



The Integrative Effect of Growth Promoting Rhizobacteria (PGPR), Organic and Mineral Fertilization on Growth and Productivity of Wheat (*Triticum aestivum* L.) in Sandy Soils

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ABSTRACT

Intensive efforts are focused on minimizing the amount of applied chemical fertilizers along with reducing the environmental hazards of pollutants. In this regard, some rhizobacteria (*Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus megaterium* and *Bacillus circulans*) were examined *in vitro* for their ability to exhibit some plant growth promotion (PGP)-related properties. Additionally, two field experiments were carried out on a sandy soil at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt, during two winter seasons of 2019/20 and 2020/21. The study concerned with the effect of integrated use of promoting rhizobacteria, organic and mineral fertilization on growth, chemical composition, yield and some yield components of wheat (*Triticum aestivum* cv. Misr 3) using sprinkler irrigation system. The experiments were laid out in randomized complete block design with four replicates. According to the laboratory experiment, all of the tested rhizobacteria were apparently able to exhibit PGP-properties. Phosphate solubilization was the common feature of all tested rhizobacteria; however, *Ps. fluorescens* and *B. megaterium* appeared to be superior to the other rhizobacteria and *Azo. brasilense* displayed the lowest capacity. The ability of all tested rhizobacteria to produce indole compounds, but *Ps. fluorescens* was more effective followed by *B. megaterium* and *B. circulans*, while *Azo. brasilense* produced lower amount of IAA. Moderate capacity (*Ps. fluorescens*) and low capacity (*B. megaterium* and *B. circulans*) to excrete ferric-specific ligands (siderophores) and biocide compound (cyanide) was detected, while *Azospirillum brasilense* failed to produce such compounds. Results of field trials revealed that the full recommended dose treatment was excelled the other tested levels of application (50% or 75% of the recommended NPK dose) in all tested wheat characters. While, wheat plants amended with compost or inoculated with mixture of PGPRs in combination with 50% or 75% NPK mineral fertilizers exerted a salient superiority in all tested wheat characters in comparison to using the same mineral fertilizers level alone. Among all fertilization treatments, the superiority of treatments comprising rhizobacterial inoculation and amended with compost in the presence of half dose or 75% of mineral fertilization, which caused significant augmentation and achieved the highest values of all wheat growth parameters (plant height, number of tillers/plant and dry weight of roots and shoots), nutrients accumulated in tissues, wheat yield and its components (number of spikes/m², number of kernels/spike, 1000-kernel weight, harvest index, biological yield, grain yield, straw yield and crude protein percentage of grain and straw). Such fertilization treatments gave values comparable to full dose of mineral NPK (100% NPK) treatment and the difference between above three fertilization treatments could not reach the level of significance. Hence, application of compost manure (10 ton fed⁻¹) in conjugation with a half dose of mineral NPK fertilizers may be acting as a good practice for sustaining wheat growth and productivity in sandy soil, particularly when this practice supported by inoculation with a mixture of effective rhizobacteria, which reduce the reliance on agrochemicals for safe and healthy food, long term sustainability and minimize environmental pollution.

Keywords: Biofertilizers, N₂-fixing bacteria, Phosphate dissolving bacteria, Silicate bacteria, Compost manure, Inorganic fertilizers, Wheat yield and its components.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major cereal crops all over the world and one of the most important crops in Egypt, which plays an important role in food and nutritional security. Increasing wheat production is an essential national target to minimize the gap between the Egyptian production and consumption by increasing the cultivated area and wheat productivity per unit area.

Nitrogen, phosphorus and potassium are known to be essential nutrients for plant growth and development. Use of nitrogen fertilizer is of great importance in agriculture, as nitrogen is the major factor limiting growth under most conditions. Phosphorus is one of the major plant growth-limiting nutrients although it is abundant in soils in both inorganic and organic forms. Phosphorus is a constituent of nucleic acids (DNA and RNA); stimulate cell division and metabolic processes (Hawkesford *et al.*, 2012 and Sharma *et al.*, 2012). Potassium (K) is a mobile element in the plant tissues and plays an important role in photosynthesis through carbohydrate metabolism, osmotic regulation and its important role in the activation of various enzymatic systems, stimulating synthesis of protein and many other compounds such as sugar, cellulose, cell wall and cell extension (Bahmanyar and Ranjbar, 2008 and Taha *et al.*, 2016). Though the use of chemical inputs in agriculture is inevitable to meet the growing demand for food in the world, which are not only so expensive but also do as agroecosystem's pollutants. So, attention is focused on friendly sustainable agricultural practices in cereals production to produce higher yield with minimal input cost and minor environmental pollution (Vessey, 2003; Muhammed *et al.*, 2013 and Meenakshi Suhag, 2016).

There is a currently considerable interest in the role of plant growth promoting rhizobacteria (PGPR) that colonize the rhizosphere and have a definite beneficial role in plant growth promotion and biological control of soil borne pathogens (Compant *et al.*, 2010 and Verma *et al.*, 2010). It is able to exert beneficial effects on plant growth and yield of many agronomic crops under a variety of environmental and soil conditions. It enhances plant growth by a variety of mechanisms like fixation of atmospheric N, production of siderophores, solubilization of minerals, enhanced stress resistance, synthesis of phytohormones and/or suppress plant pathogens (Pérez-Montañaño *et al.*, 2014; Majeed *et al.*, 2015 and Singh, 2018).

Biofertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser input of capital and energy. *Bacillus megaterium*, which also referred as "phosphate dissolving bacteria (PDB)" was used as biofertilizer for increasing the availability of accumulated phosphates by solubilization and releasing phosphorus in a more environmentally friendly and sustainable manner and increase the growth of plants by mechanisms other than P solubilization, e.g. production of phytohormones (Vessey, 2003 and Khan *et al.*, 2014). The contribution of biological agents in dissolving K-mineral in soils has attracted increased interest as certain type of microorganisms, generally referred as "silicate bacteria" were found to decompose aluminosilicate minerals, thus releasing K in an available form. One of these microorganisms is *Bacillus circulans*, which used as biofertilizer for improving K solubilization, in turn, absorption (Babalola and Glick, 2012 and Verma *et al.*, 2017). Bacteria of the genus *Azospirillum* are associative N₂-fixing rhizobacteria that are found in close association with plant roots. Microscopical evidence as to the endophytic nature of *Azospirillum* (Cassán *et al.*, 2020). However, there are several activities have been demonstrated, including production of auxins and minerals solubilization (García *et al.*, 2017 and Saleemi *et al.*, 2017). A large number of bacteria, including *Pseudomonas fluorescens* exhibit phosphate solubilization and mineralization ability, higher values of auxins production as well as production of iron-sequestering siderophores and antimicrobial compounds that hinder colonization of hosts by phytopathogens (Verma *et al.*, 2010 and Sahu *et al.*, 2018).

The application of the organic fertilizers as a soil management is of direct relevance, since it has drastic effects on some soil properties, which reflect positively on the existed grown crops, allowing a sustainable land use. It adds organic matter, which improves soil structure, aggregate formation, drought protection, stopping erosion, buffering, reduces fertilizer requirements and gave nutrients when the plants need them as well as inoculates the soil with vast numbers of beneficial microbes. Hence, it can modify soil physical properties and strongly affects its chemical and biological ones (Piqueres *et al.*, 2006 and Ahmed *et al.*, 2011). There are scientific evidences supporting the idea that the application rate of chemical fertilizers could be reduced (to achieve optimum yield levels) if they were applied along with organic fertilizers (Berecz *et al.*, 2005 and Kakraliya *et al.*, 2017).

The major objective of this study was to investigate the role of plant growth promoting rhizobacteria (PGPR) applied in combination with compost and mineral fertilizers to improve soil fertility and wheat productivity as well as hastening the nutrient-use efficiency and reduce the reliance on chemical fertilizers to minimize the environmental pollution under sandy soil conditions.

2. Materials and Methods

2.1. Bacterial strains and growth condition

Azospirillum brasilense (strain SP-245), *Pseudomonas fluorescens* (strain IFO.2034), *Bacillus megaterium* and *Bacillus circulans* (local isolates) used as plant growth promoting rhizobacteria (PGPR) were supplied by Microbiology Research Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. *Azospirillum* was grown in YEP medium (Vanstockem *et al.*, 1987) and *Bacillus circulans* was grown on nutrient broth medium (Difco Manual, 1984), while *Bacillus megaterium* and *Pseudomonas fluorescens* were grown in King's medium B (Atlas, 1995). Cultures were incubated at 28°C for three days on a rotary shaker until early log phase was developed to 10^9 viable cells ml^{-1} , and then the cultures were transferred to a sterile carrier material.

2.2. Assay of rhizobacteria for PGPR-properties *in vitro*

Rhizobacteria were tested for their ability to exhibit some PGPR-properties under *in vitro* conditions, through determination of their ability to solubilize phosphate on Di-calcium phosphate (DCP) media (Frioni, 1990); production of indole-acetic acid (IAA) (Gordon and Weber, 1951), production of siderophores (Alexander and Zuberer, 1991), as well as cyanogenesis (Bakker and Schippers, 1987). The efficiency of *B. circulans* for dissolving silicate minerals was assayed using powdered mica in the Aleksandrov's liquid medium (Zahara, 1969).

2.3. Preparation of inoculants

Neutral-powdered vermiculite supplemented with 10% Irish peat was packed in polyethylene bags (300g bag^{-1}). Bags were then sealed and sterilized by gamma irradiation (5×10^6 rads). Each bacterial culture was injected into the sterilized carrier to satisfy 60% of the maximal water holding capacity (10^7 cells/g carrier) and left for a week for curing.

2.4. Field experiments

A field experiment was conducted on a sandy soil at Ismailia Agricultural Research Station (ARC), Ismailia Governorate (Latitude 30° 35' 41. 901" N and Longitude 32° 16' 45. 843" E), Egypt, during two growing winter seasons of 2019/20 and 2020/21. The study concerned with the effect of integrated use of inoculation with promoting rhizobacteria, organic and inorganic fertilization on the growth, chemical composition, yield and some yield components of wheat (*Triticum aestivum* cv. Misr 3) using sprinkler irrigation system. The main initial physical and chemical properties of the experimental soil are shown in Table (1).

2.5. Experimental design

The experiments were laid out in randomized complete block design (RCBD) with four replicates and each plot area was 10.5 m^2 (1/400 fed).

The following fertilization treatments were conducted:

- 1- 50% from recommended inorganic- NPK dose.
- 2- 75% from recommended inorganic- NPK dose.
- 3- Full recommended inorganic- NPK dose.
- 4- Organic fertilization (compost) + 50% from recommended NPK dose.
- 5- Organic fertilization (compost) + 75% from recommended NPK dose.
- 6- Inoculation with mixture of PGPRs (*Azo. brasilense* + *Ps. fluorescens* + *B. megaterium* + *B. circulans*) +50% from recommended NPK dose.
- 7- Inoculation with mixture of PGPRs (*Azo. brasilense* + *Ps. fluorescens* + *B. megaterium* + *B. circulans*) +75% from recommended NPK dose.
- 8- Organic fertilization + PGPRs inoculation + 50% from recommended NPK dose.

9- Organic fertilization + PGPRs inoculation + 75% from recommended NPK dose.

Table 1: Initial physical and chemical properties of the experimental soil

Property	Season 2019/20	Season 2020/21
Particle size distribution (%):		
Sand	89.7	90.0
Silt	4.1	3.9
Clay	6.2	6.1
Texture grade	Sandy	Sandy
Saturation percent (S.P %)	20.10	20.30
pH (soil paste)	7.48	7.50
E.C (dS m ⁻¹ , at 25°C)	0.34	0.36
Soluble cations (meq/L):		
Ca ⁺⁺	0.58	0.66
Mg ⁺⁺	0.40	0.45
Na ⁺	1.54	1.69
K ⁺	0.79	0.77
Soluble anions (meq/L):		
CO ₃ ⁻	0.00	0.00
HCO ₃ ⁻	0.92	0.99
Cl ⁻	0.72	0.78
SO ₄ ⁼	1.67	1.80
Organic matter (%)	0.28	0.30
Total soluble- N (mg kg ⁻¹)	19.50	17.80
Available- P (mg kg ⁻¹)	5.24	5.50
Available-K (mg kg ⁻¹)	44.50	48.60
DTPA-extractable (mg kg⁻¹):		
Fe	1.80	1.66
Mn	0.38	0.35
Zn	0.51	0.47
Cu	0.22	0.20

DTPA: Di-ethylene tri-amine penta acetic acid

2.6. Fertilization

Organic fertilizer (mature compost) supplied by Soils, Water and Environ. Res. Inst. (SWERI), ARC, Giza, Egypt was applied to studied treatments during soil preparation (15 days before sowing) at the rate of 10 ton fed⁻¹. Physico-chemical and biological traits of the used compost and its seed germination test are shown in Table (2).

Phosphorus was added during soil preparation as calcium superphosphate (15.5 % P₂O₅) at the rates of 100, 150 and 200 kg/fed. The potassium was added as potassium sulphate (48% K₂O) at the rates of 50, 75 and 100kg/fed after 15 and 30 days from sowing in equal two doses. While, nitrogen fertilizer at the rates of 60, 90 and 120 kg N/fed in the form of ammonium sulphate (20.5% N) was applied in four equal doses at 10, 20, 30 and 40 days from sowing. The rates used for each mineral fertilizer mentioned above represent 50, 75 and 100% of the total recommended fertilizer dose per feddan.

2.7. Grains used and inoculation process

Grains of wheat (*Triticum aestivum* cv. Misr 3) supplied by the Wheat Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt, were sown on November 20, 2019 and November 25, 2020 in the two respective seasons. The inoculation process for wheat grains by mixture of tested PGPRs was done using coating technique at a rate of 600 g inoculum per 60 kg grains fed⁻¹, prior to sowing using gum Arabic solution (16%) as an adhesive agent.

2.8. Sampling

At 80 days from sowing, ten wheat plants were uprooted at random from each plot to assay the following vegetative parameters: plant height (cm), number of tillers/plant, dry weight of roots and shoots (g/plant) as well as their N, P and K total contents (mg/plant).

At harvest, plant samples with wooden frame (0.5 x 0.5m) were immediately collected before harvest to record data of plant height, number of spikes m⁻² (as an average of two samples for each plot area). After harvest, the following traits were also determined: number of kernels spike⁻¹, 1000-kernel weight (g) as an average of four 100-kernel random samples, biological yield (ton/fed), grain yield ardab/fed (one ardab = 150 kg) and straw yield (ton/fed) as well as harvest index (grain yield/ biological yield x100) and crude protein percentage in straw and grains.

Table 2: The main traits of used compost

Property	Value
Bulk density (kg/m ³)	560.0
Water holding capacity (%)	194.5
pH (1:10 extract)	6.75
E.C. (dS m ⁻¹ , at 25°C)	3.25
Organic-C (%)	24.52
Total-N (%)	1.38
C/N ratio	17.77
Total-P (%)	0.93
Total-K (%)	1.71
Total soluble-N (mg kg ⁻¹)	812.3
Available P (mg kg ⁻¹)	262.0
Available K (mg kg ⁻¹)	896.1
DTPA⁻-extractable (mg kg⁻¹):	
Fe	199.2
Mn	46.5
Zn	31.0
Cu	6.9
Total count of bacteria (cfu/g)	8 x 10 ⁷
Total count of fungi (cfu/g)	9 x 10 ⁶
Total count of actinomycetes (cfu/g)	2.1 x 10 ⁶
Dehydrogenase activity (mgTPF ^{**} /100g)	142.4
Germination test of cress seeds ^{***} (%)	91.0

*Di-ethylene tri-amine penta acetic acid. **Tri-Phenyl-Formazan.

***Cress seeds incubated for 48 hr.

2.9. Methods of analyses

- Soil physical and chemical properties were determined according to Piper (1950) and Page *et al.*, (1982).
- Physical properties of mature compost were determined according to Iglesias-Jimenez and Perez-Garcia (1989). Determination of chemical and microbiological traits was executed according to Page *et al.*, (1982). Seed germination test was assayed using cress seeds (*Lepidium sativum L.*, local variety) to evaluate compost maturity (Pare *et al.*, 1997).
- The oven dried plant materials were wet digested by using a mixture of pure HClO₄ and H₂SO₄ at a ratio of 1:1 according to Jackson (1973). Total nitrogen was determined using the micro-Kjeldahl method, phosphorus was determined Spectrophotometrically using ammonium molybdate and stannous chloride reagents and potassium was determined using Flamephotometer (Page *et al.*, 1982). Crude protein percentage in both grains and straw were determined by multiplying the nitrogen percentage by 5.7 factor according to A.O.A.C. (1960).
- Obtained results were subjected to analysis of variance (ANOVA) and L.S.D test was used to compare the treatment means according to the procedure of Snedecor and Cochran (1980).

3. Results and Discussion

3.1. The ability of the tested rhizobacteria to exhibit some PGP-properties *in vitro*

Data in Table (3) present some of PGP-related properties of the tested rhizobacteria. In general, all of the tested bacteria were apparently able to trigger PGP-properties under *in vitro* conditions.

Results showed that P-solubilization are the common features of all tested rhizobacteria grown on synthetic media as expressed by halo clarification zone formed around their colonies (zone diameters ranged from 1.60 to 3.10 cm). However, *Ps. fluorescens* and *B. megaterium* appeared to be superior to

the other rhizobacteria as it produced 3.10 and 2.95 cm of clear zone and *Azo. brasilense* displayed the lowest capacity (1.60 cm). The clear zone caused by the tested rhizobacteria depends on its efficiency to produce particular groups of organic acids and/or CO₂, which have high affinity to dissolve the precipitated phosphorus (Saleemi *et al.*, 2017). In this concern, Verma *et al.*, (2010), Rana *et al.*, (2011) and Kumar *et al.*, (2012) reported that the capability of the plant-growth-promoting rhizobacteria to solubilize *in vitro* some insoluble or sparingly soluble minerals via three possible mechanisms: acidification of the medium, production of chelating metabolites, and redox activity.

Table 3: Evaluation of the ability of the used rhizobacteria to produce some PGP-properties *in vitro*

Rhizobacteria	P-solubilization		IAA-production		Siderphores ^d production color Intensity	Cyanogens (HCN) Color Intensity
	Zone ^a clarification	Zone diameter (cm)	Color ^b intensity	µg/ml ^c		
<i>Azospirillum brasilense</i>	+	1.60	++	10.95	-	-
<i>Pseudomonas fluorescens</i>	+++	3.10	+++	19.77	++	++
<i>Bacillus megaterium</i>	+++	2.95	++	14.49	+	+
<i>Bacillus circulans</i>	++	2.40	++	13.85	+	+

a: Diameter of the clarification zone around colonies on DCP media plates

b,c: Intensity of the pink to red color and the quantity of IAA produced (µg/ml) in liquid culture

d: Intensity of the orange halo around colonies on the chrome azurol S agar plates

- Negative result + low ++ moderate +++ high

The results originated from both qualitative and quantitative assays of IAA reflected the ability of all tested rhizobacteria to produce indole compounds. The four tested bacteria exhibited a pink to red color with a little variation in intensity. In the quantitative measurements, the highest value of IAA production was obtained by *Ps. fluorescens*, followed by *B. megaterium* and *B. circulans* as they produced 19.77, 14.49 and 13.85 µg ml⁻¹, respectively, while *Azo. brasilense* produced nearly the lower amount of IAA being 10.95 µg ml⁻¹. In fact, many investigators consider the indole secretion, by PGPRs, as a vital mechanism to clarify the plant promotion by stimulate root growth and provides it with more branching and larger surface area (Verma *et al.*, 2010; Majeed *et al.*, 2015 and Saleemi *et al.*, 2017).

Another interested traits of PGPR's is the ability to secrete ferric-specific ligands, (siderophores) and the ability to produce biocide compounds, including cyanide that have a poisoned the phytopathogenes agents. Data in Table (3) declared that the tested rhizobacteria were able to excrete siderophores which were indicated by moderate (*Ps. fluorescens*) and low (*B. megaterium* and *B. circulans*) yellow-orange halo around their colonies to removing the color from CAS dye-Fe III-complex and cyanide expressed as a moderate to weak orange-red pigmentation. While, *Azospirillum brasilense* failed to produce such compounds. In fact, cyanide and siderophores has an important role in the biocontrol activity against soil borne phytopathogens, beside the essential function of siderophores in improvement of iron nutrition. These results are in line with those obtained by Verma *et al.*, (2010) and Rana *et al.*, (2011).

In view of the above results, it could be concluded that the tested rhizobacteria were able to exhibit PGP-properties, which may display several modes of beneficial action. This finding was emphasized by other investigators (Abaid-Ullah *et al.*, 2015; Majeed *et al.*, 2015; Sahu *et al.*, 2018 and Kadhum *et al.*, 2021).

3.2. Effect of rhizobacterial inoculation, organic and inorganic fertilization on some growth parameters and nutrients content of wheat plants in sandy soil

3.2.1. Growth parameters of wheat plants

Wheat plant characters, i.e., plant height (cm), number of tillers/plant and dry weight of roots and shoots after 80 days from sowing as affected by rhizobacterial inoculation, organic and inorganic fertilization are presented in Table (4). Data of the two studied seasons showed that all above tested characters were significantly affected by application of bio-, organic and mineral fertilization. As general, the data of plant growth characters indicated that all fertilization treatments exceeded the treatment received the half dose of mineral NPK fertilizers only.

The results in Table (4) revealed that the application of mineral fertilizers induced significant increase in all studied wheat growth parameters and the promotion effect increased as the rate of mineral fertilization increased. Wheat plants fertilized with 50% from recommended inorganic- NPK dose gave shorter wheat plant height (74.68 and 75.15 cm), low number of tillers (2.39 and 2.20/ plant) and lower root and shoot dry weights (0.93 and 0.97 g/plant) and (2.13 and 2.00 g/plant), respectively in both seasons. However, increasing application rate of mineral fertilization up to 100% recommended inorganic- NPK dose achieved higher values of all growth parameters in both tested seasons. The increase of vegetative growth with increasing inorganic fertilization might be due to the prominent role of N, P and K in increasing the meristematic activities, cell division and its important function as main constituent of many essential compounds in plant system. Many workers confirmed the promotion effect of increasing the application rate of mineral fertilization on wheat growth parameters (Muhammed *et al.*, 2013; Attia and Abd El Salam, 2016 and Phullan *et al.*, 2017).

Wheat plants amended with compost and/or inoculated with mixture of PGPRs in combination with inorganic- NPK fertilizers exerted a salient superiority in all tested wheat growth characters compared with un-amended and uninoculated treatments. Among fertilization treatments, both the recommended NPK treatment and treatments comprising rhizobacterial inoculation, compost and received half dose or 75% of mineral fertilization confirmed their synergistic effect to stimulate plant growth and achieved the highest values of all wheat growth characters with insignificant differences between them. The highest recorded values were (86.98, 86.10 and 86.78 cm), (6.12, 6.63 and 6.55/ plant), (1.62, 1.54 and 1.57 g/plant) and (4.30, 4.18 and 4.11 g/plant) for plant height, number of tillers/plant, dry weight of roots and dry weight of shoots, respectively. The highest values attained in the second season were (87.03, 86.15 and 86.80 cm), (5.96, 6.72 and 6.80/plant), (1.60, 1.59 and 1.58 g/plant) and (4.38, 4.25 and 4.21 g/plant), respectively, in the same above order.

Table 4: Effect of rhizobacterial inoculation, organic and inorganic fertilization on some wheat growth parameters after 80 days from sowing in sandy soil

Treatments	Season 2019/2020			
	Plant height (cm)	Number of tillers /plant	Root dry weight (g/plant)	Shoot dry weight (g/plant)
50% inorganic NPK	74.68 e	2.39 e	0.93 f	2.13 e
75% inorganic NPK	77.23 d	2.87 e	1.18 de	2.55 d
100% inorganic NPK	86.98 a	6.12 ab	1.62 a	4.30 a
Compost + 50% NPK	85.78 b	4.90 c	1.31 cd	3.50 b
Compost + 75% NPK	85.58 b	6.02 b	1.42 bc	3.63 b
Inoculation + 50% NPK	81.93 c	3.47 d	1.14 e	2.53 d
Inoculation + 75% NPK	83.25 c	4.53 c	1.30 cd	3.18 c
Compost + Inoculation + 50% NPK	86.10 ab	6.63 a	1.54 ab	4.18 a
Compost + Inoculation + 75% NPK	86.78 ab	6.55 ab	1.57 a	4.11 a
L.S.D. _{0.05}	1.378	0.541	0.131	0.192
Treatments	Season 2020/2021			
	Plant height (cm)	Number of tillers /plant	Root dry weight (g/plant)	Shoot dry weight (g/plant)
50% inorganic NPK	75.15 e	2.20 e	0.97 e	2.00 e
75% inorganic NPK	78.53 d	2.29 e	1.18 d	2.48 d
100% inorganic NPK	87.03 a	5.96 b	1.60 a	4.38 a
Compost + 50% NPK	86.48 a	4.96 c	1.34 bc	3.53 b
Compost + 75% NPK	85.58 ab	6.07 b	1.44 b	3.59 b
Inoculation + 50% NPK	82.48 c	3.52 d	1.16 d	2.50 d
Inoculation + 75% NPK	83.95 bc	4.70 c	1.26 cd	3.33 c
Compost + Inoculation + 50% NPK	86.15 a	6.72 a	1.59 a	4.25 a
Compost + Inoculation + 75% NPK	86.80 a	6.80 a	1.58 a	4.21 a
L.S.D. _{0.05}	1.686	0.395	0.107	0.177

* Values followed by the same letter do not differ significantly at 5% probability level.

50% NPK: 60 kg N fed⁻¹ (ammonium sulphate), 100 kg superphosphate fed⁻¹, 50 kg potassium sulphate fed⁻¹.

75% NPK: 90 kg N fed⁻¹, 150 kg superphosphate fed⁻¹, 75 kg potassium sulphate/ fed⁻¹.

100% NPK:120 kg N fed⁻¹, 200 kg superphosphate fed⁻¹, 100 kg potassium sulphate fed⁻¹.

Compost: 10 ton mature compost fed⁻¹

Inoculation: wheat grains inoculated with mixture of PGPRs (*Azo. Brasilense* + *Ps. Fluorescens* + *B. megaterium* + *B. circulans*).

The stimulatory effects of bio- and organic fertilization on developing all plant growth characters may be due to the prominent role of compost manure, which improves soil characteristics and inoculates the soil with numbers of beneficial microbes as well as reduces mineral fertilization requirements and enhance their use efficiency, which reflect positively on the grown crops. Further, the promotion effects of inoculation could be attributed to N₂-fixation, solubilization of minerals, enhanced stress resistance, suppress plant pathogens and/or produce some growth regulator substances, which it may play an important role in plant growth through promoting photosynthesis, translocation and accumulation of dry matter within different plant organs. Many investigators confirmed the synergy between PGPR and organic amendment increased the fertilization efficiency and creating a favorable habitat for plant growth (Rady *et al.*, 2016; Kakraliya *et al.*, 2017 and Phullan *et al.*, 2017). They concluded that the integration of inorganic fertilizers with organic manures and biofertilizers will not only help sustain the growth and crop productivity but also will be effective in improving soil health and hastening the nutrient-use efficiency.

3.2.2. Nutrients content of wheat plants

Table (5) shows the effect of rhizobacterial inoculation, organic and inorganic fertilization on roots and shoots nutrient contents of 80-day old wheat plants in sandy soil. Obtained data showed that both root and shoot N, P and K contents were significantly responded to all fertilization treatments and behaved in a similar manner as mentioned before for wheat plant growth characters. The values of accumulated N in root tissues ranged from 6.97 to 15.10 and 7.07 to 15.42 mg N/plant; root P-contents ranged from 6.88 to 12.71 and 6.67 to 12.84 mg P/plant, while root K-contents ranged from 8.76 to 18.32 and 8.71 to 18.23 mg K/plant in the first and second season, respectively. The corresponding values of shoot N-contents ranged between 24.91 to 59.88 and 24.52 to 58.84 mg N/plant; shoot P-contents ranged from 11.54 to 22.31 and 11.60 to 22.23 mg P/plant, while shoot K-contents ranged from 32.38 to 65.06 and 32.24 to 65.46 mg K/plant, respectively. In general, increasing application rate of NPK fertilizers from 50 to 100% exhibited a significant increase in nutrients accumulated in wheat tissues. Such increases in the nutrients content mainly attributed to the increases in both root and shoot dry weights as well as the increases in the percentage of N, P and K. These results stand in accordance with those obtained by Zeidan *et al.*, (2009) and Phullan *et al.*, (2017) who found that increasing the application rate of mineral fertilizers had a positive effect on root growth and the absorption sites which enhance absorption of nutrients.

It may be argued that, wheat plants inoculated with PGPRs and/or amended with compost in combination with any level of mineral fertilizers gave values higher than using the same mineral fertilizers level alone. However, the promotion of N, P and K accumulated in wheat plant tissues was observed with the recommended treatment (full dose of inorganic NPK) and treatments comprising rhizobacterial inoculation and organic fertilization combined with 50% or 75% NPK, which gave values comparable to 100% NPK treatment. These treatments confirmed their synergistic effect to stimulate nutrients accumulation and significantly surpassed other tested fertilization treatments. Such treatments recorded the highest values of root N, P and K-contents (14.70, 15.06 and 15.10 mg N/plant), (12.50, 12.71 and 12.65 mg P/plant) and (17.74, 18.32 and 17.50 mg K/plant) in the first season, respectively. The highest values in the second season were (14.48, 15.42 and 15.06 mg N/plant), (12.84, 12.82 and 12.68 mg P/plant) and (18.00, 18.23 and 17.85 mg K/plant), respectively. The highest values of shoot N, P and K-contents were (56.61, 58.02 and 59.88 mg N/plant), (21.54, 22.15 and 22.31 mg P/plant) and (63.50, 64.18 and 65.06 mg K/plant) in the first season. The highest values attained in the second season were (56.65, 58.28 and 58.84 mg N/plant), (21.54, 21.91 and 22.23 mg P/plant) and (63.14, 64.43 and 65.46 mg K/plant) in the same order, respectively.

It is clear that wheat plants cultivated in sandy soil appeared high response to applied organic materials, rhizobacteria inoculation and mineral fertilization, which reflected by the higher values of N, P and K contents. This improvement could be ascribed to the ability of these rhizobacteria to fix nitrogen in association or endophytic manners, solubilization of minerals and/or certain growth promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. However, the presence of organic materials resulting in improving the efficiency of mineral nutrition through hastens the availability and uptake of the nutrients by wheat plants. Many researchers confirmed the beneficial effect of bio and organic fertilization due to its direct nutrients supplying and/or its microbial functions, which enhance nutrient use efficiency led to

pronounce the nutrients accumulated in plant tissue (Singh *et al.*, 2015; Attia and Abd El Salam, 2016; Majeed *et al.*, 2015 and Mor *et al.*, 2019).

Table 5: Effect of rhizobacterial inoculation, organic and inorganic fertilization on the total uptake of N, P and K in both shoots and roots of wheat plants after 80 days from sowing in sandy soil

Treatments	Season 2019/2020					
	Root uptake (mg/plant)			Shoot uptake (mg/plant)		
	N	P	K	N	P	K
50% inorganic NPK	6.97 f	6.88 g	8.76 f	24.91 f	11.54 g	32.38 e
75% inorganic NPK	9.51 e	8.81 e	11.43 e	31.91 e	13.50 f	41.67 d
100% inorganic NPK	14.70 a	12.50 a	17.74 ab	56.61 b	21.54 b	63.50 a
Compost + 50% NPK	11.72 c	10.26 c	13.82 d	42.00 d	15.55 d	46.10 c
Compost + 75% NPK	13.48 b	11.38 b	15.79 c	45.99 c	17.48 c	51.25 b
Inoculation + 50% NPK	9.04 e	8.47 f	11.18 e	32.28 e	14.54 e	40.53 d
Inoculation + 75% NPK	10.38 d	9.84 d	13.28 d	40.36 d	15.53 d	45.15 c
Compost + Inoc. + 50% NPK	15.06 a	12.71 a	18.32 a	58.02 ab	22.15 ab	64.18 a
Compost + Inoc. + 75% NPK	15.10 a	12.65 a	17.50 b	59.88 a	22.31 a	65.06 a
L.S.D. 0.05	0.869	0.292	0.586	2.479	0.639	2.478
Treatments	Season 2020/2021					
	N	P	K	N	P	K
	50% inorganic NPK	7.07 g	6.67 e	8.71 e	24.52 e	11.60 e
75% inorganic NPK	9.55 g	8.64 d	11.34 d	31.17 d	13.55 d	41.60 e
100% inorganic NPK	14.48 b	12.84 a	18.00 a	56.65 a	21.54 a	63.14 b
Compost + 50% NPK	11.32 d	10.38 c	13.72 c	41.35 c	15.69 c	46.38 d
Compost + 75% NPK	13.47 c	11.28 b	15.95 b	45.19 b	17.53 b	50.96 c
Inoculation + 50% NPK	9.22 f	8.27 d	11.32 d	32.97 d	14.87 c	40.83 e
Inoculation + 75% NPK	10.33 e	9.80 c	13.03 c	39.84 c	15.46 c	45.60 d
Compost + Inoc. + 50% NPK	15.42 a	12.82 a	18.23 a	58.28 a	21.91 a	64.43 ab
Compost + Inoc. + 75% NPK	15.06 ab	12.68 a	17.85 a	58.84 a	22.23 a	65.46 a
L.S.D. 0.05	0.912	0.601	0.796	2.461	0.876	2.058

* Values followed by the same letter do not differ significantly at 5% probability level.

III- Effect of rhizobacterial inoculation, organic and inorganic fertilization on wheat yield and some yield components in sandy soil

Plant height, number of spikes/m², number of kernels/spike, 1000-kernel weight and harvest index as well as biological yield, grain yield, straw yield and crude protein percentage of grain and straw along the two consecutive seasons, as affected by rhizobacterial inoculation, organic and inorganic fertilization are given in Tables (6 and 7). Results elicited that the response of wheat yield and its attributes in both seasons followed a similar pattern to that of vegetative growth stage.

1) Some wheat yield components

Data presented in Table (6) revealed that plant height, number of spikes/m², number of kernels/spike; 1000-kernel weight and harvest index were affected by adding mineral fertilizer. In general, application of mineral fertilizers at the rate of 100% of the recommended NPK dose resulted in significant increment and gave the highest values of all wheat yield components, compared to the other tested levels of application (50% or 75% of the recommended dose) in both seasons. These results are in conformity with those of Zaki *et al.*, (2012) & Attia and Abd El Salam (2016).

As mentioned before, the treatments comprising rhizobacterial inoculation or organic fertilization combined with 50% or 75% NPK fertilizers gave values higher than using the same mineral fertilizers level alone. Here too, the results confirmed again the superiority of both recommended NPK treatment and treatments amended with compost and inoculated with PGPRs in the presence of half dose or 75% of mineral fertilization, which caused significant augmentation and achieved the highest values of all wheat yield components. The difference between above three fertilization treatments could not reach the level of significance. Such fertilization treatments recorded the highest values of all yield components (97.08, 98.63 and 98.25 cm), (343.3, 352.3 and 357.0/ m²), (49.50, 51.00 and 51.25/spike), (50.05, 51.75 and 52.11 g) and (37.74, 38.36 and 39.75%) for plant height, number of spikes/m², number of kernels/spike, 1000-kernel weight and harvest index, respectively. The highest values attained in the second season were (97.63, 98.55 and 98.28 cm), (345.0, 354.5 and 355.5/m²), (50.00, 52.25 and

51.75/spike), (50.18, 51.14 and 50.99 g) and (39.18, 39.11 and 39.02 %), respectively, in the same above order.

The increment in all wheat yield components could be due to the increase in dry weight of vegetative organs which may be considered as a criterion for photosynthetic efficiency of the plant. The previous finding were in harmony with those reported by several investigators who studied the interaction effect of mineral, organic and bio-fertilizer on wheat growth, development and productivity (Akhtar *et al.*, 2009; Zaki *et al.*, 2012 and Kakraliya *et al.*, 2017). They concluded that compost sustain soil fertility through their effect on physical, chemical and biological properties as well as preventing the loss of chemical fertilizers through desertification, volatilization and leaching by binding to nutrients and releasing with the passage of time. On the other hand, rhizobacterial inoculation might create a more favorable environment, in terms of nature and concentration of root exudates, for cell growth and metabolic activities of rhizospheric microorganisms or due to boosting growth and nitrogen fixation performance, P mobilization, phytohormone production and saving the bio-protection against phytopathogens. Many investigators confirmed the positive effect of biofertilizers on enhancing crop productivity (Muhammed *et al.*, 2013; Ahemad and Kibret 2014 and Kadhum *et al.*, 2021).

Table 6: Effect of rhizobacterial inoculation, organic and inorganic fertilization on some wheat parameters in sandy soil

Treatments	Season 2019/2020				
	Plant height (cm)	Number of spikes /m ²	Number of kernels /spike	1000-kernel weight (g)	Harvest index (%)
50% inorganic NPK	79.75 g	217.5 f	32.50 e	38.27 e	36.47
75% inorganic NPK	82.63 f	258.8 e	38.00 cde	40.43 de	36.35
100% inorganic NPK	97.08 b	343.3 b	49.50 ab	50.05 a	37.74
Compost + 50% NPK	93.95 cd	284.3 d	40.50 cd	43.11 c	37.39
Compost + 75% NPK	94.55 c	327.0 c	43.75 bc	46.27 b	37.58
Inoculation + 50% NPK	87.63 e	254.3 e	37.00 de	41.65 cd	38.38
Inoculation + 75% NPK	92.65 d	279.8 d	41.00 cd	42.43 cd	37.77
Compost + Inoc. + 50% NPK	98.63 a	352.3 ab	51.00 a	51.75 a	38.63
Compost + Inoc. + 75% NPK	98.25 ab	357.0 a	51.25 a	52.11 a	39.75
L.S.D. 0.05	1.469	13.570	6.069	2.394	N.S
Treatments	Season 2020/2021				
	Plant height (cm)	Number of spikes /m ²	Number of kernels /spike	1000-kernel weight (g)	Harvest index (%)
50% inorganic NPK	78.65 f	221.3 e	32.25 e	37.36 d	36.68 cd
75% inorganic NPK	81.75 e	264.5 d	36.75 d	40.72 cd	36.09 d
100% inorganic NPK	97.63 a	345.0 a	50.00 a	50.18 ab	39.18 a
Compost + 50% NPK	92.78 bc	286.5 c	42.25 b	43.71 c	38.58 ab
Compost + 75% NPK	94.65 b	325.3 b	44.25 b	47.19 b	36.80 cd
Inoculation + 50% NPK	87.40 d	258.0 d	38.75 cd	41.70 c	38.10 abc
Inoculation + 75% NPK	92.43 c	284.0 c	41.75 bc	42.86 c	37.30 bcd
Compost + Inoc. + 50% NPK	98.55 a	354.5 a	52.25 a	51.14 a	39.11 a
Compost + Inoc. + 75% NPK	98.28 a	355.5 a	51.75 a	50.99 a	39.02 a
L.S.D. 0.05	1.873	12.370	3.034	3.379	1.454

* Values followed by the same letter do not differ significantly at 5% probability level.

50% NPK: 60 kg N fed⁻¹ (ammonium sulphate), 100 kg superphosphate fed⁻¹, 50 kg potassium sulphate fed⁻¹.

75% NPK: 90 kg N fed⁻¹, 150 kg superphosphate fed⁻¹, 75 kg potassium sulphate/ fed⁻¹.

100% NPK: 120 kg N fed⁻¹, 200 kg superphosphate fed⁻¹, 100 kg potassium sulphate fed⁻¹.

Compost: 10 ton mature compost fed⁻¹

Inoculation: wheat grains inoculated with mixture of PGPRs (*Azo. Brasilense* + *Ps. Fluorescens* + *B. megaterium* + *B. circulans*).

2) Wheat yield and its crude protein percentage

Data regarding wheat yield (estimated by biological yield, grain yield and straw yield) and their crude protein percentage (estimated by grain and straw crude protein) showed a significant increase with increasing the application rate of mineral NPK fertilizers (Table 7). Hence, the full recommended dose treatment was excelled the other tested levels of application and recorded the highest values of all wheat yield parameters. These increases may due to the efficient use of NPK fertilizers, which plays a major role in crop production and play important role in chlorophyll, protein, nucleic acids, hormones

and vitamin synthesis as well as help in cell division and cell elongation. These results are in line with Malghani *et al.*, (2010) and Muhammed *et al.*, (2013).

Obtained results evidence that the treatments comprising rhizobacterial inoculation or organic fertilization combined with 50% or 75% NPK fertilizers exerted a valuable improvement in all wheat yield parameters in comparison to using the same mineral fertilizers level alone. For instance, the treatments fertilized with 50 or 75% inorganic NPK fertilizers combined with compost recorded in the first season significant increases in biological yield (42.80 and 19.19%), grain yield (46.49 and 23.02%) and crude protein of grains (19.06 and 13.37%) over than using the same level of mineral fertilizers only. The attained increases in the second season in all aforesaid parameters were (37.69 and 18.44%), (45.04 and 21.86%) and (20.21 and 13.02%) in the same order, respectively. These findings reflected the distinct role of organic materials in improvement of wheat productivity in sandy soil, which might be elucidated by improving soil fertility and plant nutritional status leading to support plant growth and its natural health. The prominent role of organic materials in enhancing crop productivity was proved by many investigators (Pandey *et al.*, 2009; Devi *et al.*, 2011 and Mor *et al.*, 2019). They confirmed that the combination of mineral fertilizers with organic manures increased mineral fertilizers use efficiency and helped in increasing the productivity of wheat compared to a system with only mineral fertilization. Kakraliya *et al.*, (2017) added that organic materials hold great promise due to their local availability as a source of multiple nutrients and ability to improve soil fertility and quality, especially under low input agricultural systems.

On the other hand, treatments fertilized with 50 or 75% mineral NPK fertilizers and inoculated with PGPRs exerted increases in biological yield (10.23 and 2.62%), grain yield (15.91 and 6.35%) and crude protein of grains (17.24 and 10.24%) over than using the same level of mineral fertilizers only, respectively. The increases recorded in the second season were (10.45 and 2.31 %), (14.66 and 6.52%), (16.23 and 9.64%) in the same order, respectively. The promotive action of inoculation in wheat yield is not necessarily due to the nitrogen fixation by the added inoculum only but might be due to solubilization of minerals, secretion of plant growth hormones and some other factors (Table, 3), which might be support such increase. Many other studies have confirmed the positive effects of rhizobacterial inoculation on the growth and yield of wheat (Ahmed *et al.*, 2011; Abd El-Lattief, 2013 and Muhammed *et al.*, 2013). They explained the positive effect of microbial inoculation, which producing growth promoting substances such as auxins, gibberellins and cytokinin-like substances. Taha *et al.*, (2016) and Kadhum *et al.*, (2021) reported that the combined application of bio- and mineral fertilizers were favorable in enhancing wheat yield than using mineral or biofertilizer alone.

However, the results in Table (7) ratifies that no significant variations within the estimated yield parameters between the treatments received the recommended dose of NPK and that received a half dose or 75% of the recommended NPK dose with combined application of microbial additives and compost. The splendid effect of above fertilization treatments achieved the highest values of all wheat yield attributes in both seasons. In the first season, the percentage increases recorded in biological yield was (71.59, 74.62 and 70.45%), grain yield (77.07, 85.02 and 85.80%), straw yield (68.45, 68.45 and 61.31%), while the increases in crude protein of grains (34.37, 38.01 and 39.83%) and crude protein of straw (26.37, 31.51 and 32.15%) over than 50% NPK treatment, respectively. During the second season, biological yield recorded increases (71.27, 70.52 and 72.76%), grain yield (83.05, 81.83 and 83.82%), straw yield (64.12, 63.53 and 65.88%), crude protein of grains (32.67, 36.02 and 35.50%) and crude protein of straw (28.48, 33.01 and 30.74%), respectively.

In fact, PGPRs and compost have been shown to greatly improve the productivity and quality of wheat, when they integrated with 50% or 75% NPK fertilizers. The increase in grain yield could be attributed to a significant increase in the number of spikes/m², number of kernels/spike and 1000-grain weight in both seasons (Zeidan *et al.*, 2009; Attia and Abd El Salam, 2016 and Kadhum *et al.*, 2021). The increases in grain and straw crude protein are attributed to increasing the available nitrogen in the root zone and at the same time the increase of the N absorption rate by plant (Akhtar *et al.*, 2009 and Zaki *et al.*, 2012). Hence, the increments in all wheat yield attributes as a result of the integrated promoting rhizobacteria with mineral and organic fertilization could be ascribed to adequate quantities and balanced proportions of nutrients supplied to the plant led to higher dry matter accumulation and its translocation in plant parts, which in turn resulted in higher yield. Our findings are in conformity with many researchers, who confirmed the beneficial effect of bio-organic materials in improving the

productivity and quality of wheat due to its direct nutrients supplying and/or its microbial functions (Devi *et al.*, 2011; Singh *et al.*, 2015; Rady *et al.*, 2016 and Kakraliya *et al.*, 2017).

Table 7: Effect of rhizobacterial inoculation, organic and inorganic fertilization on biological yield, grain yield, straw yield and crude protein percentage in grains and straw of wheat plants in sandy soil

Treatments	Season 2019/2020				
	Biological yield (ton/fed)	Grain yield (ardab/fed)	Straw yield (ton/fed)	Crude protein of grains (%)	Crude protein of straw (%)
50% inorganic NPK	2.64 f	6.41 f	1.68 e	9.34 g	3.11 f
75% inorganic NPK	3.44 d	8.34 d	2.19 d	10.25 f	3.43 e
100% inorganic NPK	4.53 a	11.35 a	2.83 a	12.55 b	3.93 b
Compost + 50% NPK	3.77 c	9.39 c	2.36 cd	11.12 de	3.49 e
Compost + 75% NPK	4.10 b	10.26 b	2.56 bc	11.62 c	3.74 c
Inoculation + 50% NPK	2.91 e	7.43 e	1.79 e	10.95 e	3.57 de
Inoculation + 75% NPK	3.53 d	8.87 cd	2.20 d	11.30 d	3.66 cd
Compost + Inoc. + 50% NPK	4.61 a	11.86 a	2.83 a	12.89 a	4.09 a
Compost + Inoc. + 75% NPK	4.50 a	11.91 a	2.71 ab	13.06 a	4.11 a
L.S.D. _{0.05}	0.213	0.661	0.232	0.311	0.151
Treatments	Season 2020/2021				
	Biological yield (ton/fed)	Grain yield (ardab/fed)	Straw yield (ton/fed)	Crude protein of grains (%)	Crude protein of straw (%)
50% inorganic NPK	2.68 e	6.55 f	1.70 e	9.55 g	3.09 e
75% inorganic NPK	3.47 cd	8.28 d	2.22 c	10.37 f	3.44 d
100% inorganic NPK	4.59 a	11.99 a	2.79 a	12.67 b	3.97 a
Compost + 50% NPK	3.69 bc	9.50 c	2.27 c	11.48 cd	3.46 cd
Compost + 75% NPK	4.11 ab	10.09 b	2.60 b	11.72 c	3.69 b
Inoculation + 50% NPK	2.96 de	7.51 e	1.83 d	11.10 e	3.62 bc
Inoculation + 75% NPK	3.55 c	8.82 d	2.23 c	11.37 d	3.68 b
Compost + Inoc. + 50% NPK	4.57 a	11.91 a	2.78 a	12.99 a	4.11 a
Compost + Inoc. + 75% NPK	4.63 a	12.04 a	2.82 a	12.94 a	4.04 a
L.S.D. _{0.05}	0.536	0.579	0.119	0.254	0.160

* Values followed by the same letter do not differ significantly at 5% probability level.

- One ardab = 150 kg

4. Conclusion

It is worth to note that wheat plants exhibited significant response to rhizobacteria inoculation, organic and mineral fertilization with respect to plant growth, wheat yield and yield attributes. There is considerable evidence that the combined application of compost and promoting rhizobacteria could be used to increase the NPK fertilizer use efficiency in sandy soil. There are no significant variations were observed within the treatments received the recommended dose of NPK and that received a half dose or 75% of the recommended NPK and amended with compost, particularly when this practice supported by inoculation with a mixture of effective rhizobacteria. Hence, rhizobacterial inoculation (as grain coating) in conjugation with compost manure (10 ton fed⁻¹) and half dose of mineral NPK fertilizers may be acting as a good practice not only for sustaining wheat yield in sandy soil but also hastening the nutrient-use efficiency and reduce the reliance on chemical fertilizers in agriculture, to improve soil fertility and minimize the environmental pollution. Further studies are necessary for more data by using different wheat varieties under different soil conditions to confirm the results for successful large-scale use and to reach the level of recommendation.

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