

# Effect of Two Isolates of *Trichoderma harzianum* on Total Nitrogen, Chlorophyll a & b Contents and Yield of Wheat (*Triticum aestivum*.L) Class Eba'a-95

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**Abstract:** This study was conducted to evaluate the effect of using two isolates of biocontrol fungi - *Trichoderma harzianum*. (*T.h.a*), and *Trichoderma harzianum* (*T.h.t*) with rice straw on improvement of growth (total nitrogen and chlorophyll a,b contents in the leaves) and productivity (plant height, weight of 1000 grains and yield) of a local wheat - class (Eba a -95). To attaining these aims, two experiments were carried out, the first was a laboratory experiment that included isolation, purification fungi from wheat field while the second experiment was conducted in at AL- Mishkhab region wheat field, / AL-Najaf province for growing season 2013. The results appear that *Aspergillus nieger* has the highest frequency 28.31% at the pre-planting period, while *Trichoderma harzianum* reached the highest frequency 43.20% at the end of growing season. The field treatment (Soil with rice straw that treated by the spores suspension ( $10^4$ ) of bio agent *T.h.t*.) gave a significant differences of total nitrogen in wheat leaves that reached 2.652 %, in compare with control 2.450%, the results, also showed a significant differences in contents of wheat leaves of chlorophyll a, b which amounted 3.320, 1.303 mg/gm in compare with control 2.911, 1.110 mg/gm, respectively. The field treatment (Soil with arice straw treated by the spores suspension ( $10^4$ ) of bio agent *T.h.t*.) gave a significant differences in productivity parameters of wheat crop, plant height reached 110.85 cm in compare with control 107.09 cm, weight of 1000 grains gave 33.719 gm than the control 32.131 gm and finally the wheat significantly gave yield that reached 6.710 t.h<sup>-1</sup> in compare with control 6.140 t.h<sup>-1</sup>.

**Keywords:** AL- Mishkhab region, biocontrol, Wheat -Eba a -95, *Trichoderma harzianum*, rice straw

## Acknowledgment:

1. (*T.h.a*): *Trichoderma harzianum* (austaralian isolate).
2. (*T.h.t*): *Trichoderma harzianum* (tahadi – local isolate).

## 1. Introduction

Wheat crop (*Triticum aestivum* L.) occupy a first rank in terms of area and production in the world, and one of the most important and oldest crops in Iraq, so the need to wheat grain are growing with the increasing of population, the production of wheat in Iraq was improved in the year 2013 up to 3 million and a half million tons, while the production reached 709 million tons in the world in the year 2013[1].

Several factors lead to increase the productivity of this crop, one of these methods, the treatment of soil with organic fertilizers that treated with growth promoting fungi which contribute in biological oxidation and reducing the number soils alkalinity and increase the nutrients availability[2]. The existence of remnants of the rice in growing areas of wheat as a result of succession planting of rice and wheat for several years, create a new bio available society [3]–[4], and the use of the most efficient fungi in the analysis of plant waste as a result of their enzymatic capabilities to increase the availability of some nutrients and one of vital means to raise the nutritional value of the waste[5]. *T.harzianum* fungi have been used in the treatment of waste of a numbers of plants such as sawdust, straw, barley, buckwheat and millet [6]–[7].

Scientific research centers and agricultural companies have been oriented to employ all of its programs and its research to develop alternative and effective solutions instead of chemical pesticides, as well as it is being benefit to the environment and human health, and it has the ability to eliminate the plant diseases and their pathogenicity [8], and it can be indicated to the clear progress in using of microorganisms in this regards, especially in the field of control of a number of pathogen fungi [9], *Trichoderma harzianum*, *Chaetomium. elatum* and *Penicillium* spp has been able to compat a types of *Fusarium* spp and *Rhizopus* spp, that causing seedling death disease and seeds rot [10]. *Trichoderma harzianum* were known as a fungus since 1930 for its ability to promote the growth of plant[11], the use of types of this fungus in biological control of pathogenic fungi due to an important mechanisms, including competition, antagonisms and secretion of enzymes and hormones[12]–[13].

The nitrogen contributes in the growth of plants clearly[14], it is important in the work of plant hormones (auxines and cytokinines)[15], which increases the divisions of merestimic cells, so it is reflected positively on the vegetation and the production of flowers [16]–[17], as well as increased and improved the root system[18], which contributes on increasing of the efficiency of the plant to absorb water and necessary nutrients from soil and its metabolization

especially phosphorus and potassium [19]. While chlorophyll helps to increase leaves content of nitrogen and reduce the number of dead leaves [20]

The disappearance of chlorophyll is often at the final stage in the life of the plant as result of anabolism operations [21]. Chlorophyll is composed of several kinds, the most important chlorophyll are chlorophyll (a), (b) and (c) [17]. The maintaining of the content of chlorophyll (a and b) in the weight unit may help in the formation of the plant by making physiological operations work better through increasing the metabolic processes of carbon, because the increasing of chlorophyll content was associated with the operations of metabolism of carbohydrates compounds by metabolize CO<sub>2</sub>, so the chlorophyll (a) is the key of carbon metabolism because all of the meta operate in the nucleus and chloroplasts to identify the proteins responsible for this process [22].

This study aimed to test two isolates of biological control fungi in analysis of rice straw and its role in improvement of growth and productivity of wheat, to achieve this goal, the following points were studied.

- 1) Isolation and identification of fungi in the wheat fields soils before and after cultivation.
- 2) The impact of biological control fungi in the proportions of nitrogen and chlorophyll a and b in wheat leaves before flowering
- 3) The addition of rice straw- treated with growth promoting fungi - to the field's treatments and its impact on plant height, 1000-grains weight and yield of wheat crop.

## 2. Methods and Materials

### 2.1 Study Area

This study was conducted in Mishkhab, AL-Najaf province for the agricultural season 2013, the soil had tilled, softened and divided into boards, this area was divided into three sectors by 21 experimental unit (board), distance of each plate (3×3) m<sup>2</sup>, each board separates with another by a distance amount 70 cm, seeds of wheat (Local class -Eba 'a-95) [23] were scattered at 15/11/2013 homogeneously, by the recommended amount (100) grams per experimental unit or 30 kg /d [24]. Then series agricultural operations were used in the cultivation and production of wheat in the region. soil was fertilized before sowing with NPK compound fertilizers (18:18:0) at a rate of 100 kg / sq.m, also urea fertilizers were added at a rate of 70 Kg / sq.m, in two installments, at the first month after sowing (beginning stage of the branching) and the second addition was after one month from latest addition (elongation phase of the plant) [24]. The two isolates fungus of *Trichoderma harzianum* (*T.h.a*) Australian and (*T.h.t*) tahadi isolates was obtained from supreme laboratory of Plant Protection Department in the College of Agriculture / University of Kufa.

### 2.2 The Physical and Chemical properties of Studied Soils

- 1) The distribution of sizes of soil aggregates:-Estimated by hydrometer method the soil suspension filtrate used (1:1) [25].
- 2) pH:-Estimated for saturated paste extract (Richard, 1954) by pH-meter [26].
- 3) Degree of electrical conductivity (ECe): Estimated for saturated paste extract By EC -meter [26].
- 4) Determination of total nitrogen in leaves of wheat (before flowering):-Estimated by modified method by using a (Kjeldal) device, available nitrogen been extracted by KCl (2N) solution [26].
- 5) Determination of chlorophyll A, B in the leaves of wheat (before flowering) [27], method was adopted by using a spectrophotometer device.

### 2.3 Isolation and identification the fungus from soil planted with wheat

Soil samples were collected from the study region before and after planting for a depths of 0-30 cm, the fungi were grown by using dilution plate method on P.D.A media in order to isolate the fungal genus and species in one gram soil. Petri-dishes were incubated at a temperature of 25 ± 2 C<sup>0</sup>, four days later the fungus colonies were been isolated, purified and identified on P.D.A and W.A media [28], also the percentage of fungal species frequency were calculated in those soils at the beginning and the end of the season. The fungus were identified according to approved taxonomic keys [29] -[30].

### 2.4. Effect of two isolates of *Trichoderma harzianum* on the some of yield parameters of wheat crop

The selected growth promoting fungi *T.h.a* and *T.h.t*. were added to sterilized rice straw, as suspension by addition of 5 ml of sterile water to each petri dish containing that fungus to make fungal suspension (1×10<sup>4</sup>) that had added to 50 gm of rice straw in polyethylene bags.

Before cultivation of wheat, 30 gm of treated rice straw with growth promoting fungus *T.h.a* and *T.h.t*. were added to field experimental units (3×3) m<sup>2</sup> according to experiment design [31]. Also, the studied fungal suspensions has been sprayed on the field soil boards before one day of cultivation with out addition of rice straw in same concentration (1×10<sup>4</sup>), the wheat seeds were scattered in the studied field according to the recommended amount of seeds, as well as the recommended fertilizers were added (Urea). At the end of the agriculture season, the plant height (measured from the place of connection stalk with soil even the highest peak of it), weight of 1000 grains and productivity of each board (taken after manual harvesting of area 1 m<sup>2</sup> of each replicate and a randomly assigned to all treatments and the weight of yield), were calculated. The studied treatments were 7 treatments with three replicates. The field experiment was designed as randomized complete block design (R.C.B.D), treatment means has been compared according to less significant difference (L.S.D) at 0.05 [32], the experiment treatments were :-

- 1) Soil only (control).
- 2) Soil with rice straw.
- 3) Soil with NPK fertilizer (18-18-0)
- 4) Soil sprayed by *T.h.t.* (Suspension).
- 5) Soil sprayed by *T.h.a.* (Suspension).
- 6) Soil with rice straw treated by *T.h.t.*
- 7) Soil with rice straw treated by *T.h.a.*

### 3. Result and Discussion

The results in table (1) appears that the surface layer texture of the studied soil was Silty – Clay – Loam, the values of reaction degree (pH) for samples was 7.71, while the value of electrical conductivity (ECe) was 2.3 dSm.m<sup>-1</sup>, so this salinity values were a suitable due to the proper washing operations and agricultural rotations which used in this region [33].

#### 3.1 Fungi species and the frequency of isolated fungi

The results of the isolation and identification of fungi from the wheat rhizosphere zone before and after the cultivations appear existence of 10 species of the fungi, whereas fungus *Aspergillus niger* was the most frequency of those species before, cultivation, which reached 28.31%, figure, (1- a), while *Trichoderma. harzianum* was surpassed at the end of the season as frequency percentage reached 43.20 % figure (1- b), the reason was due to their high competition range with other fungi, through suitable bio-control mechanisms such as secretion of antibiotic and enzymes [34]–[35], this results had coincided with the previous studies which have been isolated a number of fungi from soils cultivated with economic plants [36]–[37], the results were indicated to the domination of the biological control fungi at the end of the season and their ability to eliminate pathogens that their frequency ratio were decreased clearly, which may contributed to improvement the growth and productivity of wheat crop.

#### 3.2 Total Nitrogen

The results in the figure (2-a) shown that the two treatments, soil with rice straw that treated by *T.h.t* and soil with *T.h.t.*(suspension) gave a significant increasing in the proportion of the total nitrogen in the leaves of wheat which reached 2.652,2.619 %,respectively, in compared with control treatment (soil only),which amounted to 2.450 %,this was consistent with what indicated by [38] about the role of the mass of microbial organisms in the analysis of organic matter, which in turn increases the content of this soil with mineral nitrogen ,also this is noted by [39] that treated the rice straw with *T.harzianum* fungus was contributed in increase the nutritional value of the straw. As well as, *T.harzianum* have a role in improving the efficiency of nitrogen absorption by plant roots [40].

#### 3.3 Chlorophyll a & b Contents

The results in the figure (2-b,c) shown that the treatment of rice straw with biocontrol fungi *T.h.t* led to achieve a significant difference in the of chlorophyll a & b content (mg .gm<sup>-1</sup>) of fresh weight of wheat leaves for a following

treatments: soil with rice straw that treated by *T.h.t* and soil with *T.h.t.* (suspension),which reached 3.320, 3.271(mg .gm<sup>-1</sup>) of chlorophyll a and 1.303,1.284 (mg .gm<sup>-1</sup>) of chlorophyll b respectively, in compared with control treatment (soil only) which amounted to 2.911, 1.110 mg .gm<sup>-1</sup> of chlorophyll a, b of fresh weight respectively, this is consistent with [41] as they found that the increasing of chlorophyll correlate significantly and positively with the addition of nitrogen , also[42]–[43] that the increasing of cereal crops content of chlorophyll depends on nitrogen supplying for plant and its availability extent in in the soil solution

#### 3.4 Plant Height

The results indicate that the addition of isolates *T.h.t* had given a significant increase in the height of the plants (cm), for the two treatments: Soil with rice straw treated by *T.h.t.* and soil sprayed by *T.h.t.* (Suspension) which stood at 110.85, 109.71cm, respectively, in compared with the control treatment (soil only),which amounted to 107.09 cm figure (3- a), the cultivation of cereals with residues increase the effectiveness of micro organisms and thus obtain a significant increase in plant height[44]

#### 3.5 Weight of 1000- Grain

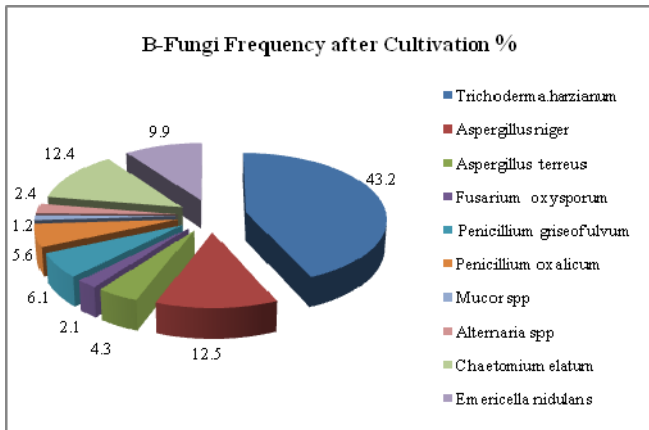
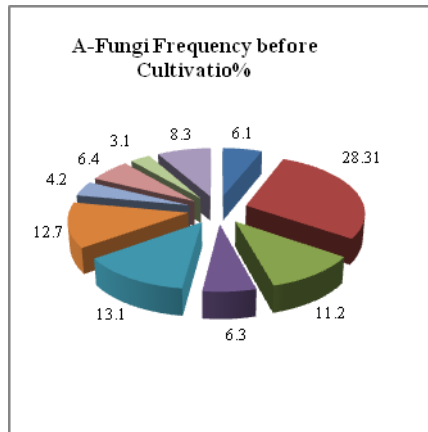
The results in figure(3-b) they were shown that the local isolate *T.h.t.* led to significant increase in 1000-grain weight, especially treatments which carried the fungus on the rice straw and *T.h.t.*(Suspension) sprayed on soil, which amounted to 33.012,32.851gm,in compared with the control treatment(soil only),which reached to 32.250gm,this explains the role of fungi in secreting of growth regulators, as pointed out that the positive impact of some isolates of *T. harzianum* in stimulating the growth of plants [45],because secretes the botanical growth regulators corresponding with other mechanisms, including the increasing of availability the absorption of plant nutrients, as well as indicated to the increasing in the rates of growth of sorghum plants which inoculated with *T.harzianum*. Also, the increasing of height of the plant affected significantly in 1000-grain weight and reducing the percentage of unfilled grains, this is consistent with [46], that indicates to the use of *T.harzianum* isolates gave a significant increasing in the weight of potato tubers

#### 3.6 Yield Weight

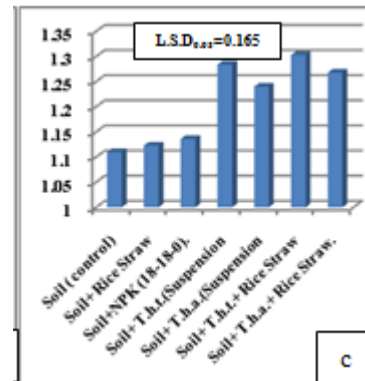
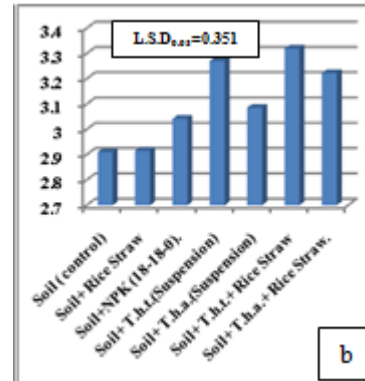
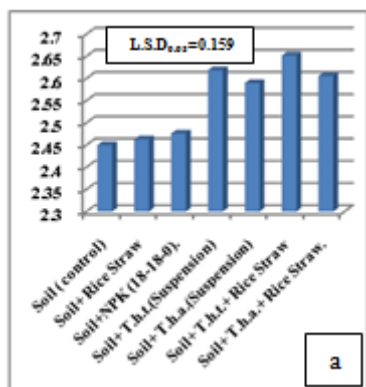
The results indicate that the addition of isolate of *T.h.t.* and its suspension had given a significant differences in the wheat yield (t.h<sup>-1</sup>), especially the two treatments: Soil with rice straw that treated by *T.h.t.*and soil sprayed by *T.h.t.* .(Suspension),which reached 6.710,6.534t.h<sup>-1</sup>,in compared with the control treatment (soil only),which amounted to 6.140t.h<sup>-1</sup>.This may be due to the role of growth promoting fungi in stimulating the secretion of hormones and increase the ability of antagonism against pathogen fungi as well as its role in improving the soil structure, which reflected positively in moisture retention and provide optimized water for plant growth[47]–[48].

**Table (1):-** Physical and Chemical Properties of Studied Soil for Agriculture season 2013

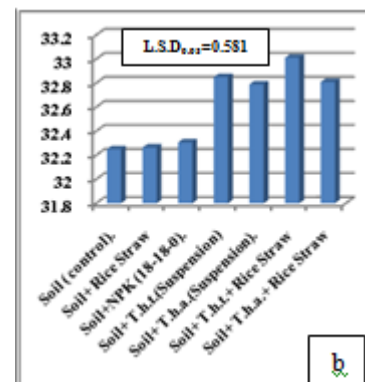
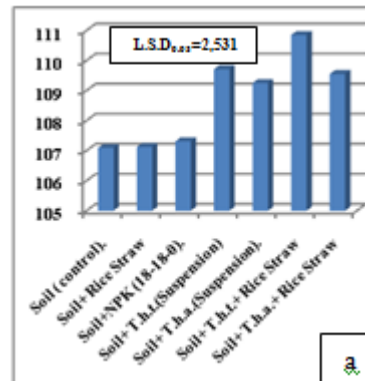
Property	Quantity	The unit of measurement
PH	7.71	-
EC	2.3	dS.m <sup>-1</sup>
Sand	120	gm.Kg <sup>-1</sup>
Silt	580	gm.Kg <sup>-1</sup>
Clay	300	gm.Kg <sup>-1</sup>
Texture	-Loam Silty- Clay	



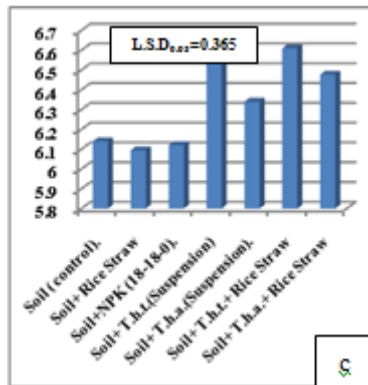
**Figure 1:** Isolated fungi speciece frequency from studied soil before & after cultivation at agriculture season 2013



**Figure (2)** The effect of addition of biological control fungi (loaded on rice straw) to the Soil on (a) - Total Nitrogen%, (b) - Chlorophyll -A, (c) - Chlorophyll - B content (mg .gm<sup>-1</sup>) in wheat leaves.







**Figure (3)** The effect of addition of biological control fungi (loaded on rice straw) to the soil on (a) - plant height(cm) , (b)-1000 –grain weight (gm) ,(c) - wheat yield t.h<sup>-1</sup> for agriculture season 2013

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