Research Article

Effective Microorganisms (EM) Co-applied with Organic Wastes and NPK Stimulate the Growth, Yield and Quality of Spinach (*Spinacia oleracea* L.)

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Abstract | Effective microorganisms (EM) are increasingly being applied to stimulate nutrient cycling and plant growth. However, information regarding its co-application with organic wastes and chemical fertilizers on enhancing spinach yield is scarce. A pot experiment was conducted at Gomal University D.I. Khan, Pakistan, during 2009-10 to study the changes in soil fertility and spinach growth after the application of EM with organic wastes and chemical fertilizers. The six treatments were; control (T_0), 10 tons (t) ha^{-1} farm yard manure (FYM) (T_1), 20 t ha^{-1} pressmud (T_2), 0.7 t ha^{-1} compost (T_3), 5 t ha^{-1} poultry manure (T_4) and mixed chemical fertilizer in the ratio of 100 : 40 : 56 kg ha^{-1} as N, P and K (T_5). Each treatment was applied alone and with EM. Results showed that EM + pressmud combination significantly improved soil physical and chemical properties as well as spinach growth compared to other fertilizers with EM. Maximum soil organic matter content (OM) and K was observed in treatment where pressmud was co-applied with EM. While greater N and P were found where compost and poultry manure were co-applied with EM respectively. Significant ($P < 0.05$) increase in plant height (11%), number of leaves (124%), fresh foliage yield (136%), dry foliage yield (16.4%), leaf length (20.2%) and leaf area (77.4%) were noted where pressmud + EM was applied as compared to its corresponding treatment without EM. Maximum Fe, Cu and Mn concentrations were found where pressmud + EM was applied. Dietary fiber, vitamin C and crude proteins were also increased in spinach with pressmud + EM application. It was concluded that EM-inoculated pressmud has higher potential to increases soil fertility as well as stimulate spinach growth and quality. Therefore, warrants further testing under field conditions.

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Introduction

In many parts of Pakistan, where subsistence farming is norm, the high cost of chemical fertilizers deters poor farmers to use them on a wide and balanced scale. In such farming system, organic wastes, like cow dung, poultry manure, pressmud and crop residues, are locally available and relatively cheap, therefore, increasingly being applied to meet crop nutrient demand (Ahmad et al., 2012). Organic wastes contain several essential plant nutrients including nitrogen (N), phosphorus (P), potassium (K), and carbon (C)

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which have the most potential to improve soil physical, chemical and biological fertility (Schroder, 2005; Zaman et al., 2004). However, majority of nutrients in these wastes are found in organic forms which are reported to get released at different rates (Zaman et al., 1998; 1999; 2002).

Since, release of nutrients from organic wastes (mineralization) is a slow process and carried out by numerous enzymes and a wide range of microorganisms (Zaman et al., 1999), therefore, co-application of organic wastes with effective microorganisms (EM) has been shown to facilitate release of nutrients such as N and P (Ahmad et al., 2012; Lack et al., 2013). EM is a suite of microorganisms which include photosynthetic bacteria, yeasts, fungi and actinomycetes.

In Pakistan, bio-fertilizer products such as EM are now commercially available. Several studies have reported positive changes in soil characteristics (Namasivayam, 2010; Chantal, 2013) and plant growth (Zhou et al., 2009; Talaat, 2014). Application of EM with organic wastes is reported to improve soil organic matter content through humification of fresh organic materials; which then leads to improved soil health and enhance microbial activities (Valarini et al., 2003). Several studies have shown that co-application of EM with organic and chemical fertilizers increased crop yield (Khaliq et al., 2006). EM applied in combination with compost increased wheat yield (Hu and Qi, 2013) and enhanced photosynthesis (Dubey, 2005).

In Pakistan, the efficiency of organic fertilizers is reported to be low probably due to lower soil fertility and slow release of nutrients from organic nutrient sources (Tahir et al., 2011; Khan et al., 2012). So, there is a need to overcome this slow mineralization problem and provide relatively cheap sources of plant nutrients to the resource poor farmers. Thus, EM technology with co-application of organic wastes was introduced in Pakistan in early 90's and pioneer research work was done by Nature Farming Research Centre, University of Agriculture, Faisalabad (Hussain et al., 1992).

In Pakistan, spinach (*Spinacia oleracea L.* a member of Chenopodiaceae family) is among the favorite food because of its nutritive value such as high Fe and vitamin K contents; however, its per hectare yield of 12588 kg is much lower than those in the developed countries such as Belgium having per hectare yield of 19700 kg (FAOSTAT, 2012). So, there is a need to improve spinach yield to meet the demand of growing population of Pakistan.

Thus, current study was carried out to assess the effect of applying organic wastes and chemical fertilizers with and without EM on soil quality and spinach growth.

Table 1: *Chemical composition of different organic wastes*

Manures	N%	P%	K%
FYM	2.02	0.58	2.75
Press mud	3.0	3.5	1.8
Compost	2.6	0.9	2.0
Poultry	3.1	1.5	2.5

Materials and Methods

A pot experiment was conducted under glasshouse conditions to assess the effect of co-application of effective microorganisms (EM) with chemical or organic fertilizers on soil quality and spinach growth and quality. Completely randomized design (CRD) was used with split plot arrangements. There were six treatments (each with and without EM) i.e. control (T_0), 10 t ha^{-1} FYM (T_1), 20 t ha^{-1} pressmud (T_2), 0.7 t ha^{-1} compost (T_3) and 5 t ha^{-1} poultry manure (T_4) and the recommended dose of NPK fertilizers at the rate of 100 : 40 : 56 kg ha^{-1} (T_5). Urea, single supper phosphate and potassium sulfate were used as the sources of mixed NPK fertilizers. Farm yard manure and poultry manure were obtained from nearby farms while pressmud was obtained from Chashma Sugar Mills, D. I. Khan. Commercial compost was purchased from Higo Organic Pvt. Ltd. Organic Fertilizers. Table 1 shows nutrient status of different organic amendments used in the experiment. Bio-Aab as source of EM was obtained from EM Foundation, Faisalabad, Pakistan. Surface soil (0-15 cm) was collected from Indus River basin, sieved through 2mm mesh and transferred to pots at the rate of 20 Kg soil pot^{-1}. Pre-sowing analysis of soil indicated 0.9% OM, 440 mg kg^{-1} N, 4 mg kg^{-1} P and 47 mg kg^{-1} K. Organic manures were saturated with liquid Bio-Aab for 15 days at room temperature (25°C) and applied one month prior to sowing. Spinach was sown at the seed rate of 10 g pot^{-1}. Pots were irrigated at regular intervals to keep optimum soil moisture content. Three spinach harvests were taken and the days taken after each harvest were recorded. Plant parameters such as plant height, number of leaves per plant, fresh and dry foliage yields, and leaf length and leaf area were recorded for each harvest. Leaf length and leaf area were determined by Graph crude method (Reddy, 2004).

Soil analysis

Post-harvested soil samples were analyzed for their physical and chemical properties. Soil water suspension of 1:1 was prepared for pH and electrical conductivity measurements (US Salinity Lab Staff, 1954; Rhoades, 1996). Organic matter was determined by potassium dichromate oxidation method- commonly known as Walkley and Black method as described by (Walkely and Black, 1934). Briefly, 1g air-dried soil was mixed with 10 mL 1N potassium dichromate and 20 mL concentrated H_2SO_4 in 500 mL beaker. After 30 minutes, 30 mL distilled water and 10 mL concentrated H_3PO_4 were added and the mixture was allowed to cool. Then, 10-15 drops of diphenylamine indicator were added and mixed thoroughly. Finally, the mixture was titrated against 0.5 M ferrous ammonium sulfate till color changed from violet blue to green. Calculation for OM was done according to standard equations. Kjeldahl nitrogen was determined by digesting and titrating soil sample according to Bremner and Mulvaney (1982). Procedure involved taking 1g dried soil sample in calibrated digestion tube and adding 5g catalyst mixture, few boiling pumice granules and 15mL concentrated H_2SO_4 and left overnight. Digestion was carried out in digestion block for 3 hours at 370°C and 15 mL distilled water was added for precipitation. Distillation was carried out at steam rate of 7-8 mL per minute with saturated H_3BO_4 and 10N NaOH, followed by titration against $0.01(NH_4)_2SO_4$ (pH 5). Extractable phosphorous (P) was determined according to the procedure of Olsen et al. (1954) and extractable potassium was determined by flame photometer (AOAC 2000). Soil saturation percentage was calculated from the water loss at 105°C by oven drying of saturated paste to a constant weight:

$$Saturation\% = ((W1 - W2)/W2)\ x100 \ldots$$

Where:
W1, mass of saturated paste before oven drying; W2, mass of dried soil paste.

Determination of micro and macro nutrients in spinach

Fully expanded spinach leaves (3rd and 4th) from the growing point were analyzed for macro nutrients (N, P, K, Na, Ca, and Mg) and micro nutrients (Zn, Cu, Fe and Mn) by using Plasma Mass Spectrometry (ICP-MS) as described by Mumtaz et al. (2014). Leaf sample (0.5 g) was dried at 80°C for 72 hours, ground to powder with mortar and pestle, and converted to ash in furnace at 500°C for 8 hours. The ash was then digested with 1:1 mixture of HNO_3 and $HClO_4$ and the extract was analyzed for various elements by ICP-MS. First, the ICP-MS system was calibrated by using external standards prepared in 1% HNO_3. The standard operating parameters of ICP-MS were adjusted to plasma gas flow: 14-16 L min^{-1}, nebulizer gas flow: 0.90 – 0.98 L min^{-1}, and sample flow at the rate of 1 mL min^{-1}.

Determination of dietary fiber, vitamin C and crude protein

The dietary fiber in spinach was determined by enzymatic-gravimetric method (AOAC, 2000). In short, powdered spinach samples were gelatinized with heat stable-α amylase and digested with protease and amyloglucosidase. Soluble and insoluble dietary fibers heat stable-α amylase and digested with protease and amyloglucosidase. Soluble and insoluble dietary fibers were precipitated and dried at 105 °C. Total dietary fiber was obtained by the sum of soluble and insoluble dietary fibers. Vitamin C was determined by using 2, 6-dichloro-indophenol (DCIP) titration method (Tee et al., 1988). Briefly, metaphosphoric acid extracts of spinach leaves were obtained and pH was adjusted to 1.2. Then, titration was done to determine the reducing capacity of extracts with DCIP. Crude protein was determined by multiplying total N content by a factor of 6.25 (Fadaei and Salehifar, 2012).

Statistical analysis

A factorial completely randomized design was followed with three replications. Analysis of variance (ANOVA) was performed to compare chemical and organic fertilizers applied with and without EM with respect to spinach yield, and other parameters. Different treatment means were compared using least significant difference (LSD) test (Steel and Torrie, 1986). All the analyses were performed using Statistix 9 software.

Results and Discussion

Date of germination and days taken to first harvest of spinach: Table 2 shows the effect of EM on germination and days taken for spinach maturity. Plants grown in the absence of EM took 65 days for first harvest while EM-treated plants took 60 days, enhancing the spinach growth rate by 5 days till harvesting as compared to non EM treatments. The enhanced growth rate is attributed to the EM, producing and inducing the plant growth hormones. Earlier, Kurepin et al. (2014) reported that bacteria-fungi- and protozoa-based seaweeds extracts act as biostimulators for inducing different phytohormones like gibberellins, cytokinines and auxins which regulate plant growth.

Plant height: Physiological traits of spinach during three harvests under different fertilizers co-applied with EM are given in Tables 3**,** 4 and 5. Plant heights were significantly influenced by the organic and inorganic fertilizers applied with EM. Significantly ($P<0.05$) greater plant to increased plant growth and crop production. Our results are also supported by earlier findings of Chantal et al. (2013) who reported that bio-organic fertilizers with EM significantly increased plant height of flue-cured tobacco.

Number of leaves per plant: Number of leaves per plant were significantly ($P<0.05$) affected by different organic and chemical fertilizers with and without EM (Tables 3, 4 and 5). The 1st, 2nd and 3rd spinach harvests indicated that maximum number of leaves of 59.6, 64.4 and 60.2 were observed where pressmud + EM was applied followed by FYM (T_1) with 32 leaves per plant that differed from the rest of treatments. The highest numbers of leaves were in 2nd harvest (138%) compared to its corresponding non-EM treatment. Organic amendments with EM were more effective compared to chemical fertilizer in promoting spinach growth. This was due to improvement in soil physical and chemical properties and in addition EM enhanced the release of nutrients from organic matter by rapid decomposition (Ibrahim et al., 2010). Muhammad and Khattak (2009) investigated the effect of pressmud on plant growth and nutrient status of maize and reported increased crop yield. Significantly ($P<0.05$) greater numbers of leaves per plant were reported in plants where EM was applied indicating the efficiency of EM. The nutrients in OM are mineralized

Table 2: *Date of germination and days taken to first harvest of spinach under different organic and inorganic fertilizers co-applied with EM*

Treatment	Without EM		With EM	
	Germination dates	First harvest time	Germination dates	First harvest time
T_0	12/11/2009	70 days	11/11/2009	68 days
T_1	09/11/2009	66 days	07/11/2009	60 days
T_2	08/11/2009	65 days	05/11/2009	60 days
T_3	09/11/2009	66 days	07/11/2009	60 days
T_4	08/11/2009	65 days	06/11/2009	60 days
T_5	11/11/2009	68 days	09/11/2009	60 days

T_0 *= Control,* **T_1** *= FYM,* **T_2** *= Pressmud,* **T_3** *= Compost,* **T_4** *= Poultry manure and* **T_5** *= NPK,* **W. EM** *= without effective microorganisms,* **EM** *= with effective microorganisms (Organic manures socked with Bio-Aab) , Data are means of three replications*

Table 3: *Physiological traits of spinach in first harvest under different fertilizers co-applied with EM*

Treatments	Plant Height (cm)		No. of Leaves (plant^{-1})		Fresh Foliage Yield (g pot^{-1})		Dry Foliage Yield (g pot^{-1})		Leaf Length (mm)		Leaf Area (mm^2)	
	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM
T_0	12.93e	12.93e	11.43e	11.43e	17.33	29.2	2.9	2.9	14.40i	14.40i	24.89	24.89
T_1	22.53bcd	30.53ab	15.67cd	27.76b	57.92	103.2	7.69	10.17	24.57de	27.73b	51.19	102.39
T_2	28.43ab	32.33a	25.10b	59.56a	95.38	118.67	9.533	10.27	27.37bc	31.33a	98.17	125.98
T_3	17.30cde	27.73ab	14.13cde	16.00c	53.67	100.18	6.98	8.30	22.53f	25.40cd	50.82	101.43
T_4	12.47e	27.10ab	13.33cde	15.07cde	37.78	53.06	5.37	5.85	20.47g	23.47ef	46.16	78.32
T_5	16.30de	24.73bc	11.80de	14.70cde	20.98	70	3.5	3.22	17.47h	19.57gh	37.67	98.72
$LSD_{0.05}$	8.22		3.88		50.30		3.31		2.18		20.43	

T_0 *= Control,* **T_1** *= FYM,* **T_2** *= Pressmud,* **T_3** *= Compost,* **T_4** *= Poultry manure and* **T_5** *= NPK,* **W.EM** *= without effective microorganisms,* **EM** *= with effective microorganisms (Organic manures socked with Bio-Aab), Data are means of three replications.*

Table 4: *Physiological traits of spinach in second harvest under different fertilizers co-applied with EM*

Treat-ments	Plant Height (cm)		No. of Leaves (plant^{-1})		Fresh Foliage Yield (g pot^{-1})		Dry Foliage Yield (g pot^{-1})		Leaf Length (mm)		Leaf Area (mm^2)	
	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM
T_0	15.43	15.43	13.17e	13.17e	20.27fg	20.27g	3.23cd	3.23d	16.43i	16.43i	26.85e	26.85e
T_1	28.66	35.67	26.63c	31.47b	77.36cd	105.25bc	9.89ab	10.63ab	26.57de	29.70b	56.51d	120.07 b
T_2	34.98	39.93	27.10bc	64.43a	117.70b	277.85a	11.19ab	13.02a	29.43bc	35.37a	104.68bc	185.69 a
T_3	20.86	30.07	19.21d	16.55de	85.18bcd	100.34bc	8.23abcd	9.545bc	24.37ef	27.00cd	53.61 d	110.05bc
T_4	14.47	27.73	15.78de	16.01de	63.37de	55.04def	7.23abcd	9.09abc	22.37fg	25.60de	52.45 d	98.12 c
T_5	18.87	27.4	15.00de	16.20de	32.71efg	77.72cd	6.15bcd	5.59bcd	19.50h	21.50gh	51.18 d	101.49 c
$LSD_{0.05}$	10.55		4.67		36.62		6.38		2.44		22.26	

T_0 = *Control,* **T_1** = *FYM,* **T_2** = *Pressmud,* **T_3** = *Compost,* **T_4** = *Poultry manure and* **T_5** = *NPK,* **W.EM** = *without effective microorganisms,* **EM** = *with effective microorganisms (Organic manures socked with Bio-Aab), Data are means of three replications*

Table 5: *Physiological traits of spinach in third harvest under different fertilizers co-applied with EM*

Treat-ments	Plant Height (cm)		No. of Leaves (plant^{-1})		Fresh Foliage Yield (g pot^{-1})		Dry Foliage Yield (g pot^{-1})		Leaf Length (mm)		Leaf Area (mm^2)	
	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM	W. EM	EM
T_0	14.93	14.93	13.17c	13.17c	18.77	18.77	3.09	3.09	15.47	15.47	25.47	25.47
T_1	26.57	35.33	26.43b	29.30b	63.38	99.30	9.23	10.27	25.77	28.20	46.96bcd	105.44b
T_2	32.37	38.70	25.93b	60.15a	116.33	254.83	10.08	10.72	28.70	34.60	92.78 bc	180.21a
T_3	19.67	26.63	19.00c	15.52c	80.30	96.77	8.22	8.54	23.50	26.53	46.31bcd	102.00bc
T_4	12.47	25.70	14.53c	13.47c	58.48	45.68	6.63	5.94	21.57	24.27	42.69 cd	92.48bc
T_5	17.30	22.47	13.00d	15.50 c	27.97	73.03	5.40	4.68	18.53	20.77	40.37 d	97.97bc
$LSD_{0.05}$	10.03		10.03		43.83		2.38		3.34		62.64	

T_0 = *Control,* **T_1** = *FYM,* **T_2** = *Pressmud,* **T_3** = *Compost,* **T_4** = *Poultry manure* **and** **T_5** = *NPK,* **W.EM** = *without effective microorganisms,* **EM** = *with effective microorganisms (Organic manures socked with Bio-Aab), Data are means of three replications.*

by EM and make them available for plant uptake. Significantly higher fresh leaf yield of flue-cured tobacco was reported by Chantal et al. (2013) when EM was applied with organic fertilizers. Our results are also supported by the findings of Gorski and Kleiber (2010) who reported higher number of leaves in plants sprayed with EM. Similar results were recorded by Lack et al. (2013) who reported maximum numbers of nods on the stem. They attributed the increased number of leaves to greater photosynthesis and increased crop yield in safflower by the application of crop growth enhancer bacteria.

Fresh foliage yield: Fresh foliage yield of spinach was significantly ($P<0.05$) influenced by organic and inorganic fertilizers with and without EM (Tables 3, 4 and 5). The 1st, 2nd and 3rd spinach harvest indicated improvement in fresh foliage yield. The highest fresh foliage yield 118.7, 277.9 and 254.8 g pot^{-1}, in 1st, 2nd and 3rd spinach harvests was observed in treatment where pressmud + EM was applied, which differed significantly from all other treatments. The significantly ($P<0.05$) greater fresh foliage yield 136% was obtained in 2nd harvest with pressmud + EM as compared to corresponding non-EM treatment. Such increasing trend of high fresh foliage yield was followed by FYM with EM. Organic wastes improve soil physical and chemical fertility through addition of carbonaceous and proteinaceous materials which increase foliage production through greater availability of nutrients during growth stage. Organic wastes with EM also enhance plant growth by producing various plant growth regulating hormones and enzymes (Kurepin et al., 2014). Effective microorganisms are also involved in the production of antibiotics. Our results are supported by Arshad et al. (2000), who supplemented soil with EM-inoculated farm yard manure and green manure with commercially prepared EM (Bio-Aab). The recorded significant increase in vegetative and reproductive growth of wheat plants.

Dry foliage yield: Dry foliage yield of spinach was significantly ($P<0.05$) affected by the application of organic wastes and chemical fertilizers with EM (Tables 3, 4 and 5). The 1st, 2nd and 3rd spinach harvest produced highest dry foliage yield of 10.3, 13 and 10.3 g pot^{-1} where pressmud + EM was applied. EM with organic sources increased biomass production and finally increased dry matter content in the crop. Our results are supported by earlier findings of Lack et al. (2013). Kurepin et al. (2013, 2014) also revealed that inoculation with *Azospirillum* sp. increased grain yield and shoot dry matter in cereals.

Leaf length: Leaf length is an important yield determining parameter in leafy vegetable. Our results showed substantial differences among the different treatments (Tables 3, 4 and 5). The 1st, 2nd and 3rd spinach harvest indicate significantly (P<0.05) maximum leaf length of 31.3, 35.4 and 34.6 cm in plants receiving pressmud + EM (T_2) which differed from all other treatments. The statistically (P<0.05) maximum leaf length of 35.4 cm which is 20.2% of the corresponding non-EM treatment, was found in plants receiving inoculated pressmud. Khan et al. (2012) reported that pressmud contained high levels of nitrogen which increased leaf length. Similarly, EM mixture contains photosynthetic bacteria which may also results in an increased leaf length. Our results were in line with those of Chantal et al. (2013) who reported increased fresh leaf yield of flue-cured tobacco after application of EM with bio-organic fertilizers.

Leaf area: Leaf area defines the photosynthetic capacity of a plant. The leaf area of spinach was significantly (P<0.05) affected by different organic and inorganic fertilizers with and without EM (Tables 3, 4 and 5). Results showed maximum leaf areas i.e. 126, 104.7 and 180.2 cm^2 in 1st, 2nd and 3rd spinach harvests respectively with pressmud + EM application. It was followed by FYM (T_1), compost (T_3), NPK (T_5) and poultry manure (T_4). It could be due to the increased nitrogen content in organic manures and the nitrogen fixation by the EM which then increase leaf area (Lack et al., 2013). Wang (1999) also found beneficial effects of pressmud when applied with EM on leaf

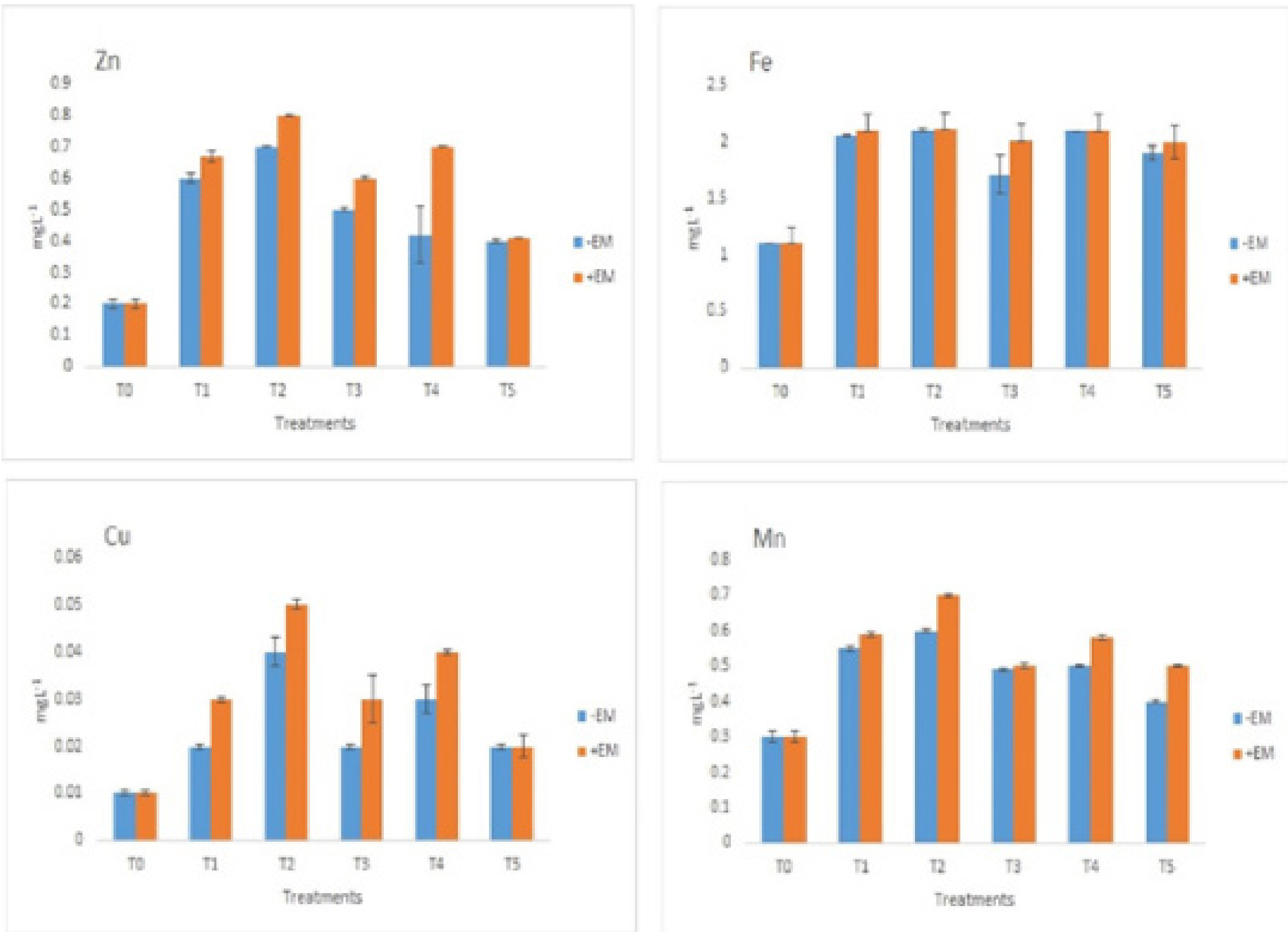

Figure 1: Effect of EM and different soil organic fertilizers on micronutrients (Zn, Fe, Cu, and Mn) of spinach
T_0 = *Control,* **T_1** = *FYM,* **T_2** = *Pressmud,* **T_3** = *Compost,* **T_4** = *Poultry manure and* **T_5** = *NPK,* **W. EM** = *without effective microorganisms,* **EM** = *with effective microorganisms (Organic manures socked with Bio-Aab)*

photosynthesis and fruit yield of tomato.

Biochemical changes in spinach as affected by different nutrient sources co-applied with and without EM

Micro-nutrients status of spinach: The effects of organic and inorganic fertilizers with and without EM on the level of micronutrients found in spinach are shown in Figure 1. The effect of EM on Zn and Mn was more pronounced compared to Fe and Cu. Maximum concentrations of Zn, Fe, Cu and Mn were observed with pressmud + EM application. However, the highest increase in Zn concentration (66.7%) due to EM was recorded with poultry manure. Maximum Fe, Cu and Mn concentrations were found in case of pressmud + EM application. The minimum values for all observed micronutrients were obtained in their respective controls. Previously, such increase in the micronutrient contents have been reported by Gorski and Kleiber (2010). This increase may be attributed to increased solubility of the nutrients at low pH: caused by rapid decomposition of the organic substances.

Macro-nutrients status of spinach: Figure 2 shows the effect of organic wastes and NPK fertilizers in the presence and absence of EM on the levels of macro nutrients in spinach. Macro-nutrient levels in the spinach were improved in all treatments receiving organic fertilizers with EM. Percent improvement of 8, 2, 6.3, 2, 6.7 and 4 for Na, Ca, Mg, N, P and K, respectively, in spinach were observed with pressmud + EM application vs. corresponding pressmud without EM application. It was followed by FYM, compost, poultry manure and NPK in the presence of EM. The lowest values were shown by the control.

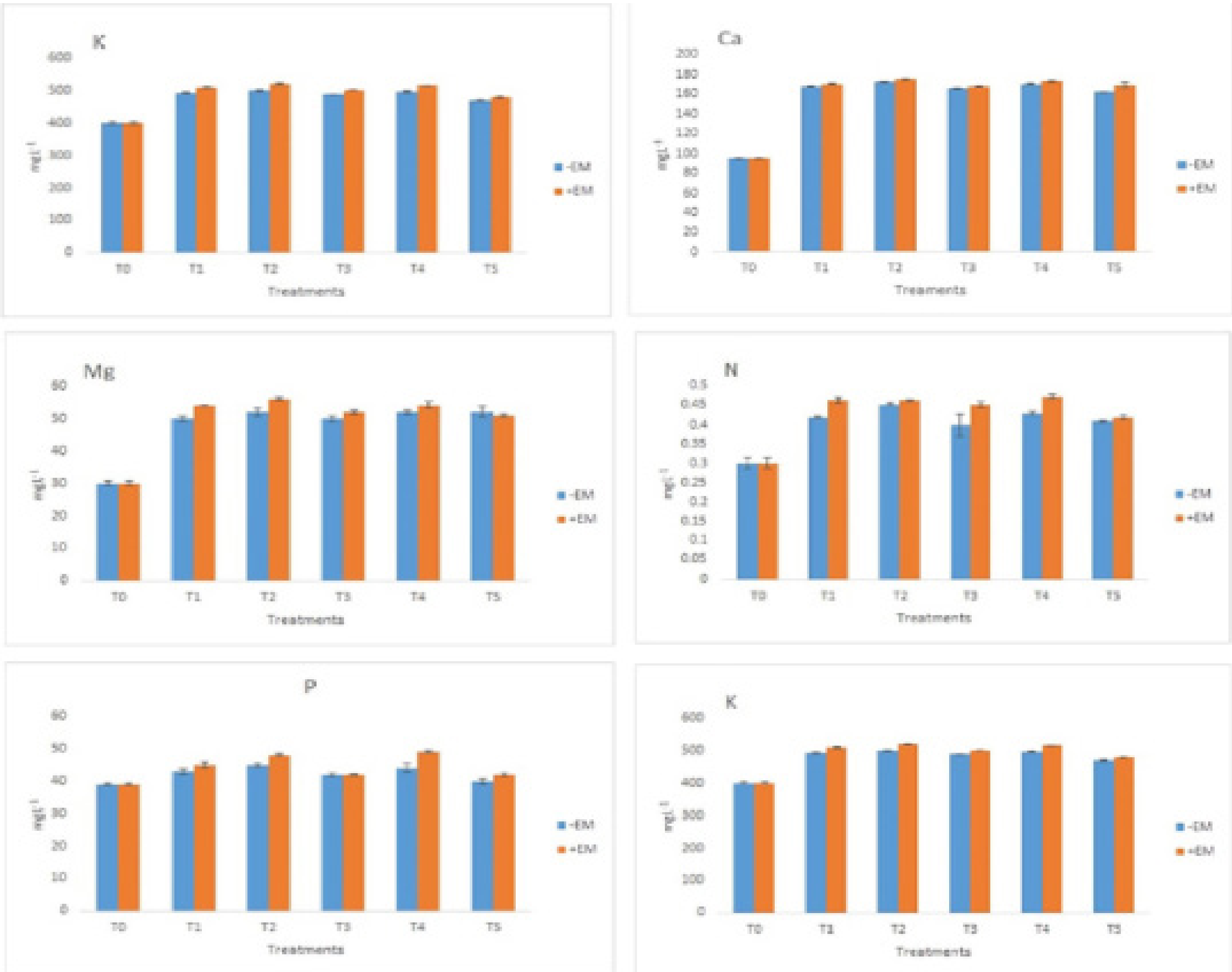

Figure 2: Effect of EM and different soil fertilizers on macronutrients (Na, Ca, Mg, N, P and K) of spinach
T_0 = Control, T_1 = FYM, T_2 = Pressmud, T_3 = Compost, T_4 = Poultry manure and T_5 = NPK, **W. EM** *= without effective microorganisms,* **EM** *= with effective microorganisms (Organic manures socked with Bio-Aab)*

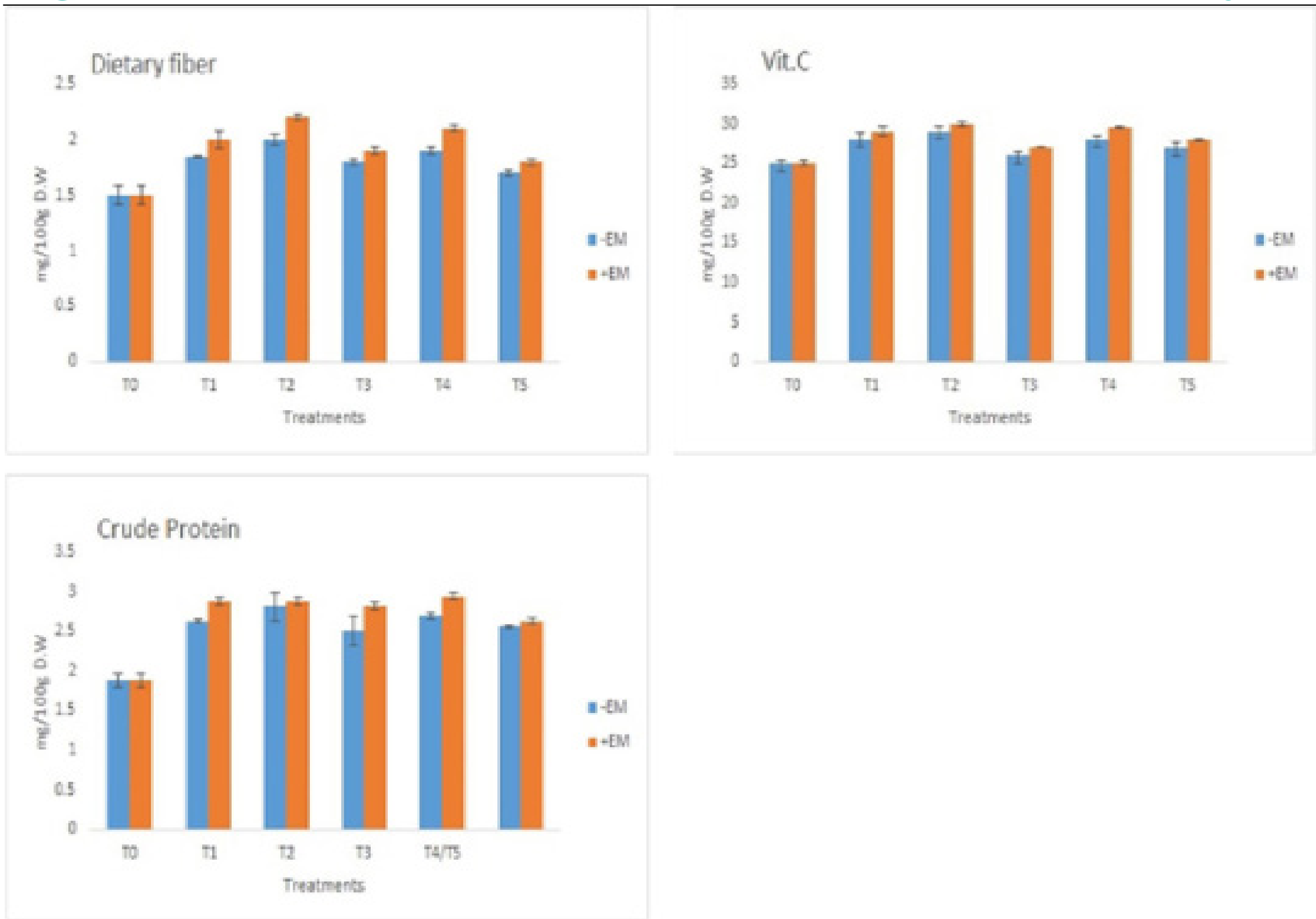

Figure 3: Dietary fiber, vitamin C and crude protein as affected by different soil fertilizers co-applied with EM
T_0 *= Control,* **T_1** *= FYM,* **T_2** *= Pressmud,* **T_3** *= Compost,* **T_4** *= Poultry manure and* **T_5** *= NPK,* **W. EM** *= without effective microorganisms,* **EM** *= with effective microorganisms (Organic manures socked with Bio-Aab)*

Our results are supported by findings of Namasivayam and Kirithiga (2010). The better results with EM + pressmud can be attributed to the fact that pressmud contains approximately 80% moisture and 0.9-1.5% sugars, organic matter, N, P, K, S etc. Both, sugars and moisture increases population and activities of effective microorganisms, leading to rapid nutrient release and subsequent uptake by spinach.

Dietary fiber, vitamin C and crude protein: Significant ($P<0.05$) increase in the dietary fiber (10 %), vitamin C (3.4%) and crude protein (2.5%) in spinach was observed in the pressmud + EM group compared to the control treatment and remaining treatments (Figure 3). Data further showed least effects of EM in combination with NPK. All the organic fertilizers in general and specially the pressmud increased the vitamin C content in spinach compared to inorganic fertilizers. This may be attributed to the high level of K and carbohydrates in organic fertilizers which favored the synthesis of ascorbic acid in spinach. Since vitamin C production is tightly linked to carbohydrate metabolism, high level of nitrogen in inorganic fertilizer increase the protein production while decrease the carbohydrate formation therefore vitamin C levels were lower in inorganic fertilizer (Qureshi et al., 2014).

Effect of EM-inoculated organic wastes and chemical fertilizers on soil properties: The effect of co-application of EM with chemical or organic fertilizers on soil fertility is shown in Figure 4. Post-harvest soil analyses showed an increase in the ECe values and a decline in pH. The highest increase in ECe value (22.4%) was noted with pressmud + EM compared to its corresponding none-EM treatment, while the highest decline (12%) in pH was observed with pressmud +EM application compared to treatments without EM. Decline in soil pH indicates production of organic acids (humic acid, amino acid, glycine and cysteine) due to mineralization (amminiza

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Figure 4: *Effect of EM and different soil organic fertilizers on electrical conductivity, pH, organic matter, saturation%, nitrogen, phosphorous and potassium in soil after spinach harvesting*
T_0 *= Control,* **T_1** *= FYM,* **T_2** *= Pressmud,* **T_3** *= Compost,* **T_4** *= Poultry manure and* **T_5** *= NPK,* **W. EM** *= without effective microorganisms,* **EM** *= with effective microorganisms (Organic manures socked with Bio-Aab)*

tion and ammonification) of organic materials by heterotrophs and nitrification by autotrophs (Sarwar et al., 2009). The ECe increased after the application of organic manure and EM. During the decomposition of organic process, microorganisms produce organic acids which react with soluble salts present in the soil solution which then lead to increased soil EC (Sarwar et al., 2008).

Organic and inorganic fertilizer applied with EM further showed improvement in soil fertility as evident from increased soil OM and saturation percentage. High OM content in soil (1.25 %) was recorded with pressmud + EM. The saturation percentage of 35% was observed with pressmud + EM compared to non-EM treatments. The improvement in key soil properties, were in the order of pressmud + EM (T_2) > poultry manure + EM (T_4) > compost + EM (T_3) > NPK (T_5) > control (T_1).

EM in combination with organic wastes and chemical

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fertilizers also increased the concentrations of N, P and K in soil. Results showed that the highest increase for N (48.5%), P (60%) and K (90%) was observed where compost + EM, poultry manure + EM and pressmud + EM were applied compared to none-EM treatments. This increasing trend may be attributed to the enhanced microbial activities by EM which led to nutrients release and availability to plants. Basically, EM enhances the rapid mineralization of organic components thus releasing plant nutrients. These results are supported by findings of Namasivayam and Kirithiga (2010) who also observed improvement in soil fertility after EM application.

Conclusion

The findings of our study suggest that EM co-applied with pressmud, has good potential to increase spinach yield and quality as well as improve soil fertility and thus warrants further testing in field conditions under agro-ecosystems of Pakistan.

Author's Contribution

This manuscript is the part of corresponding author's Ph.D dissertation. Mumtaz Khan contributed in the analysis of spinach leaves for micro and macro-nutrients by plasma mass spectrometry (ICP-MS). The research work was carried out under the supervision of Dr. Muhammad Jamil Khan. Salim Jilani provided the idea of the research. Zarina Bibi assisted in overall lab work. Muhammad Munir helped in the determination of dietary fiber and Vitamin C and Mehwish Kiran contributed in manuscript writing.

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