INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0070/2018/20–11–2401–2407
DOI: 10.17957/IJAB/15.0770
http://www.fspublishers.org

Full Length Article

Alginate-Entrapped *Enterobacter* spp. MN17 Coated Diammonium Phosphate Improves Growth, Yield and Phosphorus Use Efficiency of Wheat

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Abstract

One of the successful, effective and safe methods to enhance phosphorus use efficiency (PUE) is coating of diammonium phosphate (DAP) with alginate-entrapped bacteria (*Enterobacter* spp. MN17) which not only release microbes and phosphorus (P) slowly in soil but also protect microbes from soil stresses and P from fixation in soil. Present study was conducted with key aspects of alginate as endophytic microbes carrier, potential to maintain cell viability on DAP surface, effect on P release pattern and nutrients uptakes of wheat. MN17 survival rate was well documented in prototype solution {(alginate 1.5%) + (1% glucose + 1% glycerol)} and DAP surface. Phosphorus (419, 656, 978 and 1081 mg kg^{-1} soil) and microbe (92, 120, 118 and 110×10^6 CFU g^{-1} soil) release pattern was observed at 15, 30, 45 and 60 days (d)soil sampling from alginate-entrapped MN17 coated DAP. An enhancement of plant height (36–48%), chlorophyll contents (30–41%), grains yield (31–70%), P agronomic efficiency (57–107%), grains P concentration (39–45%) as well as micronutrients concentration in grains was recorded in treatment receiving alginate-entrapped MN17 coated DAP at recommended rate over uncoated DAP. Our study first time explores the multiples benefit of alginate entrapped endophytic bacteria coated DAP in effective microbes delivery at target site, nutrient management and quality improvement of wheat crop.

Keywords: Alginate; Microbial carrier; Slow release; Phosphorus availability; Wheat

Introduction

Phosphorus (P) plays multiple facets role in plant biometric process for proper growth and development but low availability of applied P in alkaline/calcareous soil due to fixation with reactive calcium is major drawback to obtain optimum yield (Yaseen *et al*., 2017). Without inorganic P application in agricultural crops, it is impossible to get target yield in order to meet required food demand of increasing population (Smith and Schindler, 2009). This low uptake of applied P is compelling plant scientist to tackle this problem at utmost priorities in research due to limited P fertilizers parent material. The possible ways to increase cereal crops production is the sowing of genetically modified varieties or to increase applied fertilizer efficiency. Among the strategies by which P fertilizers can be used more effectively include: improving agronomic practices, use of P solubilizing microbes and coating of fertilizer granules with polymer to enhance efficiency of applied fertilizers (Trenkel, 2012; Naveed *et al*., 2017; Yaseen *et al*., 2017). Currently, polymer coated P fertilizer application attained maximum attention to reduce P fixation and enhance P recovery efficiency by its unique features like high cation and water holding property (Dubey *et al*., 2013; Yaseen *et al*., 2017). On the other hand, microbial intervention particularly plant growth promoting endophyte (PGPE) in agriculture showed promising results to increase crop production and nutrients uptake (macro and micronutrients) by various plant-soil-microbes interactions including nutrients solubilization, disease control and hormones production (Naveed *et al*., 2017). However, microbial viability is enhanced by immobilizing into polymer with advantages such as protection from soil biotic and abiotic tresses, slow release and reduce nutrient stresses over direct inoculation by peat (Schoebitz *et al*., 2013). Previously information is available on sole application of polymer coated P fertilizer and microbes to improve phosphorus use efficiency (Naveed *et al*., 2014; Yaseen *et al*., 2017). Integration of polymer coating fertilizers and microbial interventions in agriculture is one of the significant approaches for sustainable nutrients management. This is first experiment to provide novel/new approach for inoculation of crop by coating DAP fertilizer granules by alginate entrapped endophyte strain MN17 and also investigate its effect on wheat production and nutrients uptakes either pot and field conditions. This novel

To cite this paper: Aziz, M.Z., M. Yaseen, M. Naveed and M. Shahid, 2018. Alginate-entrapped *Enterobacter* spp. MN17 coated diammonium phosphate improves growth, yield and phosphorus use efficiency of wheat. *Int. J. Agric. Biol*., 20: 2401–2407

technique, slow releases the P and microbes according to crop requirement that reduce P fixation with calcium and microbes from stresses in soil. The development of new fertilizer feature might assist not only increase the PUE and microbial survival at target site but also overcome the disinclination of farmer's community about biofertilizers due to additional labor of separate application and failure under natural soil environment.

Materials and Methods

Laboratory Experiments Description

Bacterial survival in polymer matrix and on coated surface DAP: Inoculum of the selected strain (*Enterobacter* spp. MN17) as reported for growth promotion in cereal crops P solubilization, and hormones production (Naveed, 2013; Naveed *et al*., 2014; Yaseen *et al*., 2018) was prepared in LB broth for inoculation by method described Naveed *et al*. (2014). Different concentration of polymer *i.e.*, alginate (0.5, 1 and 1.5% W/V) along with microbe culture was prepared by mixing polymer (alginate) in to inoculum. Microbial survival rate was recorded over time (one month) by addition 100 μL prototype solution (alginate + microbes) in 900 μL saline buffer solution (0.9% NaCl) and made serial dilution which was spreaded on amended LB plates to get microbe (MN17) colonies (Compant *et al*., 2005). Selected best alginate concentration 1.5% along with microbe prototype solution was amended with 1% glucose (G) and 1% glycerol (Gly) separately and both together and microbial survival rate was recorded by method described above. The prototype solution {(1.5% alginate + (1% G + 1% Gly) + MN17)} was prepared and coated on DAP granules by method described as Yaseen *et al*. (2017). Microbial survival on coated granules of DAP was measured at different time interval stored at different temperature (10, 25 and 40ºC) by dissolving the coated DAP (1 g) in saline buffer solution (0.9% NaCl) and serial dilution was made and spread on selected LB plates placed in incubator for 72 h at 28°C and data was expressed colony forming units per milliliter (CFU mL^{-1}).

Determination of Phosphorus and Microbe Release Pattern from Coated DAP

Experimental soil was collected from research area Institute of Soil and environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF) allotted for field experiment. Collected soil was analyzed for physico-chemical characteristics by US Salinity Lab. (1954) methods *i.e*., ECe (1.20), soil pH (7.6), organic matter content (0.71%), field capacity moisture contents (18%), soil textural class by hydrometer method (sandy loam), total nitrogen (0.044%), available phosphorus (6.60 mg kg^{-1} soil) by Olsen *et al*. (1954) and potassium content (135 mg kg^{-1} soil).Coated DAP fertilizer with alginate (1.5%) along with {(1% G + 1% Gly) + MN17)} was placed in cups containing 200 g soil at rate 1 100^{-1} g soil along with alone polymer (alginate) coated and uncoated DAP as control to elucidate the P release pattern in soil at field capacity (FC) moisture levels. Cups were placed in incubator (Sanyo; MIR 253) under constant temperature 25 ± 2°C. Moisture level was maintained after every 24 h using deionized water. Phosphorus and microbes in soil was determined after 15, 30, 45 and 60 d interval following the method Olsen *et al*. (1954), Naveed *et al*. (2014), respectively.

Pot Experiment Description

Pot experiment was conducted at wire house, ISES, UAF, Pakistan. Experimental soil physico-chemical characteristics were similar as described above in laboratory experiments. Six treatments consisted of different rate of alginate {(1.5% alginate + (1% G + 1% Gly) + MN17)} entrapped MN17 coated DAP at recommended rate and reduce rate 75 and 50% of recommended rate was compared with alone alginate coated, uncoated and control treatments with three replications. For wheat, recommended rate of nitrogen, phosphorus and potassium fertilizers 120-90-60 kg ha^{-1} as urea, DAP (P_2O_5) and sulphate of potash (SOP=K_2O), respectively was applied. All phosphorus and potassium containing fertilizers were applied at sowing time along with $1/3^{rd}$ nitrogen while remaining nitrogen was applied in two split at 30 and 45 d of germination. Six seeds of wheat variety Faisalabad-2008 were sown in each pot having 10 kg soil, four plants maintained till maturity. Gaseous exchange measurement (photosynthetic rate) and chlorophyll contents were measured by CIRAS-3 and chlorophyll meter as method described by Noor *et al*. (2017). Growth and yield attributes were taken before harvesting the plants from each pot. Grains and straw samples were collected for nutrients analysis. Each treatment treated equally regarding irrigation and cultural practices. Moreover, P agronomic efficiency was calculated as

$$\text{Phosphorus agronomic efficiency} = \frac{\text{YP applied} - \text{Y control}}{\text{P applied}}$$

Note: YP= Grain yield receiving P fertilizer, Y=Grain yield without any fertilizer (control)

Field Experiment Description

Field experiment was conducted at research area, ISES, UAF, Pakistan. Similar soil characteristic, treatments plan, wheat variety and fertilizer rates were used as described in above mentioned experiments. Seeds of wheat variety Faisalabad-2008 were sown at the rate of 100 kg ha^{-1} through manual drill in each experimental unit. Cultural and irrigation practices were kept same for all the treatments. Crop was irrigated with canal water five times during growing period. Wheat growth and yield parameters were collected at different growth stages. Total productivity was

calculated by multiplying the m^{-2} production. Grains and straw samples were collected for nutrients analysis.

Analytical Methods

Grains and straw samples were digested as per method described by Wolf (1982). Nitrogen, phosphorus and potassium were determined following the method of Chapman and Pratt (1961). However, micronutrients *i.e*., Zn, Fe, Cu and Mn were determined by atomic absorption spectrophotometer in digested sample.

Statistical Analysis

Collected data was statistically analyzed by Steel *et al.* (1997) and treatment means were ranked by using Tukey honestly significant difference (HSD).

Results

Laboratory Experiments

Determination of MN17 spp. survival in alginate prototype solution and on fertilizer surface: As explored the results of MN17 viability in alginate prototype solution of different concentrations *i.e.,* 0.5, 1.00 and 1.50% was analyzed during storage at room temperature. In all concentrations microbial survive was well but alginate concentration 1.5% exhibited better survival as compared to other concentrations. Linear increase of population densities in all alginate concentrations matrixes between the first to 15 d was recorded of strain MN17 (Fig. 1A), at 30 d of experiment, 1.5% alginate concentration supported maximum viability of MN17 (108×10^6 CFU mL^{-1}), followed by 1% alginate concentration (96×10^6 CFU mL^{-1}) and 0.5% alginate concentration (83×10^6 CFU mL^{-1}) prototype solution. This experiment was conducted in the light of results of above experiment (Fig. 1B). Survival of MN17 was analyzed during storage at room temperature in screened concentrations of alginate (1.5%), after preparing metrics different concentration of organic carbon sources *i.e.*, 1% glucose (G), 1% glycerol (Gly) and combination of glucose plus glycerol (1% G + 1% Gly). The maximum bacterial number was observed in formulation {(1.5% alginate + (1% G + 1% Gly) +MN17)} by 215×10^{10} CFU mL^{-1} at 15 d but 241×10^{10} CFU mL^{-1} population was recorded at 30 d, where slightly increase in population was recorded. However, 1.5% concentration of alginate prototype solution amended with 1% glucose was following treatment having number of cell 173×10^{10} CFU mL^{-1} at 30 d of incubation. So, minimum microbial survival rate was recorded in treatment {(1.5% alginate + 1%Gly + MN17)} up to 149×10^{10} CFU mL^{-1} at 30 d of experiment (Fig. 1B). This experiment was conducted to test the shelf life/storage period for microbe's survival on coated DAP surface. Data regarding microbial viability at different temperatures showed significant effect of storage temperature on microbial survival after alginate-base formulation {(1.5% alginate + (1% G + 1% Gly) + MN17)} coated on DAP throughout all the incubation periods 1, 15, 30, 60 and 90 d (Fig. 1C). The maximum microbes were recovered on alginate (1.5% alginate + (1% G + 1% Gly) + MN17 or PsJN) coated DAP surface after 1day at 10, 25, 40°C *i.e.*, $52 \pm 2.98 \times 10^7$ and $55 \pm 4.78 \times 10^7$, $71 \pm 4.01 \times 10^7$ CFU g^{-1}fertilizer of MN17, respectively. However, exponential increase in population was recorded at 30 d in sample stored at 10 and 25°C and vice versa of sample stored at 40°C. At 60 d incubation, microbial population recovered from alginate coated DAP at 25 and 40°C was decreased than 10°C stored samples, which showed MN17 $70 \pm 2.36 \times 10^7$ CFU g^{-1} fertilizers, respectively. At 10°C temperature, sustained population was observed at 90 d however, remaining stored temperatures, sample showed further decline in population (Fig. 1C).

Phosphorus and Microbes Release Pattern in Soil from Coated DAP Fertilizer

This experiment predicted P release pattern from uncoated and coated DAP fertilizer. Maximum Olsen P release upsurge attained 915 mg kg^{-1} soil at 15 d from uncoated DAP fertilizer applications. But the increase in time resulted in decreased in P availability (Olsen P). Results showed (Fig. 2) that 656, 444 and 240 mg kg^{-1} Olsen P was recovered from soil at 30, 45, 60 dafter P application, respectively from uncoated DAP. Initially P release from alone alginate coated DAP was less as compared to uncoated DAP fertilizer and by increasing time in days the Olsen P availability increase was recorded that was 319, 527, 850 and 917 mg kg^{-1} soil at 15, 30, 45 and 60 d of incubation, respectively (Fig. 2). Olsen P release from alginate formulation coated DAP results indicated that the 411, 656, 978 and 1081 mg kg^{-1} soil Olsen P was recovered after 15, 30, 45 and 60 d respectively (Fig. 2). This experiment results revealed that linear increase in cell number was observed up to two month after application of alginate entrapped MN17 coated DAP. Controlled release pattern was recoded as like controlled release fertilizer (Fig. 1D). The maximum microbe was recovered from soil after 30 and 45 d of incubation from alginate entrapped microbe coated DAP application in soil placed in incubator at 25 ± 2°C *i.e.*, 120×10^6 CFUg^{-1} soil followed by 45 d that was 118×10^6 CFUg^{-1} soil then slightly decline trend was recorded at 60 d (Fig. 1D).

Pot Experiment

Effect of alginate-entrapped MN17 coated DAPS on wheat growth and yield attributes: Alginate-entrapped bacteria coated DAP fertilizer application showed more heartening outcomes regarding growth, yield and nutrients uptakes than traditional fertilizer under wire house trial (Table 1).

It is obvious from results that highest enhancement in

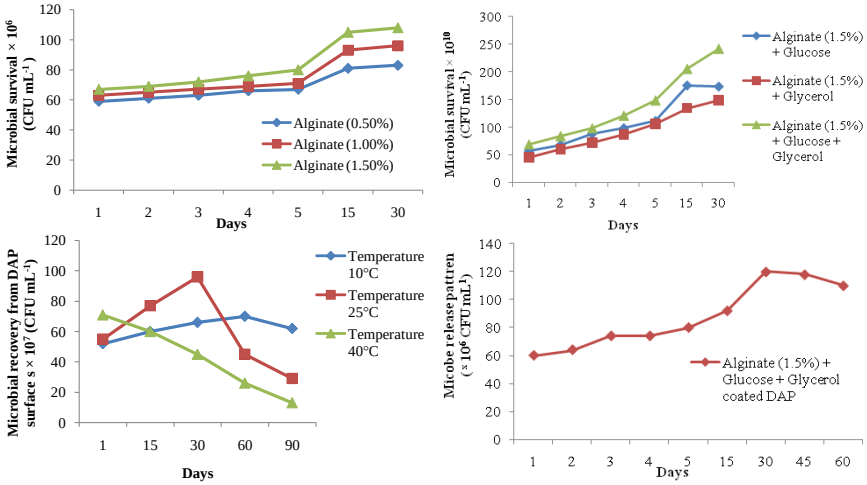
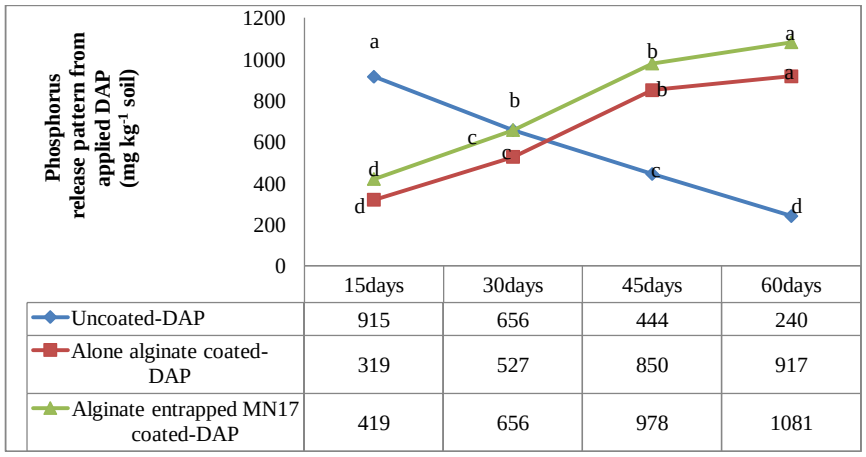


Fig. 1: (A) Viability of MN17 in different alginate concentrations formulations (B) Viability of MN17 in screened alginate concentration with carbon sources formulations (C) Viability of MN17 on coated DAP surface and (D) MN17release pattern from coated DAP in soil



	15days	30days	45days	60days
Uncoated-DAP	915	656	444	240
Alone alginate coated-DAP	319	527	850	917
Alginate entrapped MN17 coated-DAP	419	656	978	1081

Fig. 2: Phosphorus release pattern in soil from applied DAP fertilizer under controlled conditions
Note: Values followed by the same letters were not significantly different at the 5% level of significance

plant height 36.36% was recorded in the treatment alginate-entrapped MN17 coated DAP at recommended rate compared to uncoated DAP treatment (Table 1). Number of fertile tillers pot^{-1} was increased by the application of alginate entrapped MN17 coated DAP fertilizer. Alginate entrapped MN17 coated DAP at recommended rate increased 50% number of fertile tiller as compared to uncoated DAP fertilizer treatment (Table 1). Additionally, variation in chlorophyll contents and photosynthetic rate was evident from results shown (Table 3) by the application of alginate-entrapped MN17 coated DAP. Increase in chlorophyll contents and photosynthetic rate was recorded up to 41 and 47% in alginate-entrapped MN17 coated DAP at recommended rate, respectively over uncoated DAP fertilizer treatment (Table 1). Effect of different rate of alginate-entrapped MN17 coated DAP fertilizer on grains and straw yield as well as P agronomic efficiency (PAE) was though differential until now and it was conspicuous in all alginate-entrapped MN17 coated treatments compared to uncoated DAP treatment (Table 1). Results revealed that 100% recommended rate of alginate coated DAP appreciably increased grains yield *i.e.*, 71%, respectively as compared to uncoated DAP (Table 1). Furthermore, up to 45% increase in grain yield was recorded in treatment where recommended alginate-entrapped MN17 was applied as compared to sole alginate coated DAP treatment. Straw yield was increased by alginate entrapped MN17 coated DAP treatment receiving recommended rate by 39 and 18% over uncoated and alginate coated DAP treatments, respectively (Table 1). P use efficiency was estimated in term of P agronomic efficiency. Significant difference was noticed among the different treatments regarding the PAE. Results revealed that maximum PAE was recorded in treatment receiving reduce rate of alginate-entrapped MN17

Table 1: Effect of alginate-entrapped bacteria coated DAP on wheat growth and yield contributing parameters under pot conditions

Treatments	Plant height (cm)	Number of fertile tillers pot^{-1}	Chlorophyll contents (SPAD)	Photosynthetic rate (μmol m^{-2} s^{-1})	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	P agronomic efficiency (g grain g^{-1} DAP)
T_1 (control)	56e	8.0d	36e	10e	4e	9e	---------
T_2	66d	12c	44d	15d	17d	28d	27e
T_3	71c	14bc	48c	16cd	20c	33c	36d
T_4	90a	18a	62a	25a	29a	39a	56c
T_5	80b	16ab	55b	22b	26b	36b	66b
T_6	73c	11cd	46cd	17c	21c	30d	76a
HSD	**2.74**	**3.55**	**2.70**	**1.46**	**2.50**	**2.23**	**1.58**

Note: All treatments except control contain recommended doses of N and K (K_2O) as urea and SOP. Values followed by the same letters were not significantly different at the 5% level of significance. T_1= Control, T_2= Uncoated, T_3=Alginate coated, T_4=Alginate-entrapped bacteria coated DAP (100% recommended rate), T_5=Alginate-entrapped bacteria coated DAP (75% recommended rate), T_6=Alginate-entrapped bacteria coated DAP (50% recommended rate)

Table 2: Effect of alginate-entrapped bacteria coated DAP on wheat nutrient concentration under pot conditions

Treatments	N concentration (%)		P concentration		K concentration		Grains Zn	Grains Fe	Grains Cu	Grains Mn
							Concentration (mg kg^{-1})			
	Grains	Straw	Grains	Straw	Grains	Straw				
T_1 (control)	1.4f	1.1f	0.11e	0.09d	0.86f	1.12f	29f	24f	10f	12f
T_2	1.8e	1.54de	0.28d	0.20c	1.06e	1.41e	42e	37e	16e	18e
T_3	1.97c	1.69c	0.32bc	0.21c	1.21c	1.66c	45d	44cd	24c	28c
T_4	2.6a	2.12a	0.39a	0.26a	1.6a	2.12a	64a	54a	37a	44a
T_5	2.3b	1.86b	0.33b	0.25a	1.37b	1.9b	52b	49b	31b	33b
T_6	1.95cd	1.55d	0.29cd	0.23b	1.18cd	1.65cd	48c	45c	22cd	23d
HSD	**0.12**	**0.13**	**0.039**	**0.011**	**0.10**	**0.11**	**2.33**	**3.0**	**2.3**	**4.2**

Note: All treatments except control contain recommended doses of N and K (K_2O) as urea and SOP. Values followed by the same letters were not significantly different at the 5% level of significance. T_1= Control, T_2= Uncoated, T_3=Alginate coated, T_4=Alginate-entrapped bacteria coated DAP (100% recommended rate), T_5=Alginate-entrapped bacteria coated DAP (75% recommended rate), T_6=Alginate-entrapped bacteria coated DAP (50% recommended rate)

Table 3: Wheat response to applied alginate-entrapped MN17 coated DAP under field conditions

Treatments	Plant height (cm)	Number of fertile tillers (m^{-2})	Chlorophyll contents (SPAD)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	P agronomic efficiency (kg grain kg^{-1} DAP)	Grain P	Straw P	Grains micronutrients concentration (mg kg^{-1})	
							(%)		Zn	Fe
T_1 (control)	63e	179e	31c	2100d	3300d	———	0.15f	0.10f	25f	24f
T_2	76d	312d	43b	4400c	6424c	26e	0.22e	0.14e	40e	38e
T_3	81cd	355c	46b	4900b	6958b	31d	0.27c	0.16c	44cd	45c
T_4	113a	456a	56a	5800a	7714a	41c	0.32a	0.22a	60a	52a
T_5	89b	410b	51b	5200b	7124b	46b	0.29b	0.19b	50b	47b
T_6	833	320d	44b	4500c	6400c	53a	0.24d	0.15d	45c	42d
HSD	**6.39**	**31**	**4.38**	**331**	**439**	**2.52**	**0.014**	**0.02**	**2.05**	**2.78**

Note: All treatments except control contain recommended doses of N and K (K_2O) as urea and SOP. Values followed by the same letters were not significantly different at the 5% level of significance. T_1= Control, T_2= Uncoated, T_3=Alginate coated, T_4=Alginate-entrapped bacteria coated DAP (100% recommended rate), T_5=Alginate-entrapped bacteria coated DAP (75% recommended rate), T_6=Alginate-entrapped bacteria coated DAP (50% recommended rate)

coated DAP (50% of recommended rate) as compared to increasing rate receiving treatments (75 and 100% of recommended rate). Up to 181, 144 and 107% increase in PAE was recorded in treatments receiving 50, 75 and 100% recommended rate of alginate-entrapped MN17 coated DAP over uncoated DAP, respectively.

Effect of Alginate-entrapped MN17 Coated DAP on Wheat Produces Nutrients Concentration

Data given in Table 2 provide evidence that alginate-entrapped MN17 coated DAP was effective to improve the nutrients concentration in grain and straw of wheat. Correspondingly, enhancement in nitrogen concentration in grain and straw was improved by 44 and 37%, respectively in treatment alginate-entrapped MN17 coated DAP at recommended rate as compared to uncoated DAP (Table 2). As compared to recommend rate of uncoated DAP, maximum increase in grains and straw P concentration *i.e.*, 14 and 5% was recorded in treatment where sole alginate coated DAP fertilizer at recommended rate was applied, respectively (Table 2). P concentration in grain and straw was further increased by inclusion of microbes in alginate where 39 and 30% enhancement in P concentration in grain

and straw was recorded in alginate-entrapped MN17 coated DAP at recommended rate, respectively as compared to uncoated DAP. Furthermore, grain and straw potassium (K) concentration was also considerably improved by application of alginate-entrapped MN17 coated DAP fertilizer treatment. Alginate-entrapped MN17 coated DAP at recommended rate caused 51 and 50% upsurges in grains and straw K, respectively compared to uncoated DAP (Table 2). Application of alginate-entrapped bacteria coated DAP extraordinarily effected the absorption of micronutrient Zn, Fe, Cu and Mn concentration in grain of wheat (Table 2). Application of alginate-entrapped N17 coated DAP improve Zn concentration in wheat grain and estimated enhancement was recorded up to 52 and 42% over sole alginate coated and uncoated DAP treatment, respectively (Table 2). The results given in (Table 2) showed maximum increase in grain micronutrient Fe concentration was observed in alginate-entrapped bacteria coated DAP at recommended rate and this treatment effect caused 46% increase in grains Fe concentration, respectively over uncoated DAP. Furthermore, maximum increase in Cu (54%) and Mn (57%) concentrations in the wheat grain was observed in alginate entrapped bacteria (MN17) coated DAP as compared to sole alginate coated DAP (Table 2).

Field Experiment

Wheat response to applied alginate entrapped MN17 coated DAP fertilizer: Filed results showed that yield and nutrients uptakes was also increased with the application of alginate-entrapped MN17 coated DAP. Increase in plant height by application of alginate entrapped MN17 coated DAP at recommended rate was recorded, and it was 49% more than that of uncoated DAP treatment (Table 3). Enhancement in the number of fertile tillers has been recorded with the application of alginate-entrapped bacteria coated DAP (Table 3). A considerable enhancement in fertile tillers was observed with the application of 100% recommended rate of alginate-entrapped MN17 coated DAP compared to uncoated DAP whereas maximum 46% was recorded over respective uncoated treatment (Table 3). Alginate-entrapped MN17 coated DAP at 100% recommended rate showed increase in the chlorophyll contents of the wheat up to 30%compared to uncoated treatment (Table 3). Grains and straw yield altered due to alginate-entrapped MN17 coated DAP application, where 31 and 20% enhance was recorded due to 100% recommended rate of alginate-entrapped bacteria coated DAP application respectively over uncoated DAP (Table 3). A positive correlation between P agronomic efficiency and alginate entrapped MN17 coated DAP was recorded where 103% increase in PAE was estimated in treatment alginate entrapped MN17 coated DAP at half of recommended rate (Table 3). However, significant upsurge in in grains and Straw P attained *i.e*., 45 and 53%, respectively in treatment receiving recommended alginate entrapped MN17 coated DAP over uncoated DAP (Table 3). Increase in micronutrient absorption was also accelerated by application of alginate entrapped MN17 coated DAP. Maximum increase in Zn and Fe concentration in grains over uncoated DAP *i.e*., 50 and 37% was recorded, respectively in treatment receiving alginate entrapped MN17 coated DAP.

Discussion

This study first time explores the development of new formulation for the plant growth promoting endophyte *Enterobacter* spp. MN17 with a focus on long-term conservation of cell viability and modified fertilizer feature. Our methodology returned a high number of viable cells in alginate, which is an important prerequisite for successful application of product. In first laboratory study, microbe's population density decline/increase of inoculant was taken as a parameter to check the capacity of the carrier to support MN17 survival. In 1.5% alginate concentration prototype solution population increased up to 108×10^6 $CFUmL^{-1}$as compared to other two concentrations *i.e*., 0.5 and 1%, respectively (Fig. 1). Further, selected 1.5% alginate matrix amendment with carbon source (1% G + 1% Gly) finally led to cells differences of MN17over either amendment of glucose and glycerol in alginate matrix. The microbe growth was promoted in this preparation because alginate has large surface area and its microporous structure offers the possibility to inoculants (Schoebitz *et al*., 2013) with better microenvironment including protection against stresses and nutrition to microbes. Furthermore, addition carbon source showed highly viability of microbes due to modification of carrier linkages for better environment and easy source of nutrition (Cassidy *et al*., 1996; Schoebitz *et al*., 2013; Bashan *et al*., 2014). In addition, alginate entrapped microbes coated on DAP survival was investigated concerning its protecting effect at different temperature. Our results showed that enriched alginate as a protectant of microbes on surface of DAP is very efficient due to better provision of environment by carrier (Schoebitz *et al*., 2013; Bashan *et al*., 2014).

In the present study, the controlled-release P concept was also tested uncoated, alone alginate coated and alginate-formulation coated DAP fertilizer. After 15 days, Olsen P release was decreased in soil from uncoated DAP but increasing trend in Olsen P release was recorded in coated DAP treatments up to 60 days. This coating of alginate layer on DAP caused consistent release of P through diffusion that made P available for plant uptake for prolong time than that of uncoated DAP and inclusion of microbes in polymer for coating DAP further increased the available P due to the microbial activity (Trenkel, 2012; Naveed *et al*., 2014). The preceding results showed that controlled release of bacterial cells from alginate entrapped MN17 coated DAP in soil (Fig. 2D). The microbe-release pattern was related as controlled release nutrients, but sum and rate of release were diverse. After application in to soil alginate cross linkages become weak and breakable by attack of indigenous soil

microbes and hydrolytic enzymes produced by entrapped microbes which cause linear increase in the cell concentration in to the soil by slow and gradual the release. These microbes not only improved the crop yield by different mechanisms but also solubilized the fixed P by producing various organic acids and other activities (Schoebitz *et al*., 2013; Naveed *et al*., 2017).

Alginate entrapped bacteria (MN17) coated DAP application improved wheat growth and yield and nutrients contents which resulted controlled release of microbe and fertilizer according to crop need (Table 1, 2 and 3). Increase in plant root length is commonly reported in response to PGPB inoculation and coated P fertilizer application in several plant species that enhances efficient absorption of nutrients which results growth promotion of wheat crop (Trenkel, 2012; Naveed *et al*., 2017). In this experiment, grain and straw yield increased substantially in wheat which could be attributed to the production of plant growth substances produced by root colonizing bacteria in controlled release manner and availability of P fertilizer (Yaseen *et al*., 2017, 2018). Moreover, microbes release the carbonic anhydrase which helps plants to fix/assimilate higher carbon dioxide (Salantur *et al*., 2006; Naveed *et al*., 2017; Yaseen *et al*., 2018). The increase of nutrients contents in grain, straw and P use efficiency was also recorded compared to control. This promotion in nutrients contents and use efficiency might be due to an upsurge in bioavailable P fraction and reduce applied P fixation in soil (Yaseen *et al*., 2017, 18).The polymer coating increase available P through its cation exchange capacity, increase diffusion shell (Trenkel, 2012; Yaseen *et al*., 2017) and microbes including chelation, acidification and exchange reactions in rhizosphere (Naveed *et al*., 2014). Moreover, micronutrients concentration was also increased in grain that might be attributed by microbes by producing the siderophores and proton production. These metabolites enhance the available micronutrients for plant uptakes (Naveed *et al*., 2017; Yaseen *et al*., 2018). These mechanisms increase available P as well as other nutrients contents in the rhizosphere which promoted the growth, nutrients uptake and use efficiency.

Conclusion

Alginate proved good carrier for microbes and alginate-entrapped microbes coating on diammonium phosphate (DAP) plays multi-facet role like increase in available phosphorus, grain yield and nutrients uptake. Furthermore, outcomes of this study are enhanced phosphorus use efficiency and microbes in rhizosphere through controlled release. In short, polymer coated DAP fertilizer is more convenient due to its high efficiency with less application. This technology therefore not only has prospective to increase crop growth, yield and farmer income but also has encouraging consequences on probable environmental footmark of fertilizer usage.

Acknowledgements

The authors appreciatively admit to the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan, for providing facilities to conduct this research work

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(Received 16 January 2018; Accepted 30 May 2018)