



## SHORT COMMUNICATION

### Methods of plant growth-promoting fungi application to enhance the growth and yield of wheat var. Ibaa99

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#### ABSTRACT

**Aims:** The main aim of the study was to evaluate some methods of application of *Aspergillus niger* AD1 and *Trichoderma hamatum* T-113 for enhancing the growth and yield of wheat var. Ibaa99 in pots and field conditions.

**Methodology and results:** Plant growth-promoting fungi (PGPF) loaded with peat moss were used at a rate of 100, 150 and 200 mL pot<sup>-1</sup> or m<sup>-2</sup> in filed soil; seed treatment (coating) with fungi suspension  $19 \times 10^7$ , soil treatment and combination of all the three methods was employed in the study. Wheat seeds were sown in pots and field plots during 2018-2019, and data regarding various growth and yield attributes were recorded. In both pot and field trials, the results revealed that the best treatments for the desired plant growth and yield attributes were peat moss 150 mL alone or in combination with soil and seed treatments. The soil physicochemical parameters were also improved after inoculation with selected fungal isolates in different application methods compared with un-inoculated control treatment in both pot and field conditions.

**Conclusion, significance and impact of study:** The PGPF play a vital role represented phytoremediation, phytostimulation and bio-fertilization. The isolates of PGPF, which were applied with peat moss at 150 mL to the pot and in the field alone or combined with seed treatment and soil application, were significantly the best effective method for improving wheat attributes.

**Keywords:** Application methods, *Aspergillus niger*, PGPF, *Trichoderma hamatum*, wheat

#### INTRODUCTION

Developing microbial bio-inoculants for plant growth promotion, besides eradicating disease, is an alternative to enhance crop productivity. Fungi that establish a positive interaction with plants in the rhizosphere are called plant growth-promoting fungi (PGPF). These PGPF play an essential role representing as phytoremediation, phytostimulation and bio-fertilization. The main beneficial traits of PGPF include the production of some substances like plant hormones (also known as plant growth promoters) and siderophores, in addition to phosphate solubilization. PGPF are also known to protect host plants from plant diseases by suppressing pathogenic microbes.

In sustainable agriculture, the PGPF provided a new vision to the agro-economy besides its direct benefits by reducing chemical fertilizers and pesticides. Continuous fertilizers and pesticides have many negative consequences on environmental and human health. The formulation of an effective fungal strain could be a crucial

issue for inoculants and it can determine the biological agent's success or failure. The application methods used as carrier materials are an attractive option for delivering microbes to soil or the rhizosphere. Generally, the carrier materials are intended to provide a suitable environment to microbial inoculants in soil by the provision of a protective surface and provision of a specific substrate (Gupta *et al.*, 2015).

The whole process of bio-formulation, starting from the screening of the microbe and ending with the product's development and implementation, this process needs to be investigated. There are wide types of bio-fertilizer formulations, such as liquid and solid, but the main types are currently classified into dry products and suspension. The kind of crop could affect the application methods, such as annual crops that can be inoculated by broadcasting the inoculum over the soil surface, whether alone and with seeds, application in-furrow, and seed dressing or coating, while the tree can be inoculated by seedling inoculation and dipping root (Malusá *et al.*,

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2012). The choice of the carrier must depend on absorption, bulk density and hardness besides the disintegration rate in water. Peat moss is presently considered the most widely used carrier material.

PGPF is delivered through several means based on effective quality methods such as loaded on peat moss, seed and inoculants soil. PGPF, which is used as bio-fertilizers, has been found to improve nutrient content, plant growth and yield in addition to activating plant defense and is considered the best adapted for sustainable agriculture (Yadav *et al.*, 2017). Diverse studies report that inoculation with only one beneficial microorganism generally increases plant growth and decreases pathogenic agents (Chandanie *et al.*, 2006).

The successful application of fungal fertilizers reported in many studies started to promote plant growth, which helps reduce applying synthetic chemical fertilizers because of their harmful effects on humans and the environment (Sarma *et al.*, 2014). The bio-fertilizer, in some cases, can reduce the usage of chemical fertilizer by about 20 percent, besides increasing crop production. Many available commercial products of fungal bio-fertilizers are available worldwide and they can be more environmentally friendly and alternative to chemical fertilizers. However, fungal bio-fertilizers are used on a very small scale as compared to chemical fertilizers.

The application of plant growth-promoting rhizobacteria (PGPR) and PGPF alone or in combination can minimize the side effect of chemical fertilizer, in addition to improving soil health, reducing environmental stress and promoting the sustainability of agriculture (Singh *et al.*, 2017). The study aimed to choose an appropriate method of PGPF applications and their efficacy on wheat crops in both pots and field conditions.

## MATERIALS AND METHODS

The study was conducted at the Department of Plant Protection, College of Agriculture, University of Wasit, Iraq. From the previous studies, *A. niger* (AD1) and *T. hamatum* (T-113) were the best PGPF in wheat var. Ibaa99 (Al-Taie *et al.*, 2016). These strains were used to evaluate and find out the best application method to boost wheat growth and yield.

Delivery or application methods that are evaluated as a place can highly increase the growth and yield of wheat needed. The study was conducted in two experiments to select the best application methods of *A. niger* (AD1) and *T. hamatum* (T-113) for maximum effect on wheat growth and the yield in plastic pots and open fields in the 2018-2019 season.

### Maintenance of the PGPF culture

The fungi *A. niger* (AD1) and *T. hamatum* (T-113) were grown in Petri plates containing Potato Dextrose Agar (PDA) medium, which was amended with chloramphenicol 250 mg/L, then incubated at  $28 \pm 2$  °C for five days. Fungal inoculums were loaded on peat moss brand KLASMANN (Germany) because it retains

moisture to help the fungus grow and colonize. In addition, it is easy to use and available in Iraqi markets at competitive prices. After its sterilization using formalin solution 1:50 v/v, it was used at a rate of 3 L m<sup>3</sup> soil<sup>-1</sup>, placed in sealed bags for three days, then uncovered for three days to remove the toxic residue and finally added in polyethylene bags to maintain its moisture (Toajin, 1979).

### Pot culture experiment

Experiments were conducted using a plastic pot 25 × 25 × 30 cm sterilized with a 20% solution of sodium hypochlorite and then filled with sterile sandy loam soil. Each pot received N, P and K (DAP) before planting (depending on the applicable recommendations in Iraq) (Alyounis, 1993).

Wheat seeds (var. Ibaa99) were used in the study from the national program for the development of wheat in Iraq, Ministry of Agriculture, Iraq. The percentage of germination was found to be 90 percent. Five seeds were planted in each pot.

### Field experiment

The field plots were prepared by dividing the field land into five replicate plots 7 × 5 m for each treatment and each plot received N, P and K (DAP) before planting according to the Iraqi agriculture recommendation for the wheat crop.

The plots were planted with a wheat seed (var. Ibaa99) at a rate of 45 kg seeds/Donum (2500 m<sup>2</sup>) (depending on the applicable recommendations in Iraq) (Alyounis, 1993). The plots were irrigated separately to avoid contamination between the experimental units.

### Treatments

The experiment was conducted in plastic pots and field plots arranged into nine treatments: PGPF were loaded with peat moss and then used at a rate of 100, 150 and 200 mL/pot and m<sup>2</sup> for inoculation of both potting soil and field lots. Seed treatment method was seed coated with PGPF suspension  $19 \times 10^7$ ; the soil application method before seed planting by spraying PGPF suspension  $19 \times 10^7$  to the soil and mixed. Combination of all methods was as follows:

- PGPF isolate loaded in 100 mL + Peat moss + Seed treatment + Soil application
- PGPF isolate loaded in 150 mL + Peat moss + Seed treatment + Soil application
- PGPF isolate loaded in 200 mL + Peat moss + Seed treatment + Soil application

The control treatment contains soil only (uninoculated). Each treatment was replicated 5 times and arranged in a randomized completely block design (RCBD).

**Table 1:** Effect of application methods of PGPF on growth and production of wheat in plastic pots.

Attributes Treat.	No. of plant (pot)	Plant height (cm)	Plant dry weight (g)	Spike length (cm)	No. of seeds per spike	Weight of 100 kernels (g)
Peat moss 100 mL	4.03	44.53	6.08	7.90	26.67	1.67
Peat moss 150 mL	4.67	49.63	8.60	9.63	35.00	1.86
Peat moss 200 mL	4.33	46.47	6.71	8.76	27.22	1.83
Seed treatment	4.13	37.53	6.47	7.43	28.77	0.84
Soil application	4.00	35.43	5.66	6.96	19.65	0.76
Peat moss 100 mL + Seed treatment + Soil application	4.47	38.83	7.37	8.03	28.97	0.90
Peat moss 150 mL + Seed treatment + Soil application	4.69	43.17	9.30	10.80	36.97	2.23
Peat moss 200 mL + Seed treatment + Soil application	4.63	39.90	7.53	8.93	31.47	1.43
Control	3.17	30.81	4.45	6.03	14.25	0.67
Standard deviation (SD)	0.55	5.70	1.44	1.54	7.41	0.65

LSD 5% stands for: No. of plant\0.841, plant height\2.094, plant dry weight\0.806, spike length\0.790, no. of seeds per spike\6.229, weight of 100 kernels\0.300.

## Observations

Methods of application of PGPF were evaluated for maximum effect on attributes of wheat. At the end of the plant age, five plants from each pot were selected. From each field plot, plants were selected randomly from 1 m<sup>2</sup>. Wheat attributes represent no. of plants, plants height (cm), dry plant weight (g), spike length (cm), no. of seeds per spike and weight of 100 kernel (g) in addition to a grain of ten plants/m<sup>2</sup>.

## Physicochemical parameters of soil

Physicochemical parameters for pots and field soil were initially characterized. Soil site samples were taken from depths of 0-30 cm using polyethylene bags for physical and chemical laboratory analyses.

Soil analysis was done as the following procedures: (1) pH and electrical conductivity (EC) were measured in water (1:1 soil:water ratio); (2) Available nitrogen content was determined by wet-oxidation (wet digestion) procedure of the Kjeldahl method (Sahlemedhin and Taye, 2000); (3) Available phosphorus and potassium content were determined by Morgan's extraction solution (Bray and Kurtz, 1945).

## Statistical analysis

The collected data were subjected to randomized completely block design analysis and the interpretation of the data was carried out in accordance with Al-Rawi and Khalaf Allah (1980) using Genstat statistical software program. The significance level used in the least significant difference (LSD) and F test was  $p=0.05$ . The values differences were calculated whenever the F value was significant.

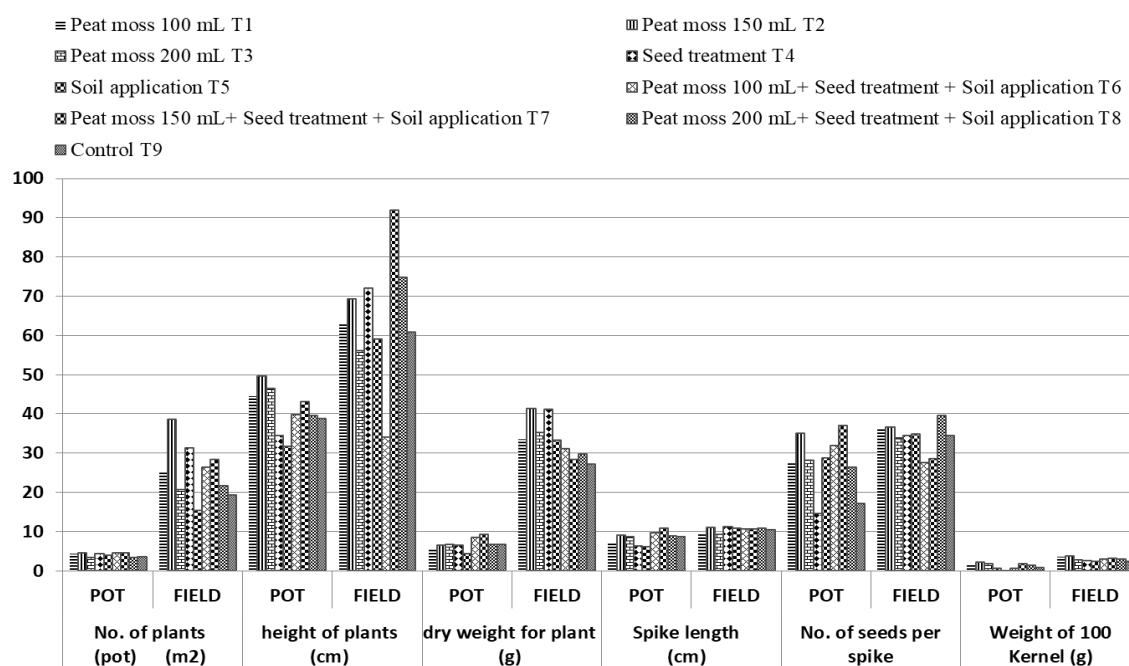
## RESULTS

Application methods of *A. niger* (AD1) and *T. hamatum* (T-113) as PGPF showed a different effect on the attributes of wheat. Plant height (cm), dry weight (g), spike length (cm), no. of seeds/spike and weight of 100 kernels (g) was determined in addition to grain yield in the field under the application. In pots, the data pertaining to the effect on plant height, dry weight, spike length, no. of seeds/spike and the weight of 100 kernels are depicted in (Table 1). Most of the attributes are significant due to application methods. The highest attributes were observed in the plants inoculated with PGPF loaded in 150 mL peat moss with an average of 49.63 cm, 8.6 g, 9.63 cm, 35 seed and 1.86 g in plant height, dry weight, spike length, no. of seeds/spike and weight of 100 kernels, respectively, compared to other application methods and control.

The results also revealed that the seed treatment application method was the second-best treatment and its effect on wheat attribute followed by the soil treatment method.

A combination of all three methods' results revealed that a combination with PGPF loaded on 150 mL peat moss was the best treatment compared with other combinations in wheat attributes. It produced an average of 43.17 cm, 9.3 g, 10.8 cm, 36.97 seed and 2.23 g in plant height, dry weight, spike length, no. of seeds/spike and weight of 100 kernels, respectively, in plastic pots (Table 1). While there were no differences between treatments in the number of plants per pot.

In the field, the results revealed that most attributes are significant due to application methods (Table 2). The highest attributes were observed in the plants inoculated with PGPF loaded in 150 mL peat moss with an average of 32.33 plants/m<sup>2</sup>, 70.32 cm, 38.94 g, 10.72 cm, 38.52 seed and 4.17 g in no. of plants per m<sup>2</sup>, plant height, dry weight, spike length, no. of seeds/spike and weight of 100



**Figure 1:** Effect if different methods of application of PGPF on growth and production of wheat in pots and field.

**Table 2:** Effect of application method of PGPF on growth and production of wheat in field condition.

Attributes	No. of plants (m <sup>2</sup> )	Plant height (cm)	Dry weight for plant (g)	Spike length (cm)	No. of seeds per spike	Weight 100 kernels (g)	Grain yield (g/m <sup>2</sup> )
Treat.							
Peat moss 100 mL	24.33	62.92	34.58	9.33	34.28	3.58	127.33
Peat moss 150 mL	32.33	70.32	38.94	10.72	38.52	4.17	240.33
Peat moss 200 mL	25.67	66.07	35.23	9.82	36.92	3.88	223.33
Seed treatment	27.33	61.98	37.10	9.18	35.89	2.70	166.33
Soil application	25.33	58.98	33.31	8.90	34.80	2.48	108.67
Peat moss 100 mL + Seed treatment + Soil application	24.83	54.13	33.20	9.65	27.65	2.95	113.33
Peat moss 150 mL + Seed treatment + Soil application	36.67	75.93	41.32	11.13	41.63	4.88	260.67
Peat moss 200 mL + Seed treatment + Soil application	21.67	64.81	31.76	8.97	35.23	3.08	124.00
Control	19.33	49.87	27.22	6.43	24.42	2.34	86.00
Standard deviation (SD)	7.03	15.71	5.08	0.64	3.79	0.55	64.50

LSD 5% stands for: No. of plant\4.696, plant height\7.286, plant dry weight\5.900, spike length\1.328, no. of seeds per spike\9.13, weight of 100 kernels\0.467, grain yield\24.14.

kernel, respectively, compared with other application methods and control.

The results also revealed that the seed treatment application method was the second-best treatment and its effect on wheat attributes followed by the soil treatment method.

A combination of all three methods' results revealed that the combination with PGPF loaded in 150 mL peat moss was the best treatment compared with other combinations in wheat attributes. It produced an average of 36.67 plants/m<sup>2</sup>, 75.93 cm, 41.32 g, 11.13 cm, 41.63 seed and 4.88 g in the no. of plants/m<sup>2</sup>, plant height, dry

weight, spike length, no. seeds/spike and weight of 100 Kernel, respectively, compared to other application methods and control (Table 2).

The results (Table 2) showed a significant increase in grain parameters with an application of single and combination methods due to applying PGPF to wheat crops in different methods. The application of PGPF loaded on 150 mL peat moss was the best single method with 240.33 g/m<sup>2</sup> and the combination methods with PGPF loaded in 150 mL peat moss were the best treatment compared with other combinations with 260.33 g/m<sup>2</sup> compared to the control treatment with 86 g/m<sup>2</sup>.

**Table 3:** Soil parameters after application of PGPF in pots.

Treatments	EC ds.m <sup>-1</sup> (1:1)	pH	N ppm	P ppm	K ppm
Peat moss 100 mL	4.33	7.60	214.00	65.67	123.67
Peat moss 150 mL	3.27	7.33	70.67	52.10	99.67
Peat moss 200 mL	4.37	7.63	151.33	67.67	128.67
Seed treatment	4.50	7.73	149.67	56.67	116.33
Soil application	4.60	7.70	142.00	61.00	125.33
Peat moss 100 mL + Seed treatment + Soil application	4.27	7.83	182.33	55.83	131.33
Peat moss 150 mL + Seed treatment + Soil application	4.43	7.13	137.67	49.20	100.33
Peat moss 200 mL + Seed treatment + Soil application	4.60	7.60	167.00	43.10	126.67
Control	5.57	7.93	192.33	71.00	147.67
Standard deviation (SD)	0.78	0.46	13.44	9.38	15.11

LSD 5% stands for: EC\0.262, pH\0.249, N\6.52, P\4.894, K\5.082.

**Table 4:** Soil parameters after application of PGPF in the field.

Treatments	EC ds.m <sup>-1</sup> (1:1)	pH	N ppm	P ppm	K ppm
Peat moss 100 mL	1.29	7.23	43.33	41.33	274.00
Peat moss 150 mL	1.35	6.30	42.33	32.37	149.67
Peat moss 200 mL	1.36	7.50	56.33	40.30	176.67
Seed treatment	1.52	7.27	37.00	35.67	139.83
Soil application	2.23	7.57	47.33	57.60	249.33
Peat moss 100 mL + Seed treatment + Soil application	1.52	7.47	48.00	45.03	330.00
Peat moss 150 mL + Seed treatment + Soil application	0.83	7.23	27.00	28.50	139.00
Peat moss 200 mL + Seed treatment + Soil application	1.87	7.63	55.67	27.70	368.00
Control	3.53	8.00	74.47	43.73	527.00
Standard deviation (SD)	0.78	0.46	13.44	9.38	130.50

LSD 5% stands for: EC\0.300, pH\0.307, N\5.056, P\11.88, K\68.32.

An increase significantly resulted in most wheat plants' attributes under field conditions compared with pots (Figure 1).

The soil parameters were also improved soil physiochemical parameters after being inoculated with PGPF in different methods of application compared with the control treatment, which was un-inoculated in both pots and field conditions (Tables 3 and 4).

As shown in Tables 3 and 4, there were significant differences in wheat attributes after treatments compared with the control. The pH and availability of N, P and K in the soil of pots and fields are measured.

## DISCUSSION

In the present study, the PGPF increased wheat attributes due to their application methods. Application method activity of PGPF individually and in combination increased Wheat's attributes up to 50% compared to the control. So, commonly PGPF has been used to improve plant growth by enhancing phosphorus absorption in plants such *Penicillium*, *Aspergillus*, *Chaetomium* and *Trichoderma*

species. However, applications are used based on their abilities to supply and mobilize plant nutrients, suppress plant diseases and promote and develop plant growth (Pal *et al.*, 2015). The positive effect of PGPF on plant growth can be represented by increasing the rates of seed germination, root growth, yield, leaf area; content of chlorophyll, magnesium, nitrogen and protein beside hydraulic activity, tolerance to drought and salt stress (Lucy *et al.*, 2004).

An increase in the shelf life of the product is one of the main requirements of carrier materials in addition to cell number and is also dependent on rhizobial species and environmental conditions. Peat moss is mostly used as a carrier material in many plant inoculation productions (Albareda *et al.*, 2008). The carrier materials are chosen depending on many characteristics like absorption, hardness, bulk density and product disintegration rate in water. The bio-fertilizers formulations are prepared using various carriers; however, peat moss remains the most widely used carrier material.

Malviya *et al.* (2011) found that if PGPF is added to the soil, then it increases the plants' uptake of P from a

water-soluble P. Also, the acidity of the rhizosphere is a main mechanism of P-solubilization, as well as Mn, Fe and Zn by plants; besides the production of phytohormones, vitamins and amino acids can also be involved.

The PGPF applications efficacy can be improved in the field by understanding the features of soil-plant microorganism system interrelationships in different conditions, besides many other factors such as determining their rhizo-competence. The fungal strain genotype and physiological state and composition of sustained populations in the rhizosphere should be determined by many environmental factors such as water content, soil pH, mineral nutrients, species, plant genotype and physiological state in addition to the presence of other microbial species (Al-Taie *et al.*, 2016).

The general benefits of using biofertilizers, particularly for small farmers, are it serves as an affordable and safe source of agricultural inputs. Furthermore it is eco-friendly and essential for sustainable agriculture. Using fungi as a bio-fertilizer will decrease plant disease occurrence by inhibiting pathogens' growth and increasing the nutrient uptake from the soil, in addition to producing bioactive compounds such as hormones and enzymes, which stimulate plant growth. Zhang *et al.* (2019) reported significant differences in pH, organic matter and soil available nutrients N, P and K among treatments compared with the control.

The rhizosphere plant-microbes interactions are beneficial for soil fertility and plant health. The filamentous fungi are highly important in phosphate solubilization. *A. niger* and some *Penicillium* species, in addition to *Trichoderma* spp. have been tested for solubilization of phosphate and biotechnological importance such as bio-control, bio-degradation and phosphate mobilization (Elias *et al.*, 2016). In this context, Kumar *et al.* (2014) reported that coating seeds with dry powder/dust of *Trichoderma* before sowing is an easy and effective delivering method for seed/soil-borne diseases management and to improve plant growth.

Using appropriate methods of PGPF application is still a challenge. Research in this field must be supported by advanced technology to enhance biofertilizer usage and profitability for farmers (Keswani *et al.*, 2014).

## CONCLUSION

The results have shown that if PGPF is applied with peat moss at a rate of 150 mL/pot and field alone and in combination with seed treatment and soil application, were the best methods to promote wheat attributes and boost the soil physicochemical properties. The efficiency of selected PGPF isolates is promising to enhance the wheat plants' growth, development and yield.

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