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Alleviating the effects of salt stress on wheat plants using organic amendments and phosphate- solubilizing bacteria

Ahmed Fathy Ahmed, Salah M. Dahdouh, Mohamed Abu-hashim and Abdel-Rahman M. A. Merwad*

Soils Science Dept., Fac. Agric., Zagazig Univ., Egypt

Abstract

A pot experiment was carried out under controlled conditions in the green house to study the effect of different organic amendments (OM) *ie.*, Moringa seed residues(MSR), biogas manure (BM) and vermicompost (Ver) with and without phosphate-solubilizing bacteria (PSB) on growth, yield and nutrients uptake of wheat (*Triticum aestivum* L, cv. Sakha 93) plants under saline calcareous soil. Application of different OM under PSB inoculation gave increases in chlorophyll a,b, carotenoids, proline content straw , grains yield and NPK –uptake of wheat plants compared to untreated plants. The highest values of Cho a, Cho b; proline; plant height, straw and grains yield, protein content, antioxidant enzymes i.e., CAT, POX, and SOD, straw and grains NPK-uptake of wheat were obtained under application of Ver in the presences of PSB, while the lowest ones were obtained with untreated soils in absence of organic amendments without PSB. The treatments of Ver with PSB gave the highest values of available P (31.2 mg kg^{-1}), while the lowest ones (6.1 mg kg^{-1}) were found with untreated soil

Keywords: Organic amendments, phosphate- solubilizing bacteria, wheat plants, Saline Calcareous Soil.

Full length article **Corresponding Author*, e-mail: amerwad@yahoo.com

1. Introduction

Desertic soils in Egypt are mostly calcareous or saline soils. These soils, generally, attended some problems, related to their physical properties, salinity and low potentiality for productivity. There are several means for solving their problems, one of them is the use of soil conditioners [1]. Soil amendments means improve the physical conditions of such soils by using small amounts of natural or artificial products to promote germination, increasing the water holding capacity and reducing evaporation from the surface of soil under arid conditions. However, minor attention was paid to the effect of conditioners on the statues of nutritive elements as well as the chemistry of their intergradient, during the improvement of the desert soils [2, 3 and 4]. Organic materials can be considered as humus supplying and soil improving agents. The maintenance of a balanced humus regime is of particular importance to soils of the arid and semi-arid regions. The improvement of soil physical and chemical properties as well as nutrient status depends to a great extent on the rational use of organic materials as amendments [5]. Vermicompost (Ver) improves soil health, enhances crop production, and improves soil physical properties such as texture, structure and tilth that determine the agronomical potential of a land. Soil physical properties largely influence penetration of plant roots, potential rooting volume, nutrient mobility and uptake, water availability, and aeration. Soil texture is also a key factor in affecting soil moisture content and its chemical properties, such as CEC. Therefore, application of Ver to sandy soils is very beneficial, as it helps increase the soil organic matter (OM) composition, which in turn aids in improving soil aeration, sustaining good soil aggregation, protecting against soil erosion, and increasing nutrient availability [6]. Moreover, vermicompost also contains different plant nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B that enhance the nutrient uptake of plants. There have been many studies on the application of different organic fertilizers to plants for alleviating the negative effects of salinity. Ver has been shown to promote plant growth and salinity tolerance. [7] obtained that salinity stress induced a significant decrease in plant height, photosynthetic pigments, content of Fe, K, Ca, Mg and protein of plant. In contrast Ver treatment led to improvement of physiological characteristics, crop quality, productivity, absorption of nutrients, and alleviation of the effect of salt stress.

Soil salinity significantly hampers plant growth, with a major effect on productivity. Nutrient imbalances under salinity hinder plant development and yield. Crucially, salinity disrupts P, a vital nutrient, affecting element distribution and uptake. P scarcity in salt-affected soils necessitates additional fertilizers, raising environmental concerns. Extra fertilization is recommended to address phosphorus deficiency and achieve balanced nutrition alongside N. In tropical soils, phosphate availability is limited, making mineral solubilization key for healthy seedlings. Microbial allies, dwelling near plants, improve mineral access, aiding nutrient accumulation. These beneficial microorganisms hold promise in promoting growth and easing stress in crops [8]. The present work aims to study the the effect of different organic amendments (Moringa seed residues, biogas manure and vermicompost) with and without phosphate- solubilizing bacteria (PSB) on growth, photosynthetic pigments, yield and nutrients uptake of wheat plants under saline calcareous soils.

2. Materials and Methods

A pot experiment was carried out under controlled conditions in the green house to study the effect of different organic amendments (Moringa seed residues, biogas manure and vermicompost) with and without phosphate-solubilizing bacteria (PSB) on growth, yield and nutrients uptake of wheat (*Triticum aestivum* L, cv. Sakha 93) plants under saline calcareous soil. The soil was taken from the surface layers (0-30 cm) from El-Noubaria Research Station beside El-Noubaria county at Northern part of Tahreer Province, Egypt. The soil was air dried for 6 days, crushed, sieved to pass through 2 mm plastic screen, thoroughly mixed and stored in plastic bags, Main soil properties are given in Table 1. Soil properties were determined according to [9, 10 and 11] .

Plastic pots of internal dimensions 25 x 30cm were filled with ten kilograms of the tested soil samples. Previously mentioned treatments were mixed with the tested soil before planting and replicated three times. A randomized complete block design was used. Moringa seed residues, biogas manure and vermicompost were added at a rate of 2% (20 g kg^{-1} soil). Some characteristics of organic amendments are shown in Table (2).

Table 1: Some physical and chemical properties of the investigated soil.

Soil characteristics	Values
Soil particles distribution	
Sand ,%	43.55
Silt,%	26.31
Clay,%	30.14
Textural class	Loamy sand
Field capacity (FC),%	14.23
$CaCO_3$, (g kg^{-1})	195
Organic matter,(g kg^{-1})	6.50
pH*	8.02
EC,(dSm^{-1}) **	6.74
Soluble cations and anions, (mmolc L^{-1})**	
Ca^{++}	15.87
Mg^{++}	19.74
Na^{+}	17.87
K^{+}	13.92
$CO_3^{=}$	-
HCO_3^{-}	22.15
Cl^{-}	19.32
$SO_4^{=}$	25.93
Available nutrients (mg kg^{-1}soil)	
Available N	39.12
Available P	8.98
Available K	97.12

* Suspension of 1:2.5 soil: water ** Soil paste extract

Table 2: Some characteristics of organic residues

Organic residues	EC**, dSm^{-1}	pH*	Organic matter, (%)	Total nutrients, %			C/N ratio
				N	P	K	
Moringa seed residues	1.34	7.45	40.23	1.96	0.60	1.53	11.93
Biogas manure	2.51	7.85	30.56	1.76	0.27	1.96	10.10
Vermicompost	2.27	7.56	40.1	2.91	0.75	1.65	8.01

*Organic residues -water suspension 1: 5 ** Organic residues water extract 1:10

Phosphatic fertilizers were added to the soil samples as Tribe super phosphate (31% P_2O_5) at a rate of at 13 mg P kg^{-1}. Also, fertilizer nitrogen was added as ammonium sulphate (205 g N kg^{-1}) at the rate of 100 mg N kg-1 soil at three equal splits. The first was 15 days after seeding, the second and third doses were added at tillering (45 day after seeding) and booting (75 day after seeding). Before seeding, potassium fertilizers as potassium sulphate (400 g K kg^{-1}) was thoroughly mixed with the soil at a rate of 40 mg K kg^{-1}. Twenty seeds of wheat were seeded per pot. The pots were daily weighed and the soil moisture content was adjusted nearly the field capacity. After germination, plants were thinned to ten plants.

The phosphate-dissolving bacteria (PDB), (*Bacillus megaterium*) was obtained from the Microbiology Department, National Research Centre, Cairo, Egypt. The PDB was inoculated in a concentration of (1×1010 CFU ml^{-1}) at rate of 50 ml pot^{-1} to mix with soil before planting and irrigated.

2.1. Determinations of photosynthetic pigments and enzymatic antioxidants

At 80 days, ten plants were randomly selected for the measurement of growth (plant height, leaf area, pod length) and photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically [12]. Porline was determined according to [13].

Using fully expanded fresh leaves, Catalase (CAT), Peroxidase (POX), and superoxide dismutase (SOD) activities were analyzed following to [14,15,16, 17 and 18]

Plant were harvested, dried at 70 ˚C for 72 hours, weighed, ground in wiley mill and prepared to chemical analysis. The plant samples were wet digested with conc. H_2SO_4 + conc. HClO4 (4:1) by volume. Total N and P were determined according to [19]. The analyses of soil samples and soil amendments were carried out according to [10]. Total phosphorus in plant was determined colourmetrically according to [20]. At harvest, plants were separated into straw and grains.Yield and yield components were recorded. Protein percent in grains was calculated by multiplying N% × 5.70 [21]. The obtained data of plant parameters were statiscally analyzed (LSD at 0.05) according to method described by [22].

3. Results

3.1. Effects of organic amendments and phosphate-dissolving bacteria on Chlorophyll a, chlorophyll b, carotenoids (mg g^{-1}f wt) and free proline (µg g^{-1} DW) of wheat plants under saline calcareous soils

The data are given in Table (3) and illustrated in Figs. (1) show the effect of applying organic amendments (OM) with or without phosphate-dissolving bacteria (PSB) on chlorophyll a, chlorophyll b, carotenoids and free proline grown on saline calcareous soil. Application of different OM under PSB inoculation gave increases in chlorophyll a,b, carotenoids and proline content of wheat plants compared to untreated plants. Application of Ver in the presence PSB gave the highest values of chlorophyll a, chlorophyll b, carotenoids and free proline of wheat plants compared to different treatments as biogas manure (BM) or MSR. These results are similar to those of [23, 24]. The high mineral nutrients i.e., N,P and K concentration of vermicompost makes it a more effective source of plant-available N or P and K compared to conventional organic fertilizers [25]. Results show that the addition of PSB to different OM. Increased chlorophyll a,b, and carotenoids compared to the untreated ones. These increases represent 12, 24. 26 and 10 % in the case of different for untreated, MSR, BM and Ver, respectively for chlo. a; 9, 20, 21 and 8 for chlo. b and 12, 25, 27 and 10 for carotenoids. These results are in agreement with those of [26].

Table 3: Effects of organic amendments and phosphate-dissolving bacteria on Chlorophyll a, chlorophyll b, carotenoids (mg g^{-1}f wt) and free proline (µg g^{-1} DW) of wheat plants under saline calcareous soils

Treatments	Chlorophyll a (mg g^{-1}f wt),	Chlorophyll b(mg g^{-1}f wt)	Total Carotenoids (mg g^{-1}f wt)	Free Proline (µg g^{-1} DW)
Untreated	1.115±0.001h	0.556±0.002h	0.434±0.003h	27.17±0.003h
PSB	1.245±0.003f	0.608±0.001f	0.488±0.006f	27.59±0.002f
MSR	1.23±0.001fg	0.6025±0.002fg	0.482±0.001fg	33.01±0.002fg
MSR+PSB	1.525±0.001c	0.726±0.003c	0.605±0.001c	33.52±0.001c
BM	1.275±0.003e	0.62±0.01e	0.501±0.004e	33.705±0.005e
BM+PSB	1.605±0.001b	0.752±0.03b	0.638±0.001b	33.945±0.001b
Ver	1.5±0.004d	0.71±0.001d	0.595±0.003d	34.05±0.001d
Ver+PSB	1.645±0.012a	0.768±0.02a	0.655±0.005a	34.445±0.00aa

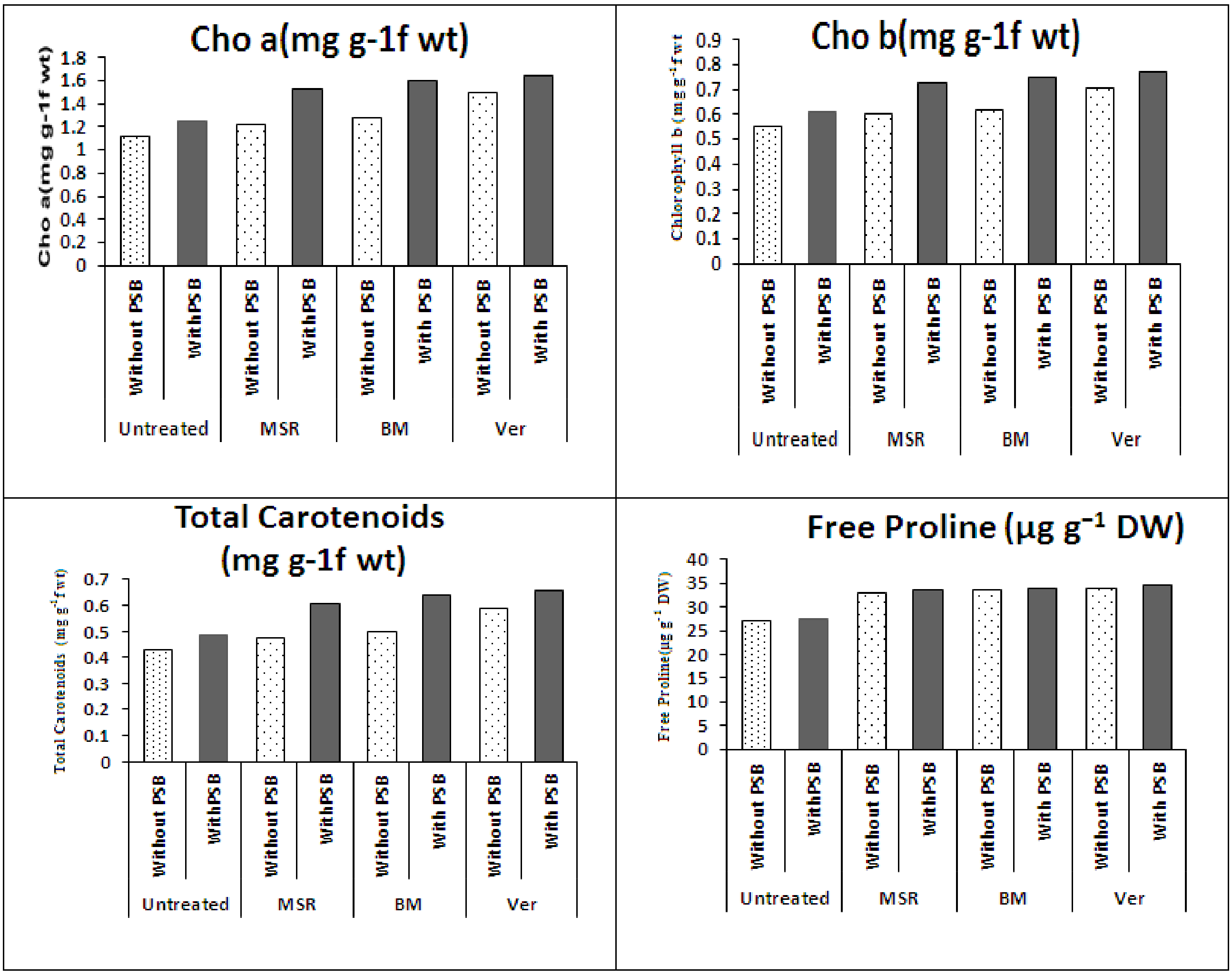

Fig. 1. Effects of organic amendments and phosphate-dissolving bacteria on Chlorophyll a, chlorophyll b, carotenoids (mg g^{-1}f wt) and free proline (μg g^{-1} DW) of wheat plants under saline calcareous soils

3.2. Effects of OM and PSB on antioxidant enzymes (A564 min^{-1} g^{-1} protein) of wheat plants under saline calcareous soils

The influence of OM i.e., BM, MSR and Ver in the presences of PSB on antioxidant enzymes in wheat cultivated in saline calcareous soil was investigated (Table 4, Figure 2). For treatments with PSB, the highest values for antioxidant enzymes *i.e.,* CAT, POX, and SOD were observed in the Ver treatment, with values of 71.43, 1.77, and 9.02, respectively, indicating enhanced antioxidant activity in the presence of Ver and PSB.

Comparison across organic amendments (MSR, BM, Ver), either alone or in combination with PSB, elucidates that the combination treatments generally offer an enhanced antioxidant response compared to their sole counterparts. Specifically, the application of Ver+PSB stands out by significantly boosting enzymatic antioxidant levels. As for the average effect of OM addition, the data show that using Ver. combined with PSB. gave higher values than MSR or BM application. This finding stands in well agreement with those of [24]. Results show that the addition of Ver. increased CAT, POX and SOD compared to the untreated ones. These increases represent 21, 36 and 103 % respectively. Results show that the applicaton of PSB to different OM. increased CAT, POX and SOD compared to the untreated ones. These increases represent 1, 2. 2 and 2 % in the case of different for untreated, MSR, BM and Ver, respectively for CAT. 3, 13, 12 and 10 for POX and 8, 6, 7 and 14 for SOD, respectively. This finding stands in well agreement with those of [27, 28, 29]. The increase of growth characteristics, chlorophyll content and antioxidant enzymes of wheat plants grown saline soil conditions reflected in increasing shoot system that might be attributed to more assimilation which correlated with macro and micro nutrients as well as amino acids [28, 30].

Table 4: Effects of OM and PSB on antioxidant enzymes (A564 min^{-1} g^{-1} protein) of wheat plants under saline calcareous soils

Treatments	CAT (A564 min^{-1} g^{-1} protein)	POX (A564 min^{-1} g^{-1} protein)	SOD (A564 min^{-1} g^{-1} protein)
Untreated	57.71±0.003h	1.18±0.003h	4.44±0.003h
PSB	58.21±0.001g	1.21±0.001g	4.78±0.0004g
MSR	66.01±0.002f	1.46±0.002f	7.15±0.01f
MSR+PSB	67.05±0.002e	1.65±0.005c	7.57±0.004d
BM	68.24±0.003d	1.55±0.001e	7.38±0.02e
BM+PSB	69.52±0.004c	1.73±0.002b	7.87±0.01c
Ver	70.39±0.002b	1.61±0.01d	7.91±0.004b
Ver+PSB	71.43±0.003a	1.77±0.001a	9.02±0.004a

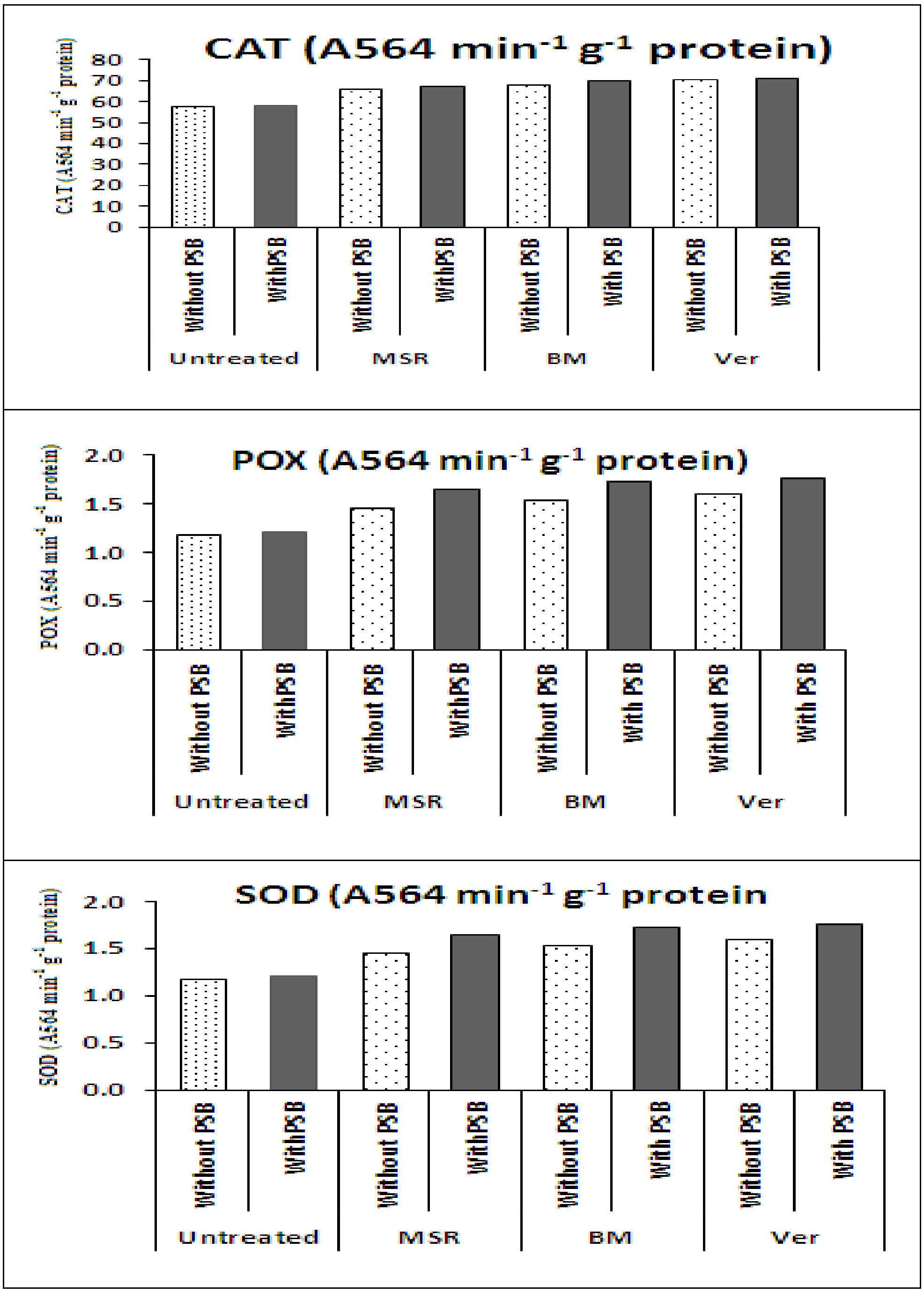

Fig. 2. Effects of organic amendments and PSB on antioxidant enzymes (A564 min^{-1} g^{-1} protein) of wheat plants

3.3. Effects of OM and PSB on plant height, straw and grains yield, 1000- grains weight and protein content of wheat plants under saline calcareous soils

Data in (Table 5) showed that the Ver treatment exhibited the tallest plant height, straw, grains weight; 1000 grains weight and protein content among all treatments either with PSB, with values of 86.67 cm; 2.40 g $pant^{-1}$; 0,50 g $pant^{-1}$; 46.27 g and 13.84%, respectively, while it was 82.5 cm; 2.2 g $plant^{-1}$; 0,40 g $pant^{-1}$; 45 g and 13.2%, respectively, without PSB. Additionally, the BM treatment showed the second highest plant height for PSB (86.67 cm). Regarding straw and grains weight, the BM treatment demonstrated the highest values across PSB (2.3 and 0.41 g plant-1, respectively), indicating enhanced biomass production. Application of Ver in the presence PSB gave the highest values of straw, grains, and biological yield and protein content of wheat plants compared to different treatments. These results are similar to those of [31,32]. Mineral fertilizers with the combination of Ver help to improve the nutrients and yield of major crops and help to enhance soil health [33]. Results show that the addition of PSB to different OM. Increased straw, grains weight; 1000-grain weight and protein content compared to the untreated ones. These increases represent 14,39. 11and 13 % in the case of different for untreated, MSR, BM and Ver, respectively for straw weight; 24, 77, 22 and 7 for grain weight; 8,2,3 and 3% for 1000- grain weight and 11, 13, 9 and 4 for protein content, respectively.

Table 5: Effects of OM and PSB on plant height, straw and grains yield, 1000- grains weight and protein content of wheat plants under saline calcareous soils

Treatments	Plant height (cm)	Straw weight (g plant $^{-1}$)	Grain weight (g $plant^{-1}$)	1000- grain weight (g)	Protein content,%
Untreated	52.00±0.006h	0.99±0.001h	0.98±0.002h	35.47±0.002h	4.61±0.008h
PSB	61.00±0.006f	1.13±0.003f	1.22±0.005f	38.23±0.00g	5.13±0.001g
MSR	57.33±0.003g	0.93±0.001g	1.16±0.004g	43.30±0.006f	10.25±0.008f
MSR+PSB	81.07±0.008d	1.29±0.002e	2.04±0.001d	44.13±0.008de	11.53±0.005de
BM	79.20±0.006e	1.34±0.003d	1.73±0.001e	44.23±0.004d	12.04±0.002d
BM+PSB	83.53±0.003b	1.48±0.002c	2.10±0.001b	45.37±0.002b	13.07±0.003c
Ver	82.50±0.004c	1.62±0.004b	2.02±0.004bc	45.07±0.004c	13.33±0.003b
Ver+PSB	86.67±0.0001a	1.83±0.0001a	2.16±0.0002a	46.27±0.003a	13.84±0.001a

3.4. Effects of OM and PSB on NPK-uptake with straw and grains of wheat plants under saline calcareous soils

The data are given in Table (6) and show the effect of applied organic amendments with or without phosphate-dissolving bacteria (PSB) on N, P and K-uptake by wheat plants grown on saline calcareous soil. Application of various organic amendments i.e. MSR or BM or Ver in the presence of PSB gave increases in N, P and K-uptake by straw and grains of wheat plants as compared to untreated plants. Similar results were obtained by [34,35,36, 37]. The highest N, P and K-uptake of wheat were obtained under application of Ver in the presences of PSB, while the lowest ones were obtained with untreated soils in absence of organic amendments in the absence of PSB. The application of organic fertilizers in the soil helps in increasing the fertility of the soil as physical condition including its water holding capacity [38].

Data showed that the application of phosphate-dissolving bacteria (PSB) to OM increased straw and grains NPK-uptake of wheat compared to the untreated ones. These increases represent 35, 60, 23 and 20% of straw N- uptake in the case of untreated, MSR, BM and Ver, respectively; 35, 83, 2 and 11 of straw P- uptake, respectively and 22, 43,13 and 16 of straw K- uptake, respectively. On the other hand, these increases represent 38, 100, 7 and 11% of grains N-uptake in the case of untreated, MSR, BM and Ver, respectively; 36, 84, 1 and 8 of grains P- uptake, respectively and 35, 82,1 and 10 of grains K- uptake, respectively. The use of PSB as inoculants simultaneously increases NPK uptake by the plant and crop yield. Strains from the genera Pseudomonas, Bacillus and Rhizobium are among the most powerful phosphate solubilizers [34, 39, 40]. The promotive effect of various organic amendments on NPK-uptake by straw and grains of wheat plants grown on alluvial soil may follow the order: Ver>BM>MSR>untreated in the presences or absence of PSB. The favourable effect of various organic amendments on nutrient content is mainly due to the positive effect of this material on increasing the available moisture content and hence increasing the availability of nutrients in the soil solution [29, 41,42].

Table 6: Effects of organic amendments and PSB on NPK-uptake with straw and grains of wheat plants under saline calcareous soils

Treatments	Straw (mg plant^{-1})			Grains (mg plant^{-1})		
	N- uptake	P- uptake	K-uptake	N- uptake	P- uptake	K-uptake
Untreated	7.76±0.001h	1.60±0.002h	10.35±0.001h	7.23±0.002h	1.61±0.001h	8.68±0.001h
PSB	10.52±0.003g	2.17±0.002g	12.62±0.004g	10.00±0.001g	2.20±0.002g	11.79±0.002g
MSR	16.97±0.002f	4.34±0.001f	17.16±0.002f	18.78±0.002f	4.37±0.007f	19.51±0.001f
MSR+PSB	27.23±0.002de	7.94±0.001e	24.54±0.001e	37.59±0.001e	8.05±0.001e	35.43±0.004e
BM	27.51±0.005d	8.41±0.002d	25.91±0.006d	40.91±0.003d	8.53±0.001c	37.15±0.001cd
BM+PSB	34.06±0.001c	8.55±0.003c	29.51±0.002c	43.84±0.001b	8.60±0.002cd	37.56±0.002b
Ver	36.51±0.002b	9.00±0.002b	32.91±0.001b	43.14±0.003bc	9.00±0.003b	37.31±0.004c
Ver+PSB	43.90±0.001a	10.02±0.002a	38.12±0.001a	47.82±0.002a	9.71±0.001a	41.09±0.001a

3.5. Available phosphorus (mg kg^{-1}) as affected by organic amendments and PSB under saline calcareous soil conditions

Under investigation, the values of available phosphorus (mg kg^{-1}) as affected by the application of organic amendments (MSR, BM and Ver) with or without PSB are show in illustrated in Fig. (3). The treatments of Ver with PSB gave the highest values of available P (31.2 mg kg^{-1}), while the lowest ones (6.1 mg kg^{-1}) were found with untreated soil. These results are in agreement with those obtained [24, 34. 43,44 ,45]. Data obtained that the addition of PSB to organic amendments i.e., untreated, MSR, BM and Ver increased available P in saline calcareous soil compared to the untreated (Fig 3). These increases represent 9, 6, 3 and 6%, respectively for the treatments of untreated; MSR, BM and Ver. These results are in agreement with those of [39]. The promotive effect of different organic amendments on available- P in calcaerous soil may follow the order: Ver> BM> MSR> untreated in the presence or absence of PSB. As a general result, the available phosphorus was clearly increased after harvest at all treatments of any organic amendments application with PSB. These increases may be due the microbial activity which has the ability to affect soil reaction in the soil microenvironment leading to solubilizing mineral phosphorus. This finding is in agreement with that obtained by [46].

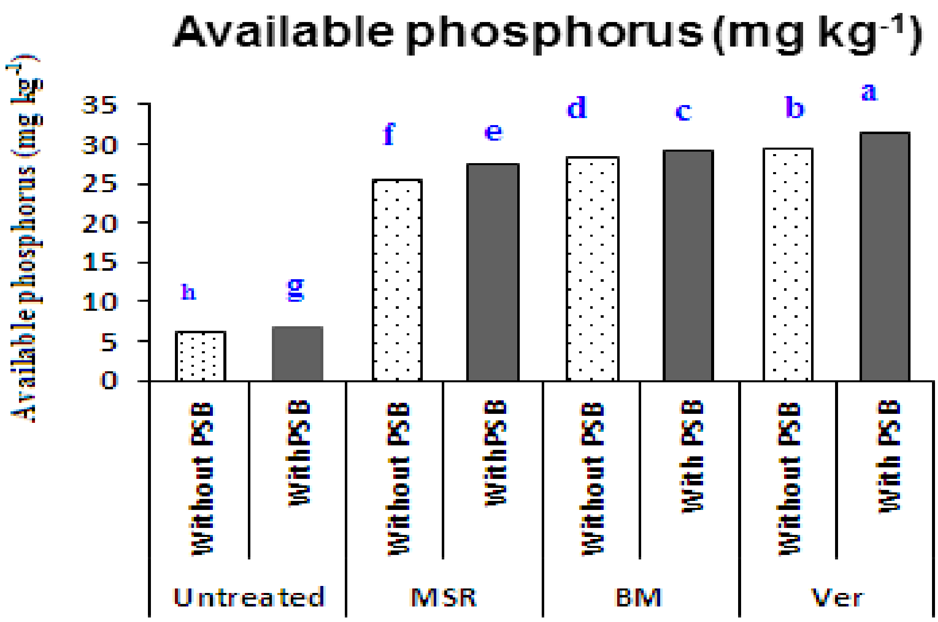

Fig. 3. Available phosphorus (mg kg^{-1}) as affected by organic amendments and PSB under saline calcareous soil conditions

4. Conclusions

The present study successfully demonstrated that the integration of organic amendments (MSR, BM, Ver) with phosphate-solubilizing bacteria (PSB), significantly enhances phosphorus availability, leading to improved growth, yield, and nutrient uptake in wheat plants grown under saline calcareous soil conditions. The combined treatment of Ver+PSB emerged as the most effective strategy, showing marked improvements in key physiological parameters, and overall plant productivity. Future research should focus on exploring the underlying mechanisms of these synergistic effects, optimizing amendment formulations, and evaluating long-term impacts on soil health and agricultural sustainability.

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