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Response of *rabi* onion (*Allium cepa*) to biofertilizers in a sandy loam alluvial soil

DIKSHA TINNA, NAVEEN GARG*, GULAB PANDOVE, SANDEEP SHARMA, NEENA CHAWLA and AJMER SINGH DHATT

Punjab Agricultural University, Ludhiana, Punjab 141 004, India

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ABSTRACT

An experiment was conducted in a factorial randomized block design during *rabi* 2016-18 at Ludhiana. There were 14 treatments consisting of two factors. Factor I comprised two levels of inorganic fertilizers [100% RDF (recommended dose of inorganic fertilizers) and 75% RDF] and factor II consisted of six combinations of biofertilizers along with a control. The data pooled over years revealed that the utilization of various biofertilizers significantly improved the plant height, number of leaves, 20-bulb weight, bulb equatorial diameter and yield over control at both levels of inorganic fertilizers. However, the per cent increase over their respective control was higher when these biofertilizers were used with 75% RDF than with 100% RDF. Biofertilizer treatments significantly improved the ascorbic acid, total soluble solids and pyruvic acid of bulbs, reduced the physiological loss in bulb weight during storage but the differences in bulb dry matter and ash content were non-significant. The effects of all biofertilizers on soil chemical and microbiological properties were beneficial but non-significant. Two treatments, i.e. 100% RDF + T_6 (*Azotobacter* + *Sphingobacterium* + *Burkholderia*) and 100% RDF + T_1 (*Azotobacter* + *Bacillus*) that exhibited 11.5% and 8.6% increment in bulb yield over control (100% RDF) have been identified. Besides, three treatments, i.e. 75% RDF + T_6, 75% RDF + T_1 and 75% RDF + T_5 (*Azotobacter* + *Bacillus* + *Burkholderia*) registered 8.3%, 7.8% and 7.3% higher bulb yield over control (100% RDF) and therefore may be recommended to save 25% inorganic fertilizers.

Key words: *Azotobacter*, *Bacillus*, *Burkholderia,* Onion, Quality

India is the second largest producer of onion (*Allium cepa* L.) in the world next to China and the third largest exporter after Netherlands and Spain. However, the onion productivity in India (16.2 t/ha) is lower than that of China (22.0 t/ha), Asia (18.6 t/ha) and the World (18.8 t/ha) (FAOSTAT 2018). The productivity can be increased by developing new high yielding varieties but their potential yields are not achieved under field conditions. This happens because the root system of onion is shallow, has low absorbing and penetrating abilities, therefore it requires an ample amount of easily accessible nutrients (Colo *et al*. 2014). Therefore, the utilization of organic and inorganic fertilizers is necessary to increase productivity but the organic manures (farmyard manure, vermicompost, poultry manure etc.) are not available in sufficient quantity to meet the entire demand of such a large area under onion cultivation. The injudicious use of inorganic fertilizers deteriorates soil health, reduces post-harvest shelf-life of bulbs, enhances cost of crop production thereby reducing profits. Therefore, it is desirable to explore other production technologies which may reduce the dependence on inorganic and organic fertilizers without any loss in yield and improve soil health for sustainable agriculture.

The utilization of biofertilizers in combination with inorganic fertilizers is a viable alternative as biofertilizers are less expensive, ecofriendly, provide plant hormones and help in sustainable crop production (Bishnoi 2015). There are a large number of reports on the beneficial effects of biofertilizers in onion (Sankar *et al*. 2009, Yeptho *et al*. 2012, Colo *et al*. 2014, Thangasamy and Lawande 2015). However, these effects vary with site and year because the inoculated bacteria will have to compete with the often better adapted native soil microflora for nutrients and space (Ruzzi and Aroca 2015). Hence, the present study was conducted to ascertain whether various bacterial biofertilizers can act as potential supplements to recommended dose of inorganic fertilizers (RDF) in improving growth, yield, quality and post-harvest shelf-life of *rabi* onion, to work out the possibility of reducing the RDF by 25% using various biofertilizers, and to study the effect of various biofertilizers on benefit: cost ratio, soil microbial and chemical properties.

MATERIALS AND METHODS

The research trials were conducted during *rabi* 2016-17 and 2017-18 at Vegetable Research Farm, Punjab

*Corresponding author e-mail: naveengargpau@gmail.com

Agricultural University, Ludhiana (30° 54′ N latitude, 75°48′ E longitude and 247 m altitude). The experimental site is characterized by sub-humid climate with an average annual rainfall of 726 mm. The experimental field was different during both the years. The soil type was alluvial, sandy loam having 71.9% sand, 18.3% silt and 9.8% clay. The trials were conducted in a factorial randomized complete block design with three replications. The fourteen treatments comprised two factors. Factor I included two levels of inorganic fertilizers, i.e. 100% recommended dose of inorganic fertilizers (100% RDF), i.e. 100 kg N, 50 kg P_2O_5 and 50 kg K_2O per ha and 75% RDF. Factor II comprised seven combinations of biofertilizers i.e. T_0: control (without biofertilizer), T_1: *Azotobacter* sp. + *Bacillus* sp., T_2: *Azotobacter* sp. + *Sphingobacterium* sp., T_3: *Azotobacter* sp. + *Burkholderia* sp., T_4: *Azotobacter* sp. + *Bacillus* sp. + *Sphingobacterium* sp., T_5: *Azotobacter* sp. + *Bacillus* sp. + *Burkholderia* sp. and T_6: *Azotobacter* sp. + *Sphingobacterium* sp. + *Burkholderia* sp. The various bacterial biofertilizers procured from Department of Microbiology, Punjab Agricultural University, Ludhiana, were mixed in equal ratio to make a constant final volume of microbial inoculants for each treatment. It was mixed with sterilized charcoal in the ratio 1:2.5 (v:w) to prepare charcoal based formulation of microbial inoculants which was used @ 2.5 kg/ha after diluting in 250 litre of water. The roots of seedlings of onion variety Punjab Naroya were dipped in these cultures for 30 min before transplanting which was done at a spacing of 15 cm × 7.5 cm in first fortnight of January. The net plot size was 1.5 m × 1.8 m accommodating 12 rows each having 20 plants. The crop was harvested in last week of May.

The observations were recorded on 10 plants per plot for plant height (cm), number of leaves/plant (at 60 and 90 days after transplanting) and equatorial diameter of bulb (cm). Bulb yield (kg/plot) was recorded from 10 competitive rows per plot and converted in to t/ha. Twenty bulbs per plot were randomly chosen to record 20-bulb weight (g). After curing the bulbs for two weeks, five bulb quality parameters, viz. total soluble solids (TSS) (°brix), ascorbic acid (mg/100 g fresh weight), pyruvic acid (mg/100 g fresh weight) (Hart and Fisher 1971), dry matter (%) and ash content (g/100 g dry matter) were estimated using standard methods. The harvested bulbs were stored for 120 days under ambient conditions with proper ventilation and storage observations were recorded periodically at 15 days interval. The physiological loss in weight (PLW) was estimated by using standard formula (Sankar *et al*. 2009).

The soil samples (at 0-15 cm depth) were taken before transplanting and after harvest, and were subjected to analysis of *p*H, electrical conductivity (EC), organic carbon (OC), available nitrogen (alkaline potassium permanganate method), phosphorus and potassium (neutral ammonium acetate method) as per standard procedures (Jackson 1967). The microbial properties of soil, i.e. total bacterial, actinomycetes and fungal counts were also estimated on nutrient agar, starch casein agar and glucose yeast extract agar medium, respectively, using serial dilution spread plate technique and expressed as colony forming units (cfu) per gram of dry soil (Foght and Aislabie 2005). The economic analysis was done as per the cost of different treatments and the prevailing market value. The benefit: cost ratio (B: C ratio) was calculated by dividing gross returns with total cost of cultivation. The data generated for both growing seasons were analyzed according to standard statistical methods.

RESULTS AND DISCUSSION

Growth attributes: The levels of inorganic fertilizers significantly influenced the plant height but exerted non-significant effects on number of leaves (Table 1). Higher dose of inorganic fertilizer (100% RDF) produced significantly higher plant height than with lower dose (75% RDF). This implied that onion, being a shallow rooted crop, is highly responsive to application of inorganic fertilizers which improve the vegetative growth of plant thereby resulting in higher plant height at 100% RDF. Biofertilizer treatments also produced significant differences in plant height and number of leaves. All biofertilizer treatments recorded low to moderate increase in plant height (2.05-9.96% and 3.74-11.57%) and number of leaves (1.96-12.85% and 5.48-13.14%) over uninoculated control at 60 and 90 DAT, respectively. The maximum plant height was recorded by T_6 that was at par with T_1 and T_5. The treatment T_6 also registered the maximum number of leaves which was significantly higher than all other treatments. Talwar *et al*. (2016) have also reported significant increase in growth parameters of onion with the use of biofertilizers. Biofertilizers improve plant growth parameters by increasing the levels of auxins and cytokinins and decreasing ethylene and abscisic acid in plants, which may result in enhanced cell division and elongation. Besides, they increase the availability of nitrogen and phosphorus to plants by fixation and solubilization, respectively, resulting in better root and shoot development and hence enhanced uptake of water and nutrients by plants (Ruzzi and Aroca 2015). The interaction effects among levels of inorganic fertilizers and biofertilizer treatments were non-significant for both the growth attributes which implied that the effects of biofertilizer treatments were consistent at 100% and 75% RDF.

Yield attributes: Higher dose of inorganic fertilizer (100% RDF) produced significantly higher bulb yield and 20-bulb weight than with lower dose (75% RDF) (Table 1). This may be due to significantly higher plant height with 100% RDF than with 75% RDF, leading to higher synthesis of photosynthates and their better translocation to the sink, as the rate of photosynthesis is significantly correlated with growth of onion (Devi and Ado 2005). All biofertilizer treatments were either statistically at par or superior to control (T_0) in respect of bulb yield, 20-bulb weight and bulb equatorial diameter (Table 1). Biofertilizer treatment T_6 registered maximum bulb yield, 20-bulb weight and bulb equatorial diameter which were statistically at par with T_1. Indira and Singh (2014) have also observed the maximum bulb yield (23.15 t/ha) of onion by seed treatment

Table 1 Effect of levels of inorganic fertilizers and various biofertilizers on growth, yield and quality of *rabi* onion at Ludhiana (pooled data of two seasons)

Treatment	Growth attributes				Yield attributes			Quality attributes				
	PH		NOL		BY	BW	ED	AA	TSS	PA	DM	AC
	60 DAT	90 DAT	60 DAT	90 DAT								
Inorganic fertilizer (IF)												
100% RDF	39.90	43.12	4.88	6.87	23.57	876.2	4.79	22.46	11.65	40.60	13.18	4.26
75% RDF	38.28	41.91	4.89	6.84	22.92	860.6	4.76	23.43	11.38	37.91	12.52	4.07
CD (IF) (P=0.05)	0.72	1.02	NS	NS	0.60	12.73	NS	0.73	0.26	1.58	0.29	0.17
Biofertilizer (BF)												
T_0	37.13	39.83	4.59	6.39	21.79	817.2	4.60	21.42	10.88	37.21	12.63	4.10
T_1	40.33	43.76	5.00	6.95	24.50	896.4	4.86	24.52	11.72	38.48	12.99	4.28
T_2	39.40	42.55	4.90	6.86	22.72	854.1	4.72	22.13	11.48	38.23	12.82	4.11
T_3	38.50	42.40	4.88	6.87	22.58	859.3	4.75	22.17	11.53	42.66	12.77	4.08
T_4	37.89	41.32	4.68	6.74	22.38	851.8	4.67	22.69	11.42	38.80	12.68	4.07
T_5	39.65	43.29	4.95	6.93	23.88	884.7	4.84	23.15	11.60	41.57	12.84	4.16
T_6	40.83	44.44	5.18	7.23	24.88	915.6	4.98	24.52	11.97	37.82	13.23	4.37
CD (BF) (P=0.05)	1.35	1.90	0.15	0.16	1.12	23.8	0.13	1.36	0.48	2.95	NS	NS
CD (IF × BF) (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

To, Control; T_1, *Azotobacter* sp. + *Bacillus* sp; T_2, *Azotobacter* sp. + *Sphingobacterium* sp.; T_3, *Azotobacter* sp. + *Burkholderia* sp.; T_4, *Azotobacter* sp. + *Bacillus* sp. + *Sphingobacterium* sp.; T_5, *Azotobacter* sp. + *Bacillus* sp. + *Burkholderia* sp., T_6, *Azotobacter* sp. + *Sphingobacterium* sp. + *Burkholderia* sp.; PH, Plant height (cm); NOL, Number of leaves per plant; BY, Bulb yield (t/ha); BW, 20-bulb weight (g); ED, Equatorial diameter of bulb (cm); AA, Ascorbic acid (mg/100 g fresh weight); TSS, Total soluble solids (^{o}brix); PA, Pyruvic acid (mg/100 g fresh weight); DM, Dry matter (%); AC, Ash content (g/100 g dry matter); DAT, Days after transplanting

with *Azotobacter* and minimum (19.90 t/ha) with chemical fertilizers only.

The utilization of various biofertilizers along with inorganic fertilizers resulted in 2.71-14.18% increase in bulb yield, 4.23-12.04% in 20-bulb weight and 1.52-8.26% increase in bulb equatorial diameter over uninoculated control. However, their utilization with 75% RDF recorded higher per cent increase than their use with 100% RDF over their respective uninoculated control treatments. This may be due to the reason that growth promotion by some biofertilizers is actually enhanced under non-fertilized or less-fertilized conditions (Ruzzi and Aroca 2015). The interaction effects among levels of inorganic fertilizers and biofertilizer treatments were non-significant for bulb yield, 20-bulb weight and equatorial diameter of bulb. Two treatments, i.e. 100% RDF + T_6 and 100% RDF + T_1 exhibited 11.48% and 8.61% increment in bulb yield over 100% RDF + T_0. Therefore, these two low cost biofertilizer cultures offer the scope of increasing bulb yield of onion by a significant margin. Three treatments, viz. 75% RDF + T_6, 75% RDF + T_1 and 75% RDF + T_5 registered 8.30%, 7.82% and 7.33% higher bulb yield over 100% RDF + T_0 manifesting the scope of utilization of these biofertilizer cultures to save 25% inorganic fertilizers without any reduction in bulb yield. Devi and Ado (2005) have also observed that utilization of *Azospirillum* and phosphotica along with less nitrogen and phosphorus had beneficial effect in improving growth and yield of multiplier onion besides saving the recommended nitrogen and phosphorus up to 17 and 35%, respectively.

Quality attributes: The 100% RDF resulted in significantly higher TSS, pyruvic acid, dry matter and ash content and lower ascorbic acid in bulbs than with 75% RDF (Table 1). Biofertilizer treatments produced significant differences in all quality parameters except dry matter and ash content. The minimum values of all quality parameters were recorded in control (T_0). The various biofertilizer treatments improved the ascorbic acid, TSS and pyruvic acid by 3.31-14.47%, 4.96-10.02% and 1.64-14.65% over control, respectively. The maximum ascorbic acid and TSS was recorded by T_6 which was at par with T_1. The previous studies have also reported an increase in ascorbic acid and TSS of onion bulbs with integrated use of inorganic, organic and biofertilizers than with inorganic fertilizers alone (Yeptho *et al*. 2012, Thangasamy and Lawande 2015, Talwar *et al*. 2016). This may be due to higher availability and uptake of nutrients which in turn might have led to increased plant metabolism. The highest pyruvic acid was registered by T_3 that was on a par with T_5. The increase in pyruvic acid (pungency) with the use of biofertilizer treatments may be due to their effect in increasing sulphur availability to plants as many previous studies have reported positive correlation between the two (Thangasamy *et al*. 2013, Thangasamy and Lawande 2015). The interaction

Table 2 Effect of levels of inorganic fertilizers and various biofertilizers on chemical and microbial properties of soil after harvesting of *rabi* onion at Ludhiana (pooled data of two seasons)

Treatment	Chemical properties of soil						Microbial properties of soil		
	pH	EC	OC	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	BC	AC	FC
Inorganic fertilizer (IF)									
100% RDF	7.35	0.21	0.29	133.0	26.4	338.8	8.41	5.14	3.92
75% RDF	7.32	0.21	0.28	130.6	26.0	335.7	8.41	5.12	3.89
CD (IF) (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Biofertilizer (BF)									
T_0	7.31	0.22	0.27	127.7	25.3	335.3	8.38	5.11	3.89
T_1	7.29	0.21	0.29	133.5	26.6	338.2	8.42	5.14	3.92
T_2	7.34	0.21	0.28	131.7	26.2	336.7	8.40	5.12	3.90
T_3	7.38	0.21	0.28	130.7	25.8	336.7	8.40	5.13	3.91
T_4	7.33	0.21	0.28	131.5	26.1	337.0	8.39	5.12	3.90
T_5	7.35	0.22	0.28	133.5	26.4	338.2	8.41	5.13	3.91
T_6	7.34	0.21	0.29	134.0	27.0	338.8	8.43	5.16	3.94
CD (BF) (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (IF × BF) (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Initial value	7.46	0.23	0.27	126.4	24.3	320.4	8.34	5.05	3.84

To, Control; T_1, *Azotobacter* sp. + *Bacillus* sp; T_2, *Azotobacter* sp. + *Sphingobacterium* sp.; T_3, *Azotobacter* sp. + *Burkholderia* sp.; T_4, *Azotobacter* sp. + *Bacillus* sp. + *Sphingobacterium* sp.; T_5, *Azotobacter* sp. + *Bacillus* sp. + *Burkholderia* sp., T_6, *Azotobacter* sp. + *Sphingobacterium* sp. + *Burkholderia* sp.; EC, Electrical conductivity (dS/m); OC, Organic carbon (%); BC, Bacterial count ($\log_{10}$ cfu/g dry soil); AC, Actinomycetes count ($\log_{10}$ cfu/g dry soil); FC, Fungal count ($\log_{10}$ cfu/g dry soil)

effects among levels of inorganic fertilizers and biofertilizer treatments were non-significant for all quality traits.

Chemical and microbial properties of soil: The levels of inorganic fertilizers, biofertilizer treatments and interaction between them exhibited non-significant differences on all the chemical and microbial properties of soil (Table 2). However, the values of available N, P and K after harvesting of onion from biofertilizer treatment plots were slightly higher than control plots. Similarly, plots incorporated with 100% RDF recorded higher values of available N, P and K than those with 75% RDF. Thangasamy and Lawande (2015) have also reported that the use of organic manures and biofertilizers (*Azospirillum* and PSB) along with inorganic fertilizers in onion caused non-significant differences in soil chemical properties after harvest.

The minimum number of bacterial, actinomycetes and fungal cells were recorded in control (T_0) and the maximum values were observed with treatment T_6 followed by T_1 (Table 2). With the use of various biofertilizer treatments (T_1 to T_6), these cell counts in the soil after harvesting exhibited slight but non-significant increase as compared to cell counts before transplanting. The microbial diversity in the rhizosphere depends on root exudates, soil properties, agrotechnical measures and ecological factors. Onion roots exude amino acids, sugars and organic acids which positively influence the rhizosphere (Colo *et al*. 2014). However, for significant improvement in soil microbial count, long term application of suitable biofertilizer is required which could not happen in the present study because the experimental field in second year was different from that in first year.

Physiological loss in bulb weight (PLW) (%): The levels of inorganic fertilizers and their interaction with biofertilizer treatments manifested non-significant differences in PLW throughout the storage period except at 15 days of storage. The biofertilizer treatments exhibited significant differences in respect of PLW throughout the storage period. The maximum PLW (7.43-28.43%) was registered with control (T_0) throughout the storage period (15 to 120 days of storage). Sankar *et al*. (2009) and Shinde *et al*. (2016) have also reported the maximum storage losses in onions produced using inorganic fertilizers only. The minimum PLW (5.38-25.71%) was recorded by T_4 that was statistically at par with T_6 (5.51-25.88%) and significantly lower than control (T_0) throughout the storage period. This may be due to the effect of biofertilizer treatments in increasing the TSS and dry matter of bulbs. Onions with high dry matter are firmer, have thicker, better adhering skin and lose water less than those which have low dry matter, high water content and thinner bulbs (Sankar *et al*. 2009).

Economics: The utilization of inorganic fertilizers alone (75% RDF and 100% RDF) resulted in the lowest benefit: cost ratio (1.55 and 1.66) and net returns (74773 and 90269 ₹/ha), respectively. However, the integrated use of any biofertilizer treatment along with inorganic fertilizers resulted in higher benefit: cost ratio and net returns over that with inorganic fertilizers alone. The maximum benefit: cost ratio (1.85) was expressed by 100% RDF + T_6 followed by 75% RDF + T_6 (1.82), 75% RDF + T_1 (1.81), 100% RDF + T_1 (1.80) and 75% RDF + T_5 (1.80). Therefore, the use of these biofertilizer treatments along with inorganic fertilizers may be considered economically beneficial over the use of inorganic fertilizers alone. In *kharif* onion, Yeptho *et al.* (2012) have also observed that the application of *Azotobacter chroococcum* along with poultry manure (20 t/ha) resulted in highest net returns and benefit: cost ratio as compared to all other treatments.

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