

Original Research Article

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The Influence of Some Fungi Bio-genic on Promoting Growth and Yield of Wheat-Var. Ibaa99

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ABSTRACT

The goal of study was to investigate the effect of certain species of *Aspergillus* and *Trichoderma hamatum* and their combination on growth and yield of wheat- Ibaa 99. The Study conducted in Wasit province during 2014-2015 and 2015-2016 agricultural seasons and the impact of fungi were evaluated according to the following treatments: *Aspergillus niger* (A.n.), *Aspergillus fumigatus* (A.f.) and *Trichoderma hamatum* (T.h.) and combination of both (A. n. + T. h.) and (A. f. + T. h.) and (A. n. + A. f. + T. h.). The wheat (var.Ibaa99) seeded in blocks with five replicates per treatment, the fungi loaded on peatmoss before applied as inoculum at a rate of 2 liters per block. The experimental data considered of average plants height (cm), dry weight (gm), spike length (cm), spike's weight (gm) and grain weight (gm) for ten plants per square meter. The results showed that examined fungi and their combination have significant effects on growth and yield of wheat in all treatment compared with the control in two agriculture seasons, The best treatments was combination of (A. n. + T. h.) and (A. n.), which gave the highest amount of grain yield was 674.25 and 638.82 gm/m², respectively, compared with control treatment 475.65 gm/ m². The results also showed that the highest effective fungi in this study (A. n. + T. h.) and (A. n.) have positive effect on wheat characteristics in addition to growth and yield in both seasons, such as increasing emergence ratio by No. of plant per m² that gave 232.5 and 236.6 plant/m² respectively compare with control treatment that gave 226.5 plant/m² and average spike's weight (gm) that gave 2.9 and 2.7 gm and 2.1 gm in control. In the agriculture season 2015-2016, the results showed significant effect for treat (A.n.+ T.h) in all growth attributes and yields of wheat compare with control treatment. The antifungal activity indicated that fungal isolates have inhibitory efficiency towards the two pathogens fungi *Fusarium oxysporum* and *Rhizoctonia solani*. And the Antifungal activity of (A.n.) was highest 66.71% against pathogens fungi *Fusarium oxysporum* while (T.h.) was the highest 77.77% against pathogens fungi *Rhizoctonia solani*. The Physiological and chemical characteristics of wheat grain shown that (A.n.+ T.h) contained the highest percentage of gluten 25.13% and ash 2.04%, also it was highest in G.W. 80.97 kg\hl and W.T.K. 30.53 gm in addition to increasing falling number Fa.No.in 629 sec. indicating highest in enzymic activated among the tested sample of treatment. While the treat (A.n.) had highest percentage of protein 13.8% followed by (A.n.+ A.f.+T.h) 13.73% and (T.h.) 13.3 % where (A.f.) had the lowest value 12.9%. These results records possibilities of these effective fungi can depend on promoting growth and yield of wheat to make it as bio-fertilizer.

Keywords

Aspergillus,
Trichoderma hamatum,
plant growth promoting
fungi (PGPF), wheat,
physiological and
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Introduction

Many soil microorganisms have ability to improve plant growth either directly through movement of nutrient and producing plant hormones, or indirectly through repressive of plant pathogens, or by stimulating systemic resistance in plants in addition to enhance the availabilities of plant elements by dissolving phosphorus, which can be used as bio-fertilizer (Xiao *et al.*, 2008). Alan (2007) reported that microorganisms affect directly on ability of plants to get the phosphorus from soil using many mechanisms, including: increasing surface area of roots, increase movement of organic phosphorus formats as well as stimulating metabolic processes that are moving directly to the solubility and metal phosphorus from organic and inorganic formats.

Fungi considered as major component of soil ecosystem, as it affects effectively with surrounded by elements of this system and a special biotic part which is plant. Depending on the nature of the relationship between fungi and plants, the fungi have the ability to set up an symbiosis relationship with plant leading to increase its ability to absorb certain nutrients and water, as well as afford the environmental stress conditions, beside their ability to makes the phosphorus component available for absorption by the plant (Rashid *et al.*, 2004).

The filamentous fungi, particularly some species belonging to genus *Aspergillus*, *Penicillium* and *Trichoderma*, endemic in the root zone (Rhizosphere) used to solve the phosphate compounds and release phosphorus through its ability for producing organic acids, reducing pH of soil and production of enzymes (Barroso *et al.*, 2006). Many of the genus *Aspergillus* species is characterized by its propensity to dissolve inorganic phosphorus such as *A. flavus* and *A. niger* and *A. terreus*, beside

production organic acids, such as Citric, gluconic, glucolic, oxalic and succinic (Akintokun *et al.*, 2007).

A lot of soil-borne fungi have multiple characteristics useful for movement of nutrients and produce substances that promoting plant growth (PGPS) plant growth promoting substances as well as biological control agents in addition to have a major impact in agricultural production (Reddy *et al.*, 2002). Also, many filamentous fungi is known to produce many extracellular hydrolytic enzymes viz., β -1, 3glucanase, chitinase, cellulose xyloase etc. by which they cause lysis in many plant pathogenic fungi (Pan and Bhagat, 2008).

The goal of study was to investigate the effect of certain species of *Aspergillus* and *Trichoderma hamatum* that promoting growth which have reported highly efficient in promoting plants that inoculated with in a previous study (Al-Tai, 2014) and their combination on growth and yield (quantity and quality) of wheat- var. Ibaa 99 for two seasons in Wasit province, western of Iraq.

Materials and Methods

Inoculums with fungi *Aspergillus niger* (*A.n*), *Aspergillus fumigates* (*A.f*) and *Trichoderma hamatum* (*T.h*) were growth on PDA media amended with Chloramphenicol 250 mg / l in Petri plates, then incubated at a 28 ± 2 ° C for seven days. Fungal inoculums then loaded on peatmoss type KLASMANN (Germany) which sterilized using commercial formalin solution, 1:50 V/V and used at rate of 3 L m³ soil⁻¹ which placed in sealed bags for 3 days then uncovered for 3 days to removed it's toxic residue, then kept in polyethylene bags to maintain its moisture (Toajin, 1979).

One Petri dish of each fungus colony and their combination were mixed with

peatmoss, then used it at rate of 2 L for inoculation the field plots after divided into small plots 7 X 5 m in five replicates. The control treatment was inoculated with peatmoss only. These plots planted with wheat (var. Ibaa99) at rate of 45 kg seeds / Donum (2500 m²) (depending on the applicable recommendations in Iraq) (Alyounis, 1993), and irrigated separately to avoid contamination between the experimental units.

The fungus ability to promote growth and performed by select 1m² randomly from each plots and take the following attributes: (No. of plants, height of plants, dry weight of a plant, length and weight of Spike, No. of seeds per spike, the grain yield for ten plants per square meter).

Repeat the field experiment in the next agriculture season 2015-2016 in a same way that conducted in previous season but in different field plots; chosen the best fungal isolate influence on wheat crop in the previous agriculture season 2014-2015.

In vitro antifungal activity (antagonistic potential) of *Aspergillus* sp. and *Trichoderma hamatum* were evaluated by dual culture technique (Fokkema1978), against both fungal pathogens *Fusarium oxysporum* and *Rhizoctonia solani*. The growth of pathogen and antagonist were recorded after 7th day of inoculation. The percentage of growth inhibition (PGI) calculated according to the equation: $PGI(\%) = (KR-R1) / KR \times 100$ (Korsten and Jager,1995)

(KR) - Diameter of pathogenic fungal growth in control treat. (cm)

(R1) - Diameter of pathogenic fungal growth in dual culture treat. (cm)

The chemical and physical characteristics of wheat grain obtained for all treatment, the characteristics include: Protein %, Ash %, Glutin %, Gravity Weight (G.W.) kg\hl, Weight of Thousand Kernel (W.T.K.) gm and Falling Number (Fa.No.) sec., these characteristics measured according to American Association of cereals chemistry (AACC, 2000).

The design of study was RCBD and the Data were analyzed using Genstat statistical software program and means were compared using $LSD \leq 0.05$ for field trail and ≤ 0.01 for lab test (Al-Rawei and Khalaf Allah, 1980).

Results and Discussion

Plant growth promoting fungi (PGPF)

The study showed a highly efficient in promoting wheat crop that inoculated with fungi and their combination on all growth attributes and yields of wheat during 2014-2015 agriculture season (Table-1). results showed significant increase in number of total plant per m² were 236.6, 234.5 and 233 when plant inoculated with *A.niger* (*A.n.*), *A. fumigatus*(*A.f.*) and (*A.n.+ A.f.+T.h*) respectively followed by the combination of three fungi (*A.n.+ T.h*) that attained to 232.5 plants compared with 226.5 plant in the control, beside there is no any differences between fungi and their combination in height of plant, but there are differences between them and control treatment, and this results are the same with average dry weight for single plant too, were for 4.6 gm for (*A.n.+ A.f.+T.h*) and 4.5 gm for (*T.h.*) and 4.4 gm for (*A.f.+T.h*) then 4.3 gm for (*A.f.*) compared with 3.3 gm in control treatment.

The yield attribute, results shown no statistical differences between treatment in average spike length and weight but there are differences between them and control treatment and the treatments (*A.n.*) and (*A.n.+ T.h*) were the highest in each of them respectively. The same results shown that the treatments (*A.n.*) and (*A.n.+ T.h*) were the highest average in grain yield by 674.25 and 638.82 gm/m² respectively compare with control treatment were 475.65 gm/m². This effect represent improve of metabolism and encourage minerals and nutrition uptake (Bashir, 2003). In the agriculture season 2015-2016, the results showed significant effect for treat (*A.n.+ T.h*) in all growth attributes and yields of wheat compare with control treatment (table-2), but its shown less from previous agr. season. This effect may refer to extend the field plot from the previous agriculture season, so the differences may appear the biological and environment effects inside soil community but effects of fungal isolates still positive significant.

Antifungal activity

The results presented in the antifungal activity (antagonistic potential) in (table-3) indicated that fungal isolates have inhibitory efficiency towards the two pathogens fungi *Fusarium oxysporum* and *Rhizoctonia solani*. The results show significant differences between the diameter growth of the two pathogenic fungal compared with to control treatment, where the average percentage of growth inhibition (PGI) by fungal be the highest in the efficiency of inhibitory toward pathogenic fungus *F. oxysporum* from *Rhizoctonia solani*.

The Antifungal activity of (*A.n.*) was highest 66.71% against pathogens fungi *Fusarium oxysporum* while (*T.h.*) was the highest 77.77% against pathogens fungi *Rhizoctonia*

solani. It was observed that maximum growth inhibition of both pathogens i.e. *Fusarium oxysporum* and *Rhizoctonia solani* was recorded in all fungal treatments

Physiological and chemical (quality) characteristics

Physiological and chemical characteristics i.e. protein, ash, gluten, G.W., W.T.K. and Fa.No. of wheat grains obtained from different fungal treats were evaluated (Table-4). The results showed that (*A.n.+ T.h*) contained the highest percentage of gluten 25.13% and ash 2.04%, also it was highest in G.W. 80.97 kg/hl and W.T.K. 30.53 gm among the tested sample of treatment. The results (Table-2) also indicate that increasing falling number Fa.No.in (*A.n.+ T.h*) 629 sec. indicating highest in enzymic activated. The treat (*A.n.*) had highest percentage of protein 13.8% followed by (*A.n.+ A.f.+T.h*) 13.73% and (*T.h.*) 13.3 % where (*A.f.*) had the lowest value 12.9%, on the other hand (*A.n.+ A.f.+T.h*) was the lowest value of ash, falling number, W.T.K 1.8%, 480 sec., 29.2 gm respectively compare with other treats.

The increasing in dry weight may be correlated with facility solubilization via these fungi absorption by plants, and available appropriate environment to plant growth and increase vegetation growth and yield and dry weight of wheat plants (Masunaka *et al.*, 2011).this increases results refer to secretion plat growth hormones (gibrilin,IAA and saitanin) by these fungi which have a great role in the promoting growth in addition to prevent pathogenic fungi attack seeds and then increase number of plants as suggested by (Al-Tai, 2014 and Chuang *et al.*, 2007).

It must be notice that fungi and its combination have significant effects growth

and yield These (PGPS) plant growth promoting fungi provide a suitable environment for plant by producing hormones, reducing pH and secretion such organic acids as (citric, formic and butyric

acids) and phosphatase. These mechanisms are effective availability of nutrition especially phosphate, then the plant growth will improve and reflex positively on plant grain yield (Brink *et al.*, 2014).

Table.1 Effect of investigated fungi treatment on growth and production of wheat during 2014-2015 agriculture season

Attributes	No. of plants (m ²)	height of plants (cm)	dry weight for single plant (gm)	Spike length (cm)	spike weight (gm)	No. of seeds per spike	grain yield (gm/m ²)
Treat.							
<i>A. n.</i>	236.6	88.9	3.5	10.5	2.7	45	638.82
<i>T. h.</i>	228.0	87.0	4.5	11.2	2.3	62.3	524.40
<i>A. f.</i>	234.3	90.5	4.3	10.5	2.3	61.5	538.89
<i>A. n. + T. h</i>	232.5	90.0	3.4	10.3	2.9	51.4	674.25
<i>A. f. + T. h</i>	221.5	89.0	4.4	11.2	2.5	58.0	553.25
<i>A. n. + A. f. + T. h</i>	233.0	91.5	4.6	10.5	1.9	52.9	442.70
Cont.	226.5	83.1	3.3	10.3	2.1	51.7	475.65
LSD%5	5.20	2.81	0.29	0.33	0.22	1.29	51.01

Table.2 Effect of best isolated fungi treatment on growth and production of wheat during 2015-2016 agriculture season

Attributes	No. of plants (m ²)	height of plants (cm)	dry weight for single plant (gm)	Spike length (cm)	spike weight (gm)	No. of seeds per spike	grain yield (gm/m ²)
Treat.							
<i>A. n. + T. h</i>	228.2	84.7	3.2	9.8	2.6	56.8	647.05
Cont.	220.6	80.2	3.0	9.2	1.9	50.8	442.35

Table.3 Antifungal activity of *Aspergillus* sp. and *Trichoderma hamatum* on radial mycelial growth of *Fusarium oxysporum* and *Rhizoctonia solani*

Fungal Pathogen	<i>Fusarium oxysporum</i>		<i>Rhizoctonia solani</i>	
Treat.	Diameter of pathogenic fungal growth (cm)	percentage of growth inhibition (PGI) %	Diameter of pathogenic fungal growth (cm)	percentage of growth inhibition (PGI)%
<i>A. n.</i>	2.33	66.71	7.00	21.22
<i>T. h.</i>	3.07	56.14	2.00	77.77
<i>A. f.</i>	2.47	64.71	7.18	20.22
Cont.	7.00	-	9.00	-
LSD%1	0.26	5.72	0.21	3.68

Table.4 Effect of investigated fungi treatment on Physiological and chemical characteristics of wheat

Attributes	Gravity Weight kg\hl G.W	Protein %	Glutin %	falling number (sec.) Fa.No.	Ash %	Weight of 1000 Kernel (gm) W.T.K
Treat.						
<i>A. n.</i>	80.70	13.80	24.23	595.00	1.98	30.17
<i>T. h.</i>	80.00	13.30	23.03	588.33	1.95	29.57
<i>A. f.</i>	80.43	12.90	23.10	593.00	1.96	29.73
<i>A. n. + T. h</i>	80.97	13.30	25.13	629.00	2.04	30.53
<i>A. f. + T. h</i>	80.37	12.97	25.10	553.00	1.84	29.53
<i>A. n.+ A. f. + T. h</i>	80.60	13.73	24.17	480.00	1.80	29.20
Cont.	79.23	12.67	23.13	465.33	1.44	28.03
LSD%1	0.41	0.21	0.42	5.71	0.45	0.24

The volatile metabolites of *T. viride* were most effective against *F. oxysporium* and least against *R. solani* and almost reverse situation was observed when non volatile metabolites were examined (Tapwal *et al.*, 2004). Baiswar *et al.*, (2006) tested four biocontrol agents *viz.*, *Trichoderma viride*, *T. harzianam*, *T. hamatum* and *Aspergillus terms* against *Penicillium gladioli* and *Aspergillus niger* causing corn rot of gladiolus during storage and observed that mycoparasitism and production of volatile compounds were the prominent mechanisms of biocontrol by *Trichoderma* species.

The differences in physiological and chemical characteristics could be mainly attributed to the differences in treatment compare with control treatment. The wheat grain with highest W.T.K. should have a higher percentage of endosperm than ales one, and thus should have a higher flour yield. Kent and Evers (1994) reported that for flat bread flour wheat grain should by of high W.T.K. and G.W. Also the gluten are included in wheat flour specification in many countries as a primary test of wheat

flour quality (Atwell, 2001). The bread quality could be determined by the quantity and quality of gluten (Abang Aaidel *et al.*, 2009). The Fa.No test would provide an indirect estimate of alpha amylase activity. The results showed that high amylase activity in all samples of treatments.

In conclusion, on the basis of the obtained results, the following conclusions can used for further research on the highest effective fungi in this study (*A.n.+T.h.*) and (*A.n.*) that have positive effect on most wheat characteristics especially in the average of grain yield (quantity and quality), These results records possibility to be applicable by farmers in addition to make it then as bio-fertilizer.

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