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EFFECT OF *ASPERGILLUS NIGER* AND *PENICILLIUM CHRYSOGENUM* AS BIOFERTILIZERS ON GROWTH AND PRODUCTIVITY OF WHEAT CROP

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Abstract

The study was conducted in growth chamber and field to evaluate the efficacy of *Aspergillus niger* and *Penicillium chrysogenum* as biofertilizers on growth and productivity of wheat in amended soil with different levels of wheat straw and phosphate rocks. The concentrations (1.0, 2.1, 4.2) and (1.5, 3.1, 6.2) 10^5 spore / ml of *Aspergillus niger* and *Penicillium chrysogenum*, respectively were used to determine their effect on growth parameters of wheat in sandy and loamy soil. The heigher lengths of wheat plants were (29.53, 33.10 cm); more fresh shoot weights (214.00, 226.67 mg) and root weights (135.67, 166.33 mg / plant) in *A. niger* treatment at 2.1 × 10^5 spore / ml conc. While lengths (31.13, 33.37 cm); shoot weight (214.00, 226.67 mg) and root weights (146.00, 173.67 mg) for *P. chrysogenum* at 3.1×10^5 spore / ml in sandy and loamy soil respectively, compared with lengths (22.60, 24.33 cm), fresh shoot weights (121.67, 136.33 mg) and root weights (87.00, 107.33 mg) in sandy and loamy soil only, respectively. It was found the 2 straw : 4 soil + *P.chrysogenum* treatment increased the length of wheat plants to (23.33 , 25.67 cm) and the fresh weights of plants (193.30 , 211.07 mg) in sandy and loamy soil, respectively . While the lengths of wheat in 2 straw : 4 soil + *A. niger* were (22.00 , 24.33 cm) and weights (196.63 , 224.40 mg) in sandy and loamy soil respectively too , compared with lengths (10.67 , 16.33 cm) and plant weights (81.10 114.43 mg) in sandy and loamy soil treatment.

The result showed that the 1g .phosphate / l. P.S.A. medium gave a high radial growth, which reached to 8.9 and 3.3 cm for *A. niger* and *P. chrysogenum* respectively compared with 7.5 and 2.2 cm in P.S.A. without phosphate. The results showed that the lengths and weights in soil + phosphate + *P. chrysogenum* reached to 31.0 cm and 1063.3 mg and in *A.niger* treatment were 27.0 cm and 903.3 mg, respectively.

The spore viability decreased on dressed grains and in spore suspension to (0.04, 1.37 ×10^5) and (0.12 , 2.93 ×10^5) to each of *A. niger* and *P. chrysogenum* respectively at 9 months, while the vital spores at 3 and 6 months were (1.32, 1.03 ×10^5) and (20.20, 15.60 ×10^5) for *A.niger* and *P.chrysogenum* on dressed grains and spore suspension, respectively.

The field experiment appeared that : soil + straw + *P. chrysogenum* gave a high growth parameters, which reached to 6.00, 133.00 cm, 4.40 g, 79.00 g, 85.33 and 45.25 g for No. of tellers, length of plant, weight of plant, length of spike, total weight of spike , weight of spike grains, No. of grains in each spike and weight of 1000 grains, respectively. The percentage of productivity increased to 45.25 and 39.67 % in soil + straw + *P. chrysogenum* and soil + straw + *A. niger* treatments, respectively.

Key words : *A. niger*, *P. chrysogenum*, biofertilizers, wheat, straw, phosphate.

Introduction

Biofertilizers have shown great potential as supplementary , renewable and environmental friendly source of plant nutrients and are an important component of integrated nutrient management and integrated plant nutrition system (Raghuwanshi, 2012). Biofertilizers not only give a better growth and productivity for the plants, but are also harmless to humans and lead to better sustainable economic development for the farmers and their country (Mishra and Dash, 2014). 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 environment 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 genera *Aspergillus*, *Penicillium* and

Trichoderma, endemic in the root zone of plant 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).

It was found that the *Aspergillu sniger* and *Trichoderma harzianum* fungi and their combination gave significant effects on the growth and yield of wheat in all treatments (Nihad *et al*., 2015). The grain yield was 674.25 and 638.82 g /m^2 in (*A. niger* + *T. harzianum*) and *A. niger* treatments, respectively compared with 475.65 g/m^2 in control treatment (Al-Taie *et al*., 2016).

The Mixture of okra bark and Turmeric increased the radial growth and sporulation of *A.niger* and *P. chrysogenum*. It was found also the mixture of okra bark and Turmeric extraction increased the carried spores of *A.niger* and *P.chrysogenum* on the wheat grains. This result is very important to use spore suspension of biofertilizers in okra bark as adhesive substance (Dewan *et al*., 2018).

Materials and Methods

Effect of different spore levels of *A. niger* and *P. chrysogenum* in sandy and loamy soil on plant growth of wheat

The spore concentration of *A. niger* and *P. chrysogenum* were prepared by washing the new fungal colonies with sterilized distelled water. The concentration (1.0, 2.1, 4.2) and (1.5, 3.1, 6.2) $\times 10^5$ were used to *A. niger* and *P. chrysogenum*, respectively. 10 ml to each concentration added to 200 g of sandy and loam soil. The inoculated soil put in small pots (200 g/pot) individualy to each fungus and concentration. The treatments replicated 3 times. 10 wheat grains planted in each pot, irregated and put in growth chamber at 25^{o}C ± 2 and 12 h. light: 12 h. dark. The length and fresh weight to shoot and root of wheat were calculated after 21 day (Dewan, 1989).

Effect of different levels of wheat straw in inoculated sandy and loamy soil by *A. niger* and *P. chrysogenum*on growth of wheat plant

The both type of soil inoculated by 10 ml of spore suspension to 200 g soil at 2.1×10^5 and 3.1×10^5 spore / ml of *A. niger* and *P. chrysogenum* respectively. The inoculated soil mixed with wheat straw at volume levels 1:4 , 2:4 and 3:4 (straw :soil). 200 g from mixed soil with straw put in small pot according to the straw levels and control treatments. The treatments replicated 3 times. The grain wheat planting, growth conditions and calculating growth parameters of wheat plants as in (1).

Effect of phosphate rock (powder) on growth of *A. niger*, *P. chrysogenum* and wheat

a- Fungal growth

Potato sucrose Agar (P.S.A.) medium was prepared and the concentrations 1,2 and 3 g of phosphate powder were added to 1L. P.S.A. medium. The medium with phosphate was autoclaved, then pured in Petri-dish. One plug 0.5 cm of new growth to each of *A. niger* and *P. chrysogenum* were cultured on the center of medium in the Petri-dish. The inoculated petri- dishes were incubated at 25^{o}C ± 2. The radial growth of each fungus in all treatments recorded after 10 days.

b- Wheat growth

1 g of phosphate powder mixed with 1 kg loamy soil. The mixed soil with phosphate inoculated with 10 ml / 200 g soil of *A. niger* and *P. chrysogenum* suspension at concentration 2.1 and 3.1×10^5 spore/ml, respectively. The mixed soil with phosphate and spores of biofertilizers were divided to three replicates according to the treatments, 200 gm put in pot, planted by 10 wheat grains to each pot. The planted pots irrigated by distilled water and inocubated in growth chamer as in (1). The vegetative length and fresh weight of wheat plats were taken after 28 days.

Viability of *A. niger* and *P. chrysogenum* spore on wheat grains and in extraction of okra bark and Turmeric mixture in laboratory conditions for different periods 32 and 10 g of okra bark and Turmeric powder respectively were added to 1L sterilized distilled water. The components boiled to 20 min and after that filtrated during 3 layers of smothmousseline cloth. The completed growth in Petri-dish of *A. niger* and *P. chrysogenum* were washed by 30 ml/petri-dish of okra bark and turmeric filetration. The spores removed by smothbrush. The spore suspension divided to two part .One part (15ml) put in the inoculation apparatus (Dewan *et al*., 2018) to dress the grain wheat. 500 g wheat grains dressed by 10 ml of spore suspension in mixture of okra bark and Turmeric . The treated wheat grain pull out from the apparatus and put on towel paper to remove the free water . The second part (15ml) of spore suspension put in sterilized tube. 1g of dressed wheat grain and 1ml of spore suspension to *A. niger* and *P. chrysogenum* were taken and put in 9 ml of sterilized distilled water individualy. Serial of dilutions done to 10^{-5} to determine the viability of spore to each fungus after treatment (directly), 3, 6 and 9 months. 1 ml of 10^5 dilution put in Petri-dish, 20 ml of P.S.A. pured on it and gently moved to mix the spore suspension with medium. The plates inocubated at 25^{o}C ± 2 for 24 h. The formed colonies were calculated as the vital spores =

No. of colonies $\times 10^5$ (Clark, 1965).

Effect of mixed wheat straw with inoculated field soil by *A. niger* and *P. chrysogenum* on growth parameters and productivity of wheat crop

The field land prepared during the season 2015-2016 and divided to small plots (4×4) m. in four replicates. The distances between the plot were 1m to prevent the contamination from other treatments, some plot soil mixed with 800g of wheat straw/plot and inoculated by 640 ml 2.1 and 3.1×10^5 spore/1ml to each of *A.niger* and *P.chrysogenum* respectively as the following treatments : soil only, soil + straw, soil + *A.niger*, soil + straw + *A.niger*, soil + *P.chrysogenum*, soil + straw + *P. chrysogenum*. The plots planted with wheat (var. Ibaa 99) at rate 35 kg grain / Donum (2500m^2) depending on the applicable recommendations in Iraq (Alyounis, 1993). The plots irrigated separately to avoid the contamination between the experimental units. In the end of season, the following parameters : No. of tellering, plant length, dry weight of plant, length and weight of spike, No. of grains per spike, weight of 1000 grains and percentage of productivity increasing were taken.

The design of study was RCBD and the data were analyzed using Genstat statistical software program and means were compared using LSD ≤ 0.05 (Al-Rawei and KhalafAllah, 1980).

Results and Discussion

Effect of different spore levels of *A. niger* and *P. chrysogenum* in sandy and loamy soil on plant growth of wheat

The results in table (1a) showed that the heigher lengths of wheat plant were 29.533 and 33.100 cm for *A.niger*, and 31.133 and 33.367 cm for *P.chrysogenum* in sandy and loamy soil at 2.1 and 3.1×10^5 spore/ml concentrations respectively, compared with 22.600 and 24.333 cm in sandy and loamy soil only. The other concentrations of spore suspension to each of *A. niger* and *P. chrysogenum* also increased the lengths of wheat plant but less than from the 2.1 and 3.1×10^5 spore/ml to above fungi, respectively. In general, *P. chrysogenum* more effective than *A. niger* especialy in loamy soil. Also, the loamy soil gave more length (28.872 cm) compared with sandy soil (26.322 cm).

More fresh weights of shoots were 214.000 and 226.667 mg in sandy and loamy soil respectively for *A. niger*, while 217.000 and 232.333 mg in *P. chrysogenum* treatment compared with soil only treatment, which were 121.667 and 136.333 mg in sandy and loamy soil, respectively. The root weights also increased to 135.667 and 166.333 in sandy and loamy soil, respectively, for *A. niger*, while *P. chrysogenum* gave more increasing in root weights which reached to 146.000 and 173.667 mg in sandy and loamy soil, respectively. In general, the loamy soil and *P. chrysogenum* gave more increasing in root weights compared with sandy soil and *A.niger*.

The growth increasing of shoot and root of wheat may be return to mobilize the availability of nutrient by biological activity of microorganisms (Ismail *et al*., 2014), or some species of *Aspergillus* and *Penicillium* have heigh ability to produce Indol Acetic Acid and Gibrilins to promote the growth of plants (El-Ghany *et al.*, 2010).

Effect of different levels of wheat straw in inoculated sandy and loamy soil by *A. niger* and *P. chrysogenum* on growth of wheat plants

It was found (table 2) the 2 straw : 4 soil + *P. chrysogenum* treatment increased the length of wheat plant to 23.33, 25.67 cm and the fresh weights were 193.30, 211.07 mg in sandy and loamy soil, respectively, compared with 10.67, 16.33 cm for length and 81.10, 114.43 mg for weight in sandy and loamy soil in same above treatment, while 2 straw : 4 soil + *A.niger* treatment increased the length to 22.00, 24.33 cm and weight to 196.63, 224.40 mg in sandy and loamy respectively compared with length (10.67, 16.33 cm) and plant weights (81.10, 114.43 mg) in sandy and loamy soil only. All the other levels of straw with inoculated soil by *A. niger* and *P. chrysogenum* increased the lengths and weights of wheat plants, but less than soil without straw or soil with fungus.

The increasing length and weight plants due to the *A. niger* and *P. chrysogenum* produce many extracellular enzymes like cellulase, xyloase etc., which act as biodegradation to the wheat straw in soil. The biodegradation release the nutrients to absorb by roots (Pab and Bhagat, 2008), or produce growth promoting substances like : Indol Acetic Acid (IAA) and Gibberellic Acid (GA) (Bilkay *et al.*, 2010; Yadav *et al.*, 2011).

Effect of different levels of phosphate rock in radial growth of *A. niger* and *P. chrysogenum* on P.S.A. medium

It was found (table 3 - a) the concentration 1g/l gave a high radial growth which reached to 8.9 and 3.3 cm for *A. niger* and *P. chrysogenum*, respectively compared with 7.5 and 2.2 cm in P.S.A. without phosphate or 7.8 and 2.5 cm at 3 g phosphate/l P.S.A. to each of fungi, respectively also.

The high growth of *A.niger* and *P.chrysogenum* in 1 g phosphate / l medium return that concentration is

Table 1 : Effect of different spore levels of *Aspergillus niger* (*A.n.*) and *Penicillium chrysogenum* (*P.c.*) in sandy and loamy soil on length (1a) and fresh weight (1b) of weight at 21 day old.

1a : length (cm) of wheat plants

Fungi	Spore conct. (10^5)	Length (cm) of wheat plants		Mean
		Sandy soil	Loamy soil	
Soil only	-	22.600	24.333	23.467
P.c.	1.5	25.967	27.067	26.517
P.c.	3.1	31.133	33.367	32.250
P.c.	6.2	24.533	28.633	26.583
A.n.	1.0	24.167	26.733	25.450
A.n.	2.1	29.533	33.100	31.317
A.n.	4.2	26.633	27.933	27.283
Mean		26.322	28.876	

L.S.D.0.05 Soil = 0.808 Fungi =1.512 Interaction = N.S.

1b : Fresh shoot and root weights (mg) of wheat plants.

Fungi	Spore conct.(10^5)	Fresh shoot weight (mg)		Mean	Fresh root weight (mg)		Mean
		Sandy soil	Loamy soil		Sandy soil	Loamy soil	
Soil only	-	121.667	136.333	129.000	87.000	107.333	97.167
P.c.	1.5	177.000	187.000	182.000	116.667	141.000	128.833
P.c.	3.1	217.000	232.333	224.667	146.000	173.667	159.833
P.c.	6.2	188.000	194.667	191.333	123.667	152.667	138.167
A.n.	1.0	172.667	167.667	170.167	111.333	131.000	121.167
A.n.	2.1	214.000	226.667	220.333	135.667	166.333	151.000
A.n.	4.2	198.667	207.667	203.167	123.000	155.667	139.333
Mean		184.143	193.190		120.476	146.809	
L.S.D.0.05 Soil = 4.26 Fungi = 7.97 Inter. N.S.					Soil = 1.672 Fungi 3.127 Inter. N.S.		

Table 2 : Effect of different levels of wheat straw in inoculated sandy and loamy soil by *A. niger* and *P. chrysogenum* on length (cm) and weights (mg) of wheat plant at 21 days old.

Treatment	Length of wheat plant (cm)		Weight of wheat plant (mg)	
	Sandy soil	Loamy soil	Sandy soil	Loamy soil
Soil only	10.67	16.33	81.10	114.43
1 straw : 4 soil	15.67	18.33	109.97	137.73
2 straw : 4 soil	16.33	19.67	131.07	165.50
3 straw : 4 soil	14.33	17.67	99.97	108.83
Soil + *P.c.*	18.67	20.33	144.40	153.30
1 straw : 4 soil+*P.c.*	20.33	22.00	158.87	175.50
2 straw : 4 soil+*P.c.*	23.33	25.67	193.30	211.07
3 straw : 4 soil+*P.c.*	18.00	19.33	135.50	151.10
Soil + *A.n.*	17.67	19.33	142.17	149.97
1 straw : 4 soil+*A.n.*	20.33	22.56	166.63	189.67
2 straw : 4 soil+*A.n.*	22.00	24.33	196.63	224.40
3 straw : 4 soil+*A.n.*	20.00	20.33	128.83	137.73
L.S.D.0.05	2.247	1.376	12.000	14.380

Table 3-a : Effect of different levels of phosphate in radial growth (cm) of *A. niger* and *P. chrysogenum* on P.S.A. medium for 10 days.

Phosphate concentration g/l	Radial fungal growth (cm)		Mean
	A. niger	*P. chrysogenum*	
P.S.A.only	7.5	2.2	4.85
1	8.9	3.3	6.1
2	8.1	2.6	5.35
3	7.8	2.5	5.15
Mean	8.21	2.78	5.49

L.S.D.0.05 Phosphate conce.=0.22 Fungi = 0.15
Interaction = 0.31

suitable for fungal growth, while 2 and 3 g/l medium concentrations slightly reduced the growth of above fungi may be due to the toxic effect by excess of phosphate.

Effect of phosphate (1g) addition to inoculated soil (1 kg) with *A. niger* and *P. chrysogenum* on length and weight of wheat plants

The results cleared (table 3 - b) that the length and

Table 3-b : Effect of phosphate addition to inoculated soil with *A. niger* and *P. chrysogenum* on the length and weight of wheat plant at 28 day old.

Treatments	Plant length (cm)	Fresh plant weight (mg)
Soil only	20.6	410.0
Soil + phosphate	23.6	596.6
Soil + *P.c.*	26.6	790.0
Soil + *A.n.*	24.3	686.6
Soil + phosphate + *P.c.*	31.0	1063.3
Soil + phosphate + *A.n.*	27.0	903.3
L.S.D.0.05	1.828	56.41

Table 4 : Effect of storage periods (months) on the spore viability of *A. niger* and *P. chrysogenum* on wheat grains and in suspension.

Treatments	Fungi	Storage periods (month) of spore (10^5)				Mean
		Direct	3	6	9	
Dreassed grains	*A.n.*	1.55	1.32	1.03	0.04	0.99
	P.c.	1.94	1.87	1.56	0.12	1.37
Spore suspension	*A.n.*	21.10	20.20	15.60	1.37	14.57
	P.c.	23.40	21.80	18.10	2.93	16.56
Mean		11.99	11.30	9.24	1.12	
L.S.D.0.05 Fungi = 0.209 Periods = 0.418 Interactions = 0.591						

decreased to (1.32, 1.03×10^5) and (20.20, 15.60×10^5 spore/ml) for *A.niger* on wheat grains and spore suspension respectively in 3 and 6 month. where as were (1.87, 1.56×10^5) and (21.80, 18.10×10^5) for *P. chrysogenum* in same above treatments and storage periods, respectively. The results showed a high reduction in the viability of storage spore at 9 month, therefore, the vital spores were (0.04, 1.37×10^5) and (0.12, 2.93×10^5) to each of *A. niger* and *P. chrysogenum* on wheat grains and spore suspension respectively at 9 month. The declivity of storage spores for 9 month may be returns to the fluctuation of laboratory temperature and autolysis (Mulusa *et al*., 2016).

Effect of mixed wheat straw with inoculated field soil by *A. niger* and *P. chrysogenum* on growth parameters and productivity of wheat crop

The results appeared table 5 that the addition of wheat straw to the field soil with or without *A. niger* and *P. chrysogenum* increased the growth parameters of wheat, but the inoculated soil with fungi showed a high growth parameters especialy in : soil + straw + *P. chrysogenum* treatment which reached to 6.00, 133.00 cm, 4.40 g, 79.00 g, 85.33 and 45.25 g for No. of Tellers, length of plant, weight

Table 5 : Effect of wheat straw mixing with inoculated field soil by *A. niger* and *P. chrysogenum* on growth parameters and productivity of wheat crop.

Treatments	No. of tillers	Length of plant (cm)	Weight of plant (g)	Length of spike (cm)	Total weight of spike (g)	Weight of spike grains (g)	No. of grains in spike	Weight of 1000 grains (g)	% increasing of productivity
Soil only	1.67	72.00	8.40	7.50	2.36	43.67	50.67	31.59	0.00
Soil +straw	4.00	82.67	22.70	9.67	3.65	69.00	78.67	35.49	10.96
Soil +*P.c.*	4.33	83.00	19.16	10.33	3.15	66.67	74.33	34.26	8.32
Soil+straw+*P.c.*	6.00	113.00	31.96	13.00	4.40	79.00	85.33	45.25	30.16
Soil +*A.n.*	4.67	80.67	17.94	9.00	2.95	66.00	69.67	34.40	7.80
Soil+straw +*A.n.*	5.00	105.33	28.38	10.00	3.64	76.00	80.33	39.67	20.27
L.S.D.0.05	1.624	8.59	3.008	2.852	0.705	3.533	5.855	2.7	6.508

weight in soil + phosphate + *P. chrysogenum* reached to 31.0 cm and 1063.3mg, respectively, while in soil + phosphate + *A.niger* were 27.0 cm and 903.3 mg respectively also, compared with 20.6 cm and 410.0 mg in soil only treatment. This increasing may be belong to the *A.niger* and *P.chrysogenum* have high ability to absorb certain nutrients and make the phosphorus component available for absorption (Rashid *et al*., 2004).

Effect of storage periods (months) on the spore viability of *A. niger* and *P. chrysogenum* on wheat grains and in suspension

The viability of fungal spores (table 4) slowly

of plant, length of spike, total weight of spike, weight of spike grains, no. of grains in each spike and weight of 1000 grains, respectively. The important result was increasing of productivity percentage in all treatments. The treatments : soil + straw + *P. chrysogenum* and soil + straw + *A.niger* increased the productivity to 30.16 and 20.27%, respectively.

A. niger and *A. fumigatus* have significant effects on growth parameters and productivity of wheat crop . (Al-Taie *et al*., 2016). The fungi act to increase the ability of plants to get the phosphorus from soil using many mechanisms including : increasing surface area of roots

(Alan, 2007). The growth promoting fungi provide a suitable environment for plant by producing hormones, reducing pH and secretion such organic acids. These mechanisms are effective on the avaiability of nutrition especially phosphate, which it reflex positively on plant growth and grain yield (Brink *et al*., 2014).

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