harzianum* isolates and their interaction with half of NPK fertilizer recommendation on wheat plant height.

Isolates	NPK Levels		Mean
	(0) Without NPK	(1/2) recomm ended NPK	
T.h A	70.62 cd*	77.16 ab	73.89 a
T.h D	80.58 a	72.73 bc	76.65 a
T.h G	67.40 d	71.82 cd	69.61 b
Mean	72.87 a	73.90 a	

*Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

Chlorophyll content: The results indicated in Table 3. showed that the isolation of *T. harzianum* fungi significantly affected the leaf content of chlorophyll among them. The isolation of *T. harzianum* isolate G (Th G) was 29.37 SPAD. Th A and T.h D in which the chlorophyll content was 23.94 and 21.74 SPAD. The chlorophyll content in the treatment of half of the fertilizer recommendation was significantly superior to the treatment without the addition of NPK fertilizer, which

was 32.68 SPAD. From the same table, the interaction coefficients between T. harzianum isolates with half fertilizer recommendation or without fertilizer significantly affected the leaf content of chlorophyll. The highest value of chlorophyll when treated with Th G isolation was 37.12 SPAD and significantly superior. For all other interference factors. The lowest chlorophyll content was found when treating the isolation of T.h A and 13.82 SPAD. Which is what Alan, 2007 found that the secretion of fungicide spp. Trichoderma for growth hormones such as gibberellin, oxin and cytokinin have been instrumental in promoting plant growth. As for Sabo et al., 2013, the results showed that the result of the appropriate addition of fertilizers is to raise the amount of nutrients absorbed within the tissues of the plant, as nitrogen is the largest role in the construction of this molecule through the way it enters the synthesis of amino acids and proteins in the construction of chloroplasts It is also included in the composition of the unit of Porphyrin involved in the synthesis of chlorophyll, where 51% of the nitrogen leaves in the synthesis of chlorophyll dyes, while the element potassium contributes to the activation of many enzymes that contribute to the construction of chloroplasts (Ahmed and others, This is the reason for the best increase in plant growth indicators such as height and concentration of chlorophyll dye in leaves.

Table 3: Chlorophyll content (SPAD) of leaves of wheat plantstreated with *T. harzianum* isolates and theirinteraction with half of the recommendation of NPKfertilizer.

Isolates	NPK Levels		Mean
	(0) Without NPK	(1/2) recomm ended NPK	
T.h A	13.82 f	34.07 b	23.94 b
T.h D	16.62 e	26.87 c	21.74 c
T.h G	21.62 d	37.12 a	29.37 a
Mean	17.35 b	32.68 a	

*Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

Wet weight: The results of Table 4 showed that the treatment of *T. harzianum* isolates significantly affected the wet weight of wheat plants. A which amounted to 6.82 g. When studying the effect of fertilization with half fertilizer recommendation and comparing it with treatment without fertilization, the treatment of half fertilizer recommendation by NPK was significantly higher wet weight than treatment without fertilization at 8.31 and 5.87 g respectively. The coefficients of *T. harzianum* isolation with half fertilizer recommendation and without fertilization and without fertilization were significantly superior to the three isolates

of T. harzianum Th A, T.h D and T.h G with half of the fertilizer recommendation as their wet weights were 8.27, 8.42 and 8.25 g respectively and significantly superior to the rest of the transactions. Studies have demonstrated that Trichoderma increases root development, crop yield, proliferation of secondary roots, seedling fresh weight and foliar area. Trichoderma spp. was found to colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling off of the Trichoderma thallus. In addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake also occurs (Harman, 2006; Sharma et al., 2011). Sharma et al., (2012) reported fungal colonization stimulated plant growth by factors including increased root size and root depth, which aid in nutrient uptake (Harman et al., 2004). The auxin dependant mechanism has been explained for increase in lateral roots by Contreras-Cornejo et al., (2009) in Arabidopsis by Trichoderma virens.

Table 5: Dry weight of wheat plants treated with *T. harzianum*isolates and their overlap with half of therecommendation of NPK fertilizer.

Isolates	NPK Levels		Mean
	(0) Without	(1/2) recomm	
	NPK	ended NPK	
T.h A	1.755 b	2.328 a	2.042 a
T.h D	2.168 ab	2.113 ab	2.141 a
T.h G	1.778b	2.030 ab	1.904 a
Mean	1.901 a	2.157 a	

* Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

Dry weight: Table 5 indicates the dry weight values of wheat plants for the studied factors. Statistical analysis indicates that there was no significant differences between T. harzianum isolates by their effect on the dry weight of wheat. There were also no significant differences in NPK fertilizer application. The interaction between T. harzianum and NPK isolates was the highest dry weight of wheat plants to treat the interaction between Th A isolation and half of the fertilizer recommendation at 2.328 g with significant difference for all treatments, while the lowest dry weight was found at Th A and T.h G isolates. Without NPK fertilizer, the average dry weight was 1.755 and 1.778 g respectively. Also, the results of arcoase, 2009 showed that the higher the level of addition of urea and superphosphate fertilizers to the anvils, this leads to an increase in the values of the studied traits. Acres, where these levels were better than others in giving higher values to those traits compared to other levels added for both manure. Xue et al., (2017) found that some T. harzianum isolates significantly affected

some growth traits of the wheat plant, especially the dry weight of the plant. Similarly, *Trichoderma koningi* was found to colonize roots of *Lotus japonicus* which produced isoflavonoid phytoalexin vestitol and increased plant dry weight (Masunaka *et al.*, 2011).

Table 4: Wet Weight of Wheat Plants Treated by *T. harzianum*Isolates and their Interference with Half of NPKRecommendation.

Isolates	NPK Levels		Mean
	(0) Without NPK	(1/2) recomm ended NPK	
T.h A	5.37 c	8.27 a	6.82 b
T.h D	5.75 bc	8.42 a	7.08 ab
T.h G	6.50 b	8.25 a	7.38 a
Mean	5.87 b	8.31 a	

* Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

1000 grains weight: Table 6 shows the average weight of 1000 grain of wheat where there is no significant differences between the treatments of isolates of T. mushroom harzianum. . In the study of the interaction between T. harzianum isolate and NPK fertilizer, th A, T.h D and T.h G isolation coefficients were interfere with half of the NPK fertilizer recommendation. Respectively and significantly superior to the rest of the fungal isolates to which NPK fertilizer was not added. Also, Mohammed, 2009, found that the weight of 1000 grains was significantly superior in the application of fertilization treatment compared to the non-fertilization treatment where there was no significant superiority. The colonization of Trichoderma harzianum in the root resulted in increase in growth of root thus providing enough strength for more nutrient uptake by the roots in fields with limited irrigation facility (Sharma et al., 2012). Bae et al., (2009) reported similar results in Theobroma cacao by using Trichoderma hamatum thus enhancing crop growth in drought prone area.

Trichoderma isolates they increased 1000 grain

Table 6: Effect of wheat seed treatment with *T. harzianum* isolates and their overlap with half fertilizer recommendation of 1000 grain weight of wheat crop.

Isolates	NPK Levels		Mean
	(0) Without NPK	(1/2) recomm ended NPK	
T.h A	28.46 b	31.17 a	29.81 a
T.h D	28.75 b	31.50 a	30.13 a
T.h G	28.33 b	31.38 a	29.86 a
Mean	28.51 b	31.35 a	

* Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

weight in treated plants. *G virens* G.525 inoculated plants showed more increase in 1000 grain weight, resulting in better quantitative improvement in the crop productivity and yield (Hajieghrari and Mohammadi. 2016).

Table 7: Study of the effect of wheat seed treatment with *T*.

 harzianum isolates and their interaction with half

 fertilizer recommendation on wheat bio weight.

Isolates	NPK Levels		Mean
	(0) Without NPK	(½) recomm ended NPK	
T.h A	257.50b	391.88 a	324.69 b
T.h D	373.13 a	419.79 a	396.46 a
T.h G	282.00 b	355.83 a	318.92 b
Mean	304.21 b	389.17 a	

* Means followed by the same letter are not significantly different based on a Duncan's multiple range test (0.05).

Weight of the biological product: The results of Table 7 showed that there was a significant effect of T. harzianum isolates and NPK fertilization treatments and their interaction and their effect on the weight of wheat bio crop. The isolation of T.h D fungi was significantly superior to 396.46 wheat yields over T.h A and T.h G isolates, which had a weight of 324.69 and 318.92 respectively. The wheat bio yield at half of the fertilizer recommendation, which amounted to 389.17, was higher than the treatment without the use of NPK fertilizer. In the study of the interaction between the two factors, the isolation of T.h A, T.h D and T.h G fungi with half of the NPK fertilizer recommendation was 391.88, 419.79 and 355.83, respectively, superior to the rest of the fungal isolates and without fertilizer application. This was noted by Singh and Sharma, 2002, in their study of the interaction between biofertilizers and chemical fertilizers. He stressed the importance of adding biofertilizers with chemical fertilizer by half of the fertilizer recommendation, which will achieve the required production in order to reduce production costs and environmental pollution resulting from the use of chemical fertilizers excessive. Al-Azzawi et al., 2018, who found that the different nitrogen levels had significant effect (P < 0.01) in the characteristic of the bio-crop and some components of the studied crop. It was also observed that the interaction between the cultivars and nitrogen fertilization of the first season was not significant in the weight of 1000 grains, bio-crop and harvesting index.

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