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Efficacy of formulated carriers inoculated with plant growth promoting rhizobacteria on maize growth

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Abstract

Unavailability of a suitable carrier seriously hampered the mass production of bio-inoculant in developing countries including Pakistan. The present study was designed to evaluate the quality of formulated carriers for bio-inoculant on maize. Complete randomized design was applied with six treatments in triplicates. Formulated carriers; FC-1 (40% clay soil + 35% fly-ash + 15% press-mud + 10% lignitic coal), FC-2 (40% clay soil + 40% fly-ash + 10% press-mud + 10% lignitic coal), FC-3 (40% clay soil + 35% fly-ash + 10% press-mud + 15% lignitic coal), FC-4 (40% clay soil + 30% fly-ash + 15% press-mud + 15% lignitic coal) and BC (biozote carrier) were inoculated with broth culture of pre-isolated PGPR strains (MR8 & MR5). Un-inoculated seeds were used as control. Results revealed that all the inoculated carriers showed a significant increase over un-inoculated control related to different growth parameters but FC-4 found better followed by FC-1. The two PGPR strains demonstrated significant variation for shoot height and root length. Interaction effect (inoculated carriers × bacterial strains) remained non-significant in shoot and root. Nutrient uptake by maize plant differed significantly from control due to PGPR inoculated carriers. The highest uptake was observed with FC-4 followed by FC-1. Interaction effect (inoculated carriers × bacterial strains) was significant for N, P, K, Na, Zn, Cu and Mn uptake. Significantly higher nutrient uptake was calculated in plants inoculated with MR8 compared to MR5. Formulated carrier-4 can be utilized for biofertilizer production but further testing on other bacterial strains and crops is recommended.

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Introduction

Biofertilizer technology involves mass multiplication of microorganisms in the laboratory and inoculation in a suitable carrier which can be supplied to the growers for application in the field. Utilization of carrier for the inoculation of microbes has been experienced since a long time to keep the bacteria viable (Kaljeet *et al.,* 2011). Carrier is any matrix which maintains the population density of inoculated microorganisms acceptable high for a longer time period but the type and properties of the carrier material play important role in the succeeding performance of the inoculants. The major portion of any bio-inoculant of bio formulation is composed of carrier. The formulation of any carrier may vary; it can be in slurry or powder form but must ensure one essential and important characteristic: the ability to provide the adequate number of viable microbes in good physiological state at the appropriate period of time (Bashan, 1998). As the carrier is the delivery vehicle of viable microorganisms from the laboratory to the field but presently no universal carrier or formulation is available for the transport of microorganisms into soil (Trevors *et al.*, 1992).

Peat is the most commonly used carrier in many areas of the world but unavailability of appropriate peat in developing countries including Pakistan has triggered efforts to identify alternate carrier material for inoculants. Among substitute carrier material coal has received consideration for inoculants. Although coal did not show effectiveness like peatin sustaining microbial cells but most coal-based inoculants maintained the except population of rhizobia i.e>10^4 per seed upto 28 days after inoculation, which is comparable with the minimum standard i.e 7 x 10^4 rhizobia per seed at the time of inoculation (Crawford and Berryhill, 1983). Various studies have shown the potential use of coal as carrier, but the significance of these finding was inadequate due to restricted experimentation (Paczkowski and Berryhill, 1979). Fly-ash is normally generated in huge amount in thermal power plants; it is generally known as waste, which is environmental hazard but various trials proved that it promote crop growth (Kumar and Gupta, 2010). Gaind and Gaur(2004) reported thatfly-ash can be utilized efficiently for the formulation of carrier but bio-efficacy of product should be evaluated by various investigations. Different studies conducted in India have shown the suitability of sugar-cane press mud as a carrier for the production of bacterial inoculants. Sugarcane press mud as such cannot be utilized as a carrier for biofertilizer production however its efficacy can be improved by possibility of amending it with charcoal or soil. Press mud/charcoal (75/25) was found better combination for the shelf life of beneficial microbes in different amended carriers of press mud (Jauhri, 1990). High adsorption capacity, good colloidal structure and high amending capability of clay make it appropriate material to formulate solid carrier.

The work of different researchers described that not a single carrier material has capability of maintaining the required viable population of bacterial cells for long time buta suitable carrier can be formulated by in different combinations; in the range of 30-40% fly-ash, 10-15% press mud, 10-15% lignitic coal and 40% clay with competency of adequate population of bacterial cells for longer period of time at standard level (Tabassam *et al.,* 2015). Present study was conducted to evaluate the quality of different inoculated carrier's formulation from locally available material on growth and nutrient uptake of maize plants.

Materials and methods

A pot study was carried out at National Agricultural Research Centre (NARC), Islamabad to assess the quality of formulated carriers on maize crop. Sterilized soil, compost, sand (1:1:1) was used for pot filling. Factorial experiment with complete randomized design having six treatments replicated three times was used. The seeds were surface sterilized with 5% Sodium hypochloride and coated with formulated carriers i.eFC-1 (40% clay soil + 35% fly-ash + 15% press mud + 10% lignitic coal), FC-2 (40% clay soil + 40% fly-ash + 10% press mud + 10% lignitic coal), FC-3 (40% clay soil + 35% fly-ash + 10% press mud + 15% lignitic coal), FC-4 (40% clay soil +

30% fly-ash + 15% press mud + 15% lignitic coal) (Tabassam *et al.,* 2015) and BC (Biozote carrier; mineral soil used for biofertilizer production in NARC) inoculated with broth culture of pre-isolated plant growth promoting rhizobacteria (PGPR) strains (MR-8 & MR-5) collected from culture collection of Soil Biology and Biochemistry laboratory, Land Resources Research Institute (LRRI), NARC, Islamabad. Un-inoculated seeds were used as a control. Biozote carrier is a mineral soil having pH, 7.70; clay, 15.82%; silt, 25%; sand, 54%; organic matter, 4.6% (khalil*et al*, 1991) and was used as a reference carrier.

Growth parameters

The maize plants were uprooted after 36 days of sowing. After washing with running tap water and distilled water the samples were air dried. Different growth parameters i.e root length, shoot height and dry weight of shoot and root were recorded.

Nutrient content and uptake of maize plant

Oven dried shoot and root samples were investigated for macronutrient and micronutrient content using standard analytical methods (Ryan *et al.,* 2001). Nutrient uptake plant^{-1} was calculated from data related to dry weight and nutrient concentration of whole plant i.e shoot and root.

Statistical analysis

Recorded data was statistically analyzed with analysis of variance (ANOVA) technique, significant means were compared with LSD test method at 5% level using Statistix 8.1 computer software.

Results

The formulated carriers and Biozote carrier (reference carrier) were evaluated on maize crop. The carriers were inoculated with pre-isolated PGPR strains of maize plant. Physico-chemical characteristics of selected soil and compost were investigated (Table1, Table 2). Bio-chemical characteristics of selected PGPR strains are presented in Table. 3.

Visual observation

Visual observation revealed the increased shoot height and biomass as of PGPR inoculated carriers as compared to un-inoculated control (Figure 1). The plants treated with inoculated FC-4 seem better. Same trend is depicted in Figure 2 where root biomass of maize plants treated with inoculated carriers is more vigorous compared to un-inoculated control. Again, the FC-4 presented more biomass.

Table 1. Characteristics of selected soil.

pH	7.5
ECe(dS m^{-1})	0.25
P (mg kg^{-1})	4.54
NO_3-N (mg kg^{-1})	2.13
K (mg kg^{-1})	166
Ca^{+2} +Mg^{+2} (m mol L^{-1})	6.0
Zn (mg kg^{-1})	0.52
Cu (mg kg^{-1})	0.70
Fe (mg kg^{-1})	6.40
Mn(mg kg^{-1})	11.6
Organic matter (%)	0.86
Textural class	Sandy clay loam
Saturation (%)	32.6

Growth parameters of maize shoot

Statistical significant (P≤0.05) increase of growth parameters by PGPR inoculated carriers over control was observed for maize shoot (Table 4). Formulated carrier-4 presented maximum mean shoot length plant^{-1}with 37 % increase, fresh weight plant^{-1} with 77% increase and shoot dry weight plant^{-1} with 49% increase over un-inoculated control.

Table 2. Characteristics of selected compost.

pH (2:1)	7.57
EC (dS m^{-1})	2.50
P (%)	0.25
N (%)	1.65
K (%)	1.20
Zn (mg kg^{-1})	152
Cu (mg kg^{-1})	75
Fe (mg kg^{-1})	1300
Mg (mg kg^{-1})	400
Organic matter (%)	35
Organic carbon (%)	20
C:N	12:1

Different bacterial strains revealed significant difference for shoot height while non-significant for fresh and dry weight but MR8 performed better compared to MR5. Interaction between inoculated carriers and bacterial strains remained non-significant for all growth characteristics.

Table 3. Biochemical characteristics of selected bacterial strains.

Strain	PSB	IAA	Ammonia	Amylase	Protease	Pectinase	HCN	Catalase
MR8	+	++	+	+	+	+	+	-
MR5	+	+	+	+	+	-	-	+

Table 4. Effect of inoculated carriers on different growth parameters of maize shoot.

Formulation	Height (cm)			Fresh weight (g)			Dry weight (g)		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	24.21	24.80	24.50 ^{C}	3.72	3.24	3.48 ^{B}	0.22	0.23	0.23^{C}
FC-1	38.66	25.76	32.21 ^{AB}	6.40	5.40	5.90 ^{A}	0.38	0.39	0.38^{A}
FC-2	39.66	26.94	33.30 ^{AB}	5.80	5.14	5.47 ^{B}	0.34	0.35	0.35^{B}
FC-3	37.94	28.00	32.97 ^{AB}	6.66	5.15	5.91 ^{A}	0.40	0.35	0.38^{AB}
FC-4	37.40	29.72	33.56 ^{A}	6.65	5.66	6.16 ^{A}	0.40	0.39	0.39^{A}
BC	35.67	27.23	31.45 ^{B}	6.36	5.06	5.71 ^{A}	0.38	0.34	0.36^{AB}
Mean	33.59	27.07		5.93	4.94		0.35	0.34	
Strain	*			*			*		
LSD(P≤0.05)									
Formulation	2.08			0.72			0.08		
Formulation × Strain	ns			ns			ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Macronutrient content of maize shoot

Significant (P≤0.05) positive difference of macronutrient i.e N, P, K, Na and Mg concentration of maize shoot due to PGPR inoculation of different carriers over un-inoculated control is evident in the data (Table 5). The highest mean N, P, Mg concentration was observed with inoculated FC-4 while K was high in FC-1 which presented 47%, 81%, 24% and 47% respectively increase over control. Bacterial strains showed significant variation only for P content of maize shoot where MR8 found efficient. Interactive relation between inoculated carriers and bacterial strains remained non-significant for all analyzed macronutrients.

Table 5. Effect of inoculated carriers on macronutrient content (%)of maize shoot.

Formulation	Nitrogen			Phosphorus			Potassium			Sodium			Magnesium		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	2.20	2.05	2.12^{C}	0.12	0.13	0.12^{C}	2.07	2.22	2.15^{C}	0.18	0.22	0.20	0.15	0.14	0.14^{B}
FC-1	3.09	2.82	2.95^{AB}	0.22	0.19	0.21^{AB}	3.37	2.98	3.17^{A}	0.16	0.17	0.17	0.18	0.17	0.17^{A}
FC-2	2.91	2.76	2.83^{AB}	0.18	0.17	0.18^{AB}	3.19	2.77	2.98 ^{B}	0.18	0.18	0.18	0.17	0.17	0.17^{A}
FC-3	3.14	2.90	3.01^{AB}	0.19	0.18	0.19^{AB}	3.35	2.86	3.10^{AB}	0.17	0.16	0.17	0.17	0.18	0.17^{A}
FC-4	3.36	2.90	3.14^{A}	0.23	0.21	0.22^{A}	3.37	2.90	3.14^{AB}	0.16	0.15	0.16	0.19	0.18	0.18^{A}
BC	2.67	2.71	2.70^{B}	0.18	0.16	0.17^{BC}	3.28	2.84	3.06^{AB}	0.17	0.17	0.17	0.17	0.16	0.16^{AB}
Mean	2.90	2.69		0.19	0.17		3.10	2.76		0.17	0.17		0.17	0.16	
Strain	ns			*			ns			ns			*		
LSD (P≤0.05)															
Formulation	0.47			0.03			0.53			0.03			0.001		
Formulation × Strain	ns			ns			ns			ns			ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Micronutrient content of maize shoot

Micronutrient concentration of maize shoot revealed significant (P≤0.05) positive influence of PGPR inoculated carriers over un-inoculated control (Table 6).Formulated carrier-4 presented highest mean concentration of Zn, Cu,Mn and Fe content which showed 70%, 76%, 131% and 51% increase over un-inoculated control. Plant growth promoting rhizobacteria showed significant variation only for Zn and Fe where MR8 strain perform better for Zn while MR5 strain showed better response for Fe content. Interaction between inoculated carriers and bacterial strains remained non-significant for all determined micronutrients.

Table 6. Effect of inoculated carriers on micronutrient content (mg kg^{-1})of maize shoot.

Formulation	Zinc			Iron			Copper			Manganese		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	18.33	16.00	17.17^{C}	55.33	58.67	57.00^{C}	4.60	4.67	4.63^{D}	24.00	25.67	24.83^{C}
FC-1	30.67	26.33	28.50^{A}	77.33	82.00	79.67^{AB}	7.83	7.43	7.63 ^{AB}	43.00	47.67	45.33^{AB}
FC-2	24.00	24.00	24.00^{B}	62.67	73.67	68.17^{BC}	5.87	5.40	5.63^{CD}	47.00	43.67	45.33^{AB}
FC-3	28.67	24.67	26.67^{B}	70.33	76.33	73.33^{BC}	7.40	6.87	7.13^{AB}	46.67	49.67	48.17 ^{AB}
FC-4	30.00	28.33	29.17^{A}	82.00	90.00	86.00^{A}	8.33	8.20	8.27^{A}	59.13	55.67	53.33^{A}
BC	23.67	23.00	23.33^{B}	65.33	77.33	71.33^{BC}	6.53	6.73	6.63 ^{BC}	47.67	40.00	43.83^{B}
Mean	25.89	23.72		68.83	76.33		6.76	6.55		49.53	43.74	
Strain	*			*			ns			ns		
LSD(*P*≤0.05)												
Formulation	3.81			14.49			1.72			9.04		
Formulation × Strain	ns			ns			ns			ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (*P*≤0.05).

Table 7. Effect of inoculated carriers on different growth parameters of maize root.

Formulation	Length (cm)			Fresh weight (g)			Dry weight (g)		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	09.57	09.37	9.47 ^{D}	0.31	0.30	0.31 ^{C}	0.14	0.18	0.16 ^{D}
FC-1	13.06	11.78	12.42 ^{BC}	0.81	0.68	0.75 ^{AB}	0.29	0.25	0.27 $^{A-C}$
FC-2	11.78	11.60	11.69 ^{C}	0.63	0.58	0.61 ^{B}	0.21	0.24	0.23 ^{C}
FC-3	14.78	11.72	13.25 ^{AB}	0.86	0.66	0.76 ^{AB}	0.30	0.27	0.29 ^{AB}
FC-4	15.06	13.39	14.22 ^{A}	0.89	0.76	0.82 ^{A}	0.31	0.28	0.30 ^{A}
BC	13.16	11.72	12.44 ^{BC}	0.65	0.61	0.63 ^{AB}	0.24	0.24	0.24 ^{BC}
Mean	12.90	11.60		0.69	0.59		0.25	0.24	
Strain	*			ns			ns		
LSD(*P*≤0.05)									
Formulation	1.39			0.19			0.05		
Formulation × Strain	ns			ns			ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (*P*≤0.05).

Growth parameters of maize root

Inoculated carriers showed significant(P≤0.05) increase over control for recorded growth parameters of maize root (Table 7). The highest mean root length plant^{-1}, fresh weight plant^{-1} and dry weight plant^{-1}was recorded in the FC-4 which was 50%, 169% and 83%more than control. Effect of different PGPR strains was significant for root length and non-significant for fresh and dry weight but response of MR8 was better than MR5.

Table 8. Effect of inoculated carriers on macronutrient content (%)of maize root.

Formulation	Nitrogen			Phosphorus			Potassium			Sodium			Magnesium		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	1.63	1.64	1.63 ^{C}	0.15	0.15	0.15 ^{C}	0.84	0.86	0.85 ^{C}	0.85	0.82	0.84 C	0.088	0.071	0.079 B
FC-1	2.24	2.13	2.18 ^{B}	0.24	0.20	0.22 ^{A}	1.70	1.08	1.39 C	1.04	1.01	1.02 AB	0.104	0.094	0.099 A
FC-2	2.11	2.12	2.12 ^{B}	0.19	0.16	0.17 ^{BC}	1.01	1.06	1.04 B	1.29	1.07	1.18 A	0.104	0.092	0.098 A
FC-3	2.18	2.38	2.28 ^{B}	0.25	0.21	0.23 ^{A}	1.63	1.07	1.35 B	1.57	1.08	1.32 A	0.105	0.096	0.100 A
FC-4	2.76	2.65	2.71 ^{A}	0.26	0.25	0.25 ^{A}	1.94	1.47	1.70 A	1.33	1.18	1.25 A	0.105	0.101	0.103 A
BC	2.15	2.01	2.08 ^{B}	0.19	0.18	0.19 ^{B}	1.51	1.08	1.30 B	1.53	1.09	1.31 A	0.104	0.095	0.099 A
Mean	2.18	2.16		0.21	0.19		1.44	1.10		1.27	1.04		0.102	0.091	
Strain	ns			*			*			*			*		
LSD(P≤0.05)															
Formulation	0.39			0.03			0.23			0.16			0.007		
Formulation × Strain	ns			ns			ns			ns			Ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Table 9. Effect of inoculated carriers on micronutrient content (mg kg^{-1})of maize shoot.

Formulation	Zinc			Iron			Copper			Manganese		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	22.80	24.33	23.57 ^{C}	237.67	226.00	231.83 ^{C}	16.33	14.33	15.33 ^{D}	44.00	43.67	43.83 ^{C}
FC-1	42.00	36.67	39.33 ^{A}	336.33	303.00	319.67 ^{B}	26.80	21.47	24.13 ^{AB}	68.00	56.00	62.00 ^{B}
FC-2	34.00	35.00	34.50 ^{B}	281.33	277.67	279.50 ^{BC}	21.13	17.83	19.48 ^{C}	58.00	55.33	56.67 ^{B}
FC-3	37.33	40.67	39.00 ^{A}	376.67	266.67	321.67 ^{AB}	26.40	22.07	24.23 ^{A}	81.67	60.00	70.83 ^{A}
FC-4	42.00	39.33	40.67 ^{A}	400.00	345.00	372.50 ^{A}	27.33	24.00	25.67 ^{A}	88.67	65.33	77.00 ^{A}
BC	39.33	35.33	37.33 ^{AB}	316.00	285.00	300.50 ^{B}	22.90	18.07	20.48 ^{BC}	57.33	52.00	54.67 ^{B}
Mean	36.24	35.22		324.67	283.89		23.48	19.63		66.28	55.39	
Strain	ns			*			*			*		
LSD(P≤0.05)												
Formulation	4.31			51.57			3.68			8.25		
Formulation × Strain	ns			ns			ns			Ns		

ns = non-significant; * = significant *(P≤0.05)*

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Interaction between inoculated carriers and bacterial strains remained non-significant for all growth characteristics.

Macronutrient content of maize root

Significant(P≤0.05) increase of macronutrient content of maize root due to PGPR inoculation of different carriers over un-inoculated control is evident in the data (Table 8). Bacterial strains showed significant variation for all determined macronutrient content of maize root except N while interactive effect between inoculated carriers and bacterial strains remained non-significant for all analyzed macronutrients except K and Na. The highest mean concentration of N, P and K revealed 66%, 77% and 100% increase over un-inoculated control due to PGPR inoculated FC-4.

Micronutrient content of maize root

A significant (P≤0.05) positive response of inoculated

carrier formulations for micronutrient content (Zn, Fe, Cu and Cu) of maize root was noticed over un-inoculated control (Table 9). The highest mean concentration of Zn, Fe, Cu and Mn was observed with FC-4which presented 73%, 61%, 67% and 76% increase over un-inoculated control. Bacterial strain MR8 was significantly better from MR5for all determined micronutrient except Zn while interaction between inoculated carriers and bacterial strains remained non-significant except Mn.

Table 10. Effect of inoculated carriers on macronutrient uptake (mg plant^{-1}) of maize.

Formulation	Nitrogen			Phosphorus			Potassium			Sodium			Magnesium		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	09 ^{e}	11 ^{e}	10 ^{D}	0.5 ^{g}	0.6 ^{g}	0.6 ^{E}	07 ^{c}	08 ^{c}	08 ^{D}	2.0 ^{f}	2.4 ^{f}	2.2 ^{D}	0.5	0.6	0.5 ^{C}
FC-1	32 ^{b}	23 ^{d}	27 ^{B}	2.1 ^{ab}	1.4 ^{ef}	1.8 ^{B}	30 ^{a}	21 ^{b}	26 ^{AB}	5.5 $^{b-e}$	4.4 ^{e}	4.9 ^{C}	1.5	1.1	1.3 ^{AB}
FC-2	24 ^{d}	21 ^{d}	22 ^{C}	1.4 $^{d-f}$	1.1 ^{ef}	1.3 ^{D}	22 ^{b}	19 ^{b}	21 ^{C}	4.7 $^{c-e}$	4.3 ^{e}	4.5 ^{C}	1.2	1.1	1.1 ^{B}
FC-3	30 ^{bc}	23 ^{d}	26 ^{B}	2.0 $^{a-c}$	1.3 ^{ef}	1.7 ^{BC}	29 ^{a}	20 ^{b}	25 ^{B}	7.0 ^{a}	4.7 ^{de}	5.8 ^{A}	1.4	1.2	1.3 ^{AB}
FC-4	36 ^{a}	29 ^{bc}	33 ^{A}	2.4 ^{a}	1.9 $^{b-d}$	2.1 ^{A}	29 ^{a}	27 ^{a}	28 ^{A}	6.6 ^{ab}	5.7 $^{b-d}$	6.1 ^{A}	1.6	1.4	1.5 ^{A}
BC	27 ^{c}	21 ^{d}	24 ^{C}	1.6 $^{c-e}$	1.1 ^{f}	1.4 ^{CD}	26 ^{a}	19 ^{b}	23 ^{BC}	5.9 $^{a-c}$	4.3 ^{e}	5.1 ^{BC}	1.3	1.1	1.2 ^{B}
Mean	26	21		1.7	1.2		24	19		5.3	4.3		1.3	1.1	
Strain	*			*			*			*			*		
LSD(P≤0.05)															
Formulation	2.34			0.31			2.87			0.87			0.18		
Formulation × Strain	3.31			0.43			4.06			1.23			ns		

ns= non-significant, * = significant (P≤0.05)

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Macronutrient uptake by maize plant

It is inferred from the data that macronutrient i.e N, P, K uptake by maize plant increased significantly (P≤0.05) over control due to PGPR inoculation of different carriers except Mg. Significant higher uptake was revealed in plants inoculated with MR8 as compared to MR5 and interaction between inoculated carriers and bacterial strains was significant for N, P, K and Na (Table 10). The highest mean uptake of N, P, K, Mg was observed with FC-4 which was 230%, 133%, 187 and 140% higher than un-inoculated control.

Table 11. Effect of inoculated carriers on micronutrients uptake (µg plant^{-1}) of maize.

Formulation	Zinc			Iron			Copper			Manganese		
	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean	MR8	MR5	Mean
Cont.	10	10	10 ^{D}	50	58	54 ^{E}	03	04	04 ^{D}	12	16	14 ^{C}
FC-1	42	30	36 ^{AB}	294	280	286 ^{AB}	13	09	11 ^{B}	58	40	49 ^{B}
FC-2	28	27	27 ^{C}	128	182	155 ^{D}	08	07	07 ^{C}	47	36	41 ^{B}
FC-3	40	30	35 ^{A}	274	220	247 ^{BC}	13	09	11 ^{B}	55	41	48 ^{B}
FC-4	48	37	43 ^{A}	330	345	338 ^{A}	14	12	13 ^{A}	68	52	60 ^{A}
BC	32	26	29 ^{BC}	201	201	201 ^{CD}	09	08	09 ^{C}	46	33	39 ^{B}
Mean	33	27		213	214		10	08		48	36	
Strain		*			ns			*			*	
LSD (P≤0.05)												
Formulation		5.63			55.69			1.81			9.67	
Formulation× Strain		ns			ns			ns			ns	

ns = non-significant; * = significant (P≤0.05)

Bars sharing the same letter (s) are statistically non-significant (P≤0.05).

Micronutrient uptake by maize plant

Data revealed significant (P≤0.05) increase of micronutrient uptake by maize plant over control due to PGPR inoculation of different carriers, however, interaction effects between inoculated carriers and bacterial strains remained non-significant. Significant higher uptake of micronutrient was recorded in maize plant inoculated with MR8 as compared to MR5 except Fe(Table 11). The highest uptake was observed with FC-4 where mean uptake of Zn, Fe, Cu, and Mn

was 190%, 272%, 125% and 178% more than the un-inoculated control.

Discussion

To enhance the agricultural production it is desirable that nutrients level should be improved in the rhizosphere to boost the accessibility of essential minerals for crops. For improving the fertility status of soil and reducing the requirement for chemical fertilizer as well as pesticide, the application of beneficial microorganisms has increased enormously.

Fig. 1. Effect of inoculated carriers on growth of maize plant.

Suitable carrier formulation

For efficient utilization of beneficial microbes it is essential that target microorganisms should be inoculated at a higher rate in the soil to make them useful for plant yield enhancement. Suitable bio-inoculant formulation safeguards microbes or viable cells against unfriendly environmental conditions. It is desirable that appropriate carrier materials should be utilized for maintaining microbial viability to cope such harmful environmental conditions (Singh *et al.*, 2014). A suitable formulation must support survival of microbes to sustain a viable quantity, sufficient enough to improve growth of plants (Aeron *et al.*, 2011).It is well documented that microbial populations in the rhizosphere have significance influence on conservation of plant health, nutrient uptake as well as tolerance against disease and environmental stress. Microbial population of crop rhizosphere can be improved by inoculating growth promoting bacteria to enhance growth of plant which showed significant potential under laboratory and greenhouse conditions; however, response was variable during field studies (Bowen and Rovira, 1999). Commercial utilization of plant growth promoting rhizobacteria to improve crop health depends on the development of suitable carrier's formulation which maintains the adequate population density of living cells for a substantial time period.

Enhancement in growth and nutrient uptake

Plant growth promoting rhizobacteria have great potential to enhance various growth parameters of crops but this capability is specific to certain plant genotypes, species and cultivars (Figueiredo *et al.*, 2010).To impact the plant at different growth stages PGPR adopt numerous mechanisms. Various direct and indirect mechanisms consist of; solubilization of fixed minerals, availability of nutrients, atmospheric nitrogen fixation, hydrogen cyanide production and bio-control activity, ACC deaminase ability for salinity

tolerance and of phytohormones production to improve the growth parameters of plants (Gupta *et al.,* 2000.

Plant growth promoting rhizobacteria improve plant nutrition by enhancing the uptake of essential nutrients which exhibit significant impact on crop growth. Plant growth promoting rhizobacteria inoculated maize crop significantly increase plant weight, plant height and nutrient uptake i.e N, P, K, Fe, Cu, Zn and Mn (Jarak *et al.*, 2012).

Fig. 2. Effect of different inoculated carriers on growth of maize root.

Conclusion

Among different formulated carriers FC-4 (40% clay soil + 30% fly-ash + 15% press mud + 15% lignitic coal)inoculated with PGPR presented better growth, mineral composition as well as nutrient uptake for maize plant. This carrier formulation can be utilized for biofertilizer production but further experimentation under field condition is recommended.

References

Aeron A, Dubey RC, Maheshwari DK, Pandey P, Bajpai VK, Kang SC. 2011. Multifarious activity of bioformulated*Pseudomonas fluorescens*PS1 and biocontrol of *Sclerotiniasclerotiorum*in in Indian rapeseed (*Brassica campestris*L.).European Journal of Plant Pathology **131,** 81–93.

Bashan Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Advances **4**, 729-770.

Bowen GD, Rovira AD. 1999. The rhizosphere and its management to improve plant growth. Advances in Agronomy **66**,1–102.

Crawford SL, Berryhill DL. 1983. Survival of *Rhizobium phaseoli* in coal-based legume inoculants applied to seeds. Applied and Environmental Microbiology **45**, 703-705.

Figueiredo MVB, Seldin L, Fernando Araujo F, Mariano RR. 2010. Plant Growth Promoting Rhizobacteria: Fundamentals and Applications. In: Maheshwari DK, ED. Plant Growth and Health Promoting Bacteria. Microbiology Monographs **18**, Berlin, Heidelberg: Springer-Verlag

Gaind S, Gaur AC. 2004. Evaluation of fly-ash as a carrier for diazotrophs and Phosphobacteria. Bioresource Technology **95**,187-190. http://doi.org/10.1016/j.biortech.2003.07.014

Gupta A, Gopal M, Tilak KV. 2000. Mechanism of plant growth promotion by rhizobacteria. Indian Journal of Experimental Biology **38,** 856–862.

Jarak M, Mrkovački N, Bjelić D, Jošić D, Hajnal-Jafari T, Stamenov D. 2012. Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. African Journal of Microbiology Research **6,** 5683-5690. http://doi.org/10.5897/AJMR12.759

Jauhri KS. 1990. Modified sugarcane pressmud: a potential carrier for commercial production of bacterial inoculants. Indian Journal of Agricultural Research **24,** 189-197.

Kaljeet S, Keyeo F, Amir HG. 2011. Influence of carrier materials and storage temperature on survivability of rhizobial inoculant. Asian Journal of Plant Sciences **10,** 3923.

Khalil S, Khokhar SN, Khan MA. 1991. Evaluation of an indigenous mineral soil as Rhizobium carrier. Pakistan Journal of Agricultural Research **12,** 62-65.

Kumar V, Gupta P. 2010. Studies on shelf-life of fly-ash based *Azotobacter chroococcum* formulation and its bio-efficacy in Wheat. Research Journal of Agriculture and Biological Sciences **6,** 280-282.

Paczkowski MW, Berryhill DL. 1979. Survival of *Rhizobium phaseoli* in coal-Based legume inoculants. Applied and Environmental Microbiolgy**38,** 612.

Ryan J, Estefan G, Rashid A. 2001. Soil and Plant Analysis Laboratory Manual.Second Edition.Available from ICARDA, Aleppo, Syria.

Singh S, Gupta G, Khare E, Behal KK, Arora NK. 2014. Effect of enrichment material on the shelf life and field efficiency of bioformulation of Rhizobiumsp. and P-solubilizing *Pseudomonas fluorescens*. Science Research Reporter **4,** 44-50.

Tabassam T, Sultan T, Akhtar ME, Mahmood-ul Hassan M, Ali A. 2015. Suitability of different formulated carriers for sustaining microbial shelf life. Pakistan Journal of Agricultural Research **28,**143-151.

Trevors JT, Van-Elsas JD, Lee H, Van Overbeek LS. 1992. Use of alginate and other carriers for encpasulation of microbial cells for use in soil. Microbial Releases **1,** 61-6.