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CO-INOCULATION OF BIOGAS SLURRY AND *BACILLUS* STRAIN CIK-515 IN IMPROVING NUTRIENT CONCENTRATION AND YIELD OF POTATO (*SOLANUM TUBEROSUM*)

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Abstract

Manipulations of organic and inorganic fertilizers are known to enhance plant growth regulating factors in the soil that ultimately increase crop growth and yield. However, less has been investigated on the efficiency of both fertilizers by co-inoculating plant growth promoting rhizobacteria (PGPR). This study was designed to investigate the improvement in potato tuber yield and nutrient contents (N, P) through the integrated application of biogas slurry (BGS) and PGPR, *Bacillus* strain CIK-515, with or without the recommended dose of NPK (RNPK). The results showed a significant effect of BGS and PGPR on tuber yield ($p<0.05$) and that it was independent of the addition of RNPK. The application of BGS and CIK-515 showed a significant improvement in plant height (22-42%), total dry biomass (24-43%), tuber yield (19-51%), N and P (25-45%, 5-31% in plant biomass) in potato plant compared to RNPK, with or without. However, the combination of BGS and RNPK led to a significant increase in agronomic indices of N and P use efficiency. A better correlation had existed between tuber yield and NUE ($R^2 = 0.73$) compared to tuber yield and PUE ($R^2 = 0.66$). It could be concluded that co-inoculation of BGS and *Bacillus* strain CIK-515 could be a useful soil amendment that has the ability to improve N and P contents and tuber yield when utilized with mineral fertilizers.

Key words: Biogas slurry; PGPR; Nutrient use efficiency; Tuber yield; Mineral fertilizer.

Introduction

After the Green Revolution, the use of chemical fertilizer has substantially increased as their applications has provided a way of improving crop growth and yield (Sáez *et al*., 2012; Shahbaz *et al*., 2014). In spite of considerable use of chemical fertilizers, agricultural soils in developing countries including Pakistan are deficient in essential nutrients and led to a decrease in crop yield (Singh, 2006). However, adequate supplies of nutrients are known to increase photosynthetic activity during plant growth and that ultimately enhance crop yield (Makino, 2011). All this resulted in improved dry matter accumulation especially partitioning into economic part of the plant (Zvomuya & Rosen, 2002). Fertilizer source, soil type and environmental conditions have an influence on nutrient availability to plant (Carson & Ozores-Hampton, 2013). It may ultimately affect performance of the crop; therefore, nutrient supply should be synchronized to crop demand (Baligar *et al*., 2001; Bindraban *et al*., 2015).

However, two types of possible approaches known as horizontal expansion and vertical expansion exist to meet the ever-rising demand for food in developing countries of the World (Savci, 2012; Shahbaz *et al*., 2014). Vertical expansion of crop production involves intensive use of chemical fertilizers that is expensive both ecologically and economically (Savci, 2012). Some studies have reported that farmers can obtain the same level of crop production through sustainable use of chemical fertilizers compared to their excessive use (Bakhsh *et al*., 2012). Second approach to achieve maximum yield potential of crops is the integration of organic and inorganic fertilizers (Shahbaz *et al*., 2014; Akhtar *et al*., 2019). Organic amendments in the soil such as addition of biogas slurry (BGS) and composts are very well known sources of plant nutrients. In addition, BGS are known to provide essential nutrients to field crops after their decomposition and may act as soil conditioner (Barbosa *et al*., 2014). Field applications of integrated use of organic amendments and chemical fertilizers will not only reduce the cost of production of crops, but also improve nutrient management on sustainable basis (Sáez *et al*., 2012).

Integrated use of BGS in combination with chemical fertilizer has been evaluated worldwide and it is associated with its high nutritional value, increasing crop productivity and safe disposal to soil (Rahman *et al*., 2008; Liu *et al*., 2008; Barbosa *et al*., 2014). BGS approach is relatively inexpensive, environmentally safe and renewable energy source of nutrients for the plants in the soil (Islam *et al*., 2010). The application of BGS has also been reported to ameliorate heavy metal stress by reducing environmental impact of waste disposal (Ahmad *et al*., 2015) and improves the structure and aeration of soil and diverse nutrients for sustainable crop productivity (Yu *et al*., 2010). Other sustainable source of crop production include plant growth promoting rhizobacteria (PGPR) that are known to enhance crop growth through several mechanisms such as endogenous levels of plant growth regulating substances i.e. phytohormones (Tahir *et al*., 2019; Bashir *et al*., 2020), phosphate solubilization, 1-aminocyclopropane-1-carboxylate deaminase (ACC) and siderophore production (Khan *et al*., 2017; Hussain *et al*., 2019). The inoculation of PGPR containing ACC-deaminase in combination with different rates of nitrogen fertilizer increased crop growth and nutrient use efficiency (Shaharoona *et al*., 2008; Ahmad *et al*., 2014b).

Although, copious information are available for the use of organic matter in vegetable production, however, in the present study our main emphasis was to determine the impact of BGS and CIK-515 on tuber yield, nutrient concentration and nutrient use efficiency indices in fertilized or non-fertilized field conditions. We hypothesized that inclusion of BGS and CIK-515 assist mineral fertilizers in improving nutrient use efficiency of potato and ultimately enhance potato growth and tuber yield. Some work has been reported on BGS regarding crop yield (Ahmad *et al*., 2014b) and heavy metal stabilization in soil (Ahmad *et al*., 2015), perhaps this is the first study to elucidate the impact of BGS and PGPR on nutrient concentration and their efficiency indices in potato crop under field conditions.

Experimental

Characterization of BGS and soil: Semi-dry form of BGS from cattle dung was obtained from a biogas plant situated in the city of Faisalabad, Pakistan (30°31.5′ N, 73°74′ E) and was analyzed for various physiochemical properties [pH 7.2; organic matter 48%; nitrogen (N) 1.6%; phosphorus (P) 0.52% and potassium (K) 1.1%] before applying to the soil. Shade-dried and grinded form of BGS (C:N ratio = 37.60) was incorporated into the soil at the time of seedbed preparation. Soil samples were collected before sowing from the depth of 0-15 cm and were analyzed for soil physiochemical properties (pH 8.1; organic matter 0.7%; N 0.04%; P_2O_5 0.0006% and K_2O 0.03%). Organic matter in the soil and BGS was determined according to Walkley and Black (1934) and loss of weight on ignition method (Ahmad *et al*., 2015), respectively. Medium loam soil textural class (sand 48%; silt 29%; clay 23%) was established by using Moodie *et al*., (1959) procedure. Total N was estimated by following Gunning and Hibbard's method (Jackson, 1962) through the digestion of sulfuric acid and distillation of ammonia into boric acid (4%). Phosphorus and potassium was determined by using Olsen's method and flame photometer, respectively (US Salinity Lab. Staff, 1954). pH meter (JENCO Model-671 P) was used to determine pH of soil by making paste of soil saturated with distilled water, while pH of BGS was determined by making BGS to water ratio (1:2).

Collection and preparation of PGPR strain: Plant growth promoting *Bacillus* strain (CIK-515) was collected from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Inoculum was prepared in Erlenmeyer flasks by inoculating desired strain using sterilized nutrient broth and incubated at 28 ± 1°C for 48 h in shaking incubator (100 rpm). Optical density was measured at 540 nm and uniform population [optical density OD_{540} = 0.5; 10^7-10^8 colony forming unit (cfu) mL^{-1}] was achieved by dilution with sterilized water prior to seed inoculation.

Field experiment: A field experiment was conducted into randomized complete block design (RCBD) with plot size of 6 × 3 m^2 for each plot having row to row distance 45 cm and plant to plant distance 15 cm and was performed at the University of Agriculture, Faisalabad, Pakistan,. Five treatments were applied *vis.* BGS @ 600 kg ha^{-1} with RNPK (Recommended dose of N, P, K); CIK-515 with RNPK; both CIK-515 and BGS with RNPK; RNPK only and control (without RNPK, BGS and CIK-515). Recommended rates of N, P and K for potato production were 250, 125 and 125 kg ha^{-1} and were derived from different sources such as urea (CH_4N_2O), di-ammonium phosphate (DAP; $(NH_4)_2HPO_4$) and potassium sulfate also known as sulfate of potash (SOP; K_2SO_4), respectively. BGS was incorporated at the time of seedbed preparation while potato seedlings were grown in nursery using tuber seeds (cv. Santa, Punjab Seed Corporation Sahiwal, Pakistan). Thereafter, potato seedlings of same size was uprooted and kept in 200 mL inoculum of (10^7-10^9 cfu mL^{-1}) *Bacillus* strain (CIK-515) for 2 h. For the uninoculated control treatment, potato seedlings were immersed in 50 mL sterilized medium. Inoculated seedlings were sown in soil-plots on beds amended with different treatments (described above) and irrigated with canal water (pH = 7.86 and EC = 0.45 dS m^{-1}) when required.

Plant morphological and nutrient analysis: The morphological growth performance of potato plant was assessed. From each plot, ten plants were randomly tagged and data regarding plant height, number of leaves $plant^{-1}$, number of stems $plant^{-1}$, dry weight (vine, tuber and total plant) and tuber yield from all the plots were recorded at the stage of plant maturity attained ≈ 85 days after planting. Harvest index was calculated according to Ojulong *et al*., (2010) using the following formula.

$$\text{Harvest index (HI)} = \frac{\text{Tuber yield}}{\text{Biological yield}} \qquad (1)$$

Tuber yield was calculated from the fresh weight of potato tuber (kg ha^{-1}), while biological yield was calculated from the fresh weight of potato tuber and potato vines (vines = stem + leaves; kg ha^{-1}). Potato vines (n = 3) and tubers (n = 3) from each plot were first oven dried at 65°C until constant weight and then grinded, separately. Thereafter, 0.1 g from each grinded part was digested in the mixture of concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). N concentrations in vine and tuber were then determined by distillation of ammonia into 4% boric acid by microkjeldahl apparatus while phosphorus was determined using Barton reagent on spectrophotometer (Ashraf *et al*., 1992). Nutrient (N, P) accumulation in whole plant (vines + tubers) was estimated by summing the N and P concentrations in vines and tubers. Different forms of nutrient (N, P) efficiency indices were computed according to Zvomuya & Rosen (2002).

$$\text{NUE or PUE} = \frac{\text{TNp} - \text{TNc}}{\text{Nf} - \text{Nc}} \quad (2)$$

$$\text{ANE or APE} = \frac{\text{TDMf} - \text{TDMc}}{\text{Nf} - \text{Nc}} \quad (3)$$

$$\text{NHI or PHI} = \frac{\text{Tuber Nf} - \text{Tuber Nc}}{\text{TNp} - \text{TNc}} \quad (4)$$

$$\text{PNE or PPE} = \frac{\text{TDMf} - \text{TDMc}}{\text{TNf} - \text{TNc}} \quad (5)$$

where, NUE or PUE (N or P use efficiency); ANE or APE (Agronomic N or P use efficiency); PNE or PPE (Physiological N or P use efficiency); and NHI or PHI (N or P harvest index, which is the proportion of total plant N or P that is partitioned to potato tubers); TNp (Total N or P use by plant (vines plus tuber) in treated soil); TNc (Total N or P use by plant (vines plus tuber) in control); Nf (Amount of N or P applied in treated soil); Nc (Amount of N or P applied in control soil); TDMf (Total dry matter of plant (vines plus tuber) produced in treated soil); TDMc (Total dry matter of plant (vines plus tuber) produced in control); Tuber Nf (Total N or P use by tuber in treated soil); Tuber Nc (Total N or P use by tuber in control).

Statistical analyses

One way analysis of variance was applied to analyze the data (Steel *et al*., 1997). The treatment means were compared using Post-hoc HSD Tukey comparison test at 5% probability level. Simple linear regression analyses were used to estimate relative contribution of each treatment (fertilizer applied, BGS and/or *Bacillus* strain CIK-515) on tuber yield, nutrient concentrations and nutrient use efficiency indices by potato plant and to confer relation of tuber and dry yield with nutrient contents (N, P) in plant.

Results

Plant growth and yield: All treatments except control showed significant improvement in plant height (Fig. 1a; t-test, $p<0.05$), while BGS and CIK-515 strain, whether alone or in combinations, have significantly increased number of leaves when RNPK was applied (Fig. 1b; t-test, $p<0.05$). However, RNPK fertilization alone was found statistically non-significant in increasing the number of leaves in comparison to control. The treatments did not induce significant effect on number of stems per plant (Fig. 1c; t-test, $p>0.05$). Plant yield in terms of vine and tuber dry biomass, tuber yield and harvest indices significantly increased with the use of RNPK, BGS and CIK-515 as compared to control (Fig. 2, t-test, $p<0.05$). RNPK fertilization alone showed significant increase in vine dry biomass (>30%) and tuber yield (>26%) compared to control. Addition of BGS along with RNPK further improved vine and total plant (vine + tuber) dry biomass and tuber yield by 76, 42 and 51%, respectively. This treatment combination (NPK + BGS) produced plant dry biomass and tuber yield significantly greater than control and RNPK alone. Integration of BGS with RNPK also showed promising impact on tuber yield (Fig. 3a; t-test, $p>0.05$). Plants inoculated with strain CIK-515 along with NPK also showed significant increase in vine dry biomass (35%) and tuber yield (31%) compared to control treatment. The treatment combination of NPK + *Bacillus* strain CIK-515 + BGS showed significant improvement in vine (76%) and total plant dry biomass (32%) and tuber yield (48%) as compared to control (Figs. 2, 3, t-test, $p<0.05$). Results showed good relation of tuber yield vs. N contents in plant ($R^2 = 0.76$, Fig. 4a) as compared to P contents in plant ($R^2 = 0.64$, Fig. 4c), in case of dry yield vs. P contents in plant ($R^2 = 0.91$, Fig. 4d) better relation was existed in comparison to N contents in plant ($R^2 = 0.53$, Fig. 4b).

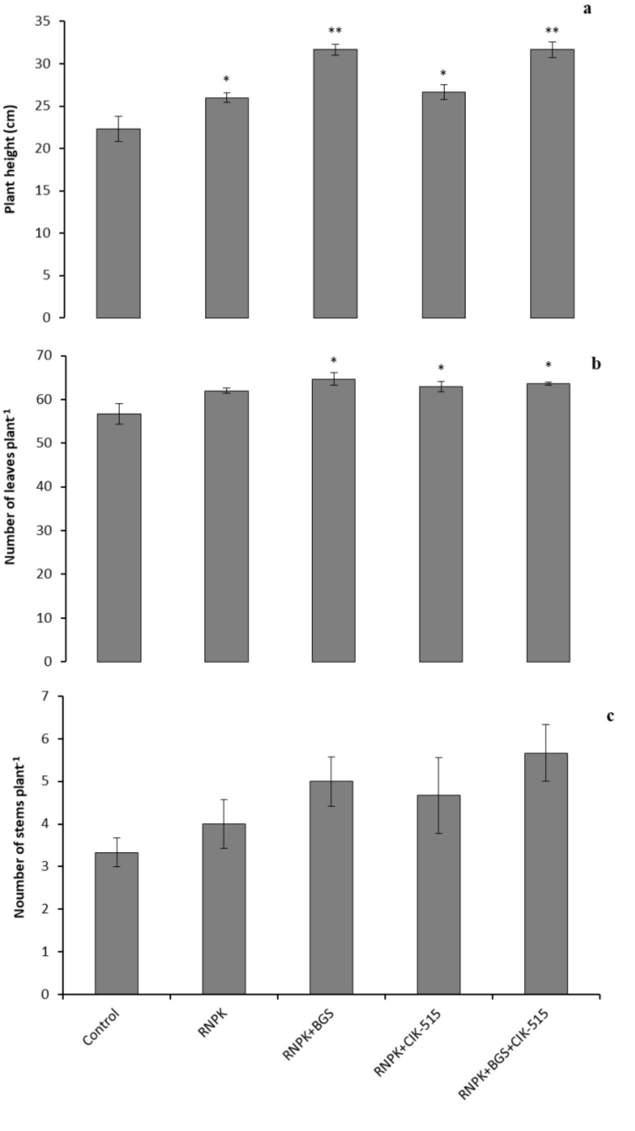

Fig. 1. Effects of biogas slurry (BGS), plant growth promoting *Bacillus* strain (CIK-515) and recommended NPK (RNPK) singly and in different combinations on plant height (a), number of leaves (b) and stems per plant (c). Different bars represent mean (±SE) and asterisks show significant ($p<0.05$) differences from respective control* and RNPK**.

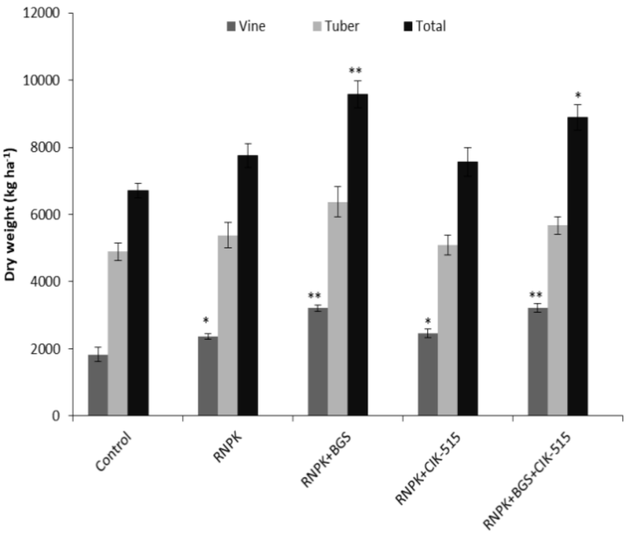

Fig. 2. Effects of BGS, CIK-515 and RNPK singly and in different combinations on dry weight of vine, tuber and total biomass. Different bars represent mean (±SE) and asterisk shows significant (p<0.05) differences from respective control* and RNPK**.

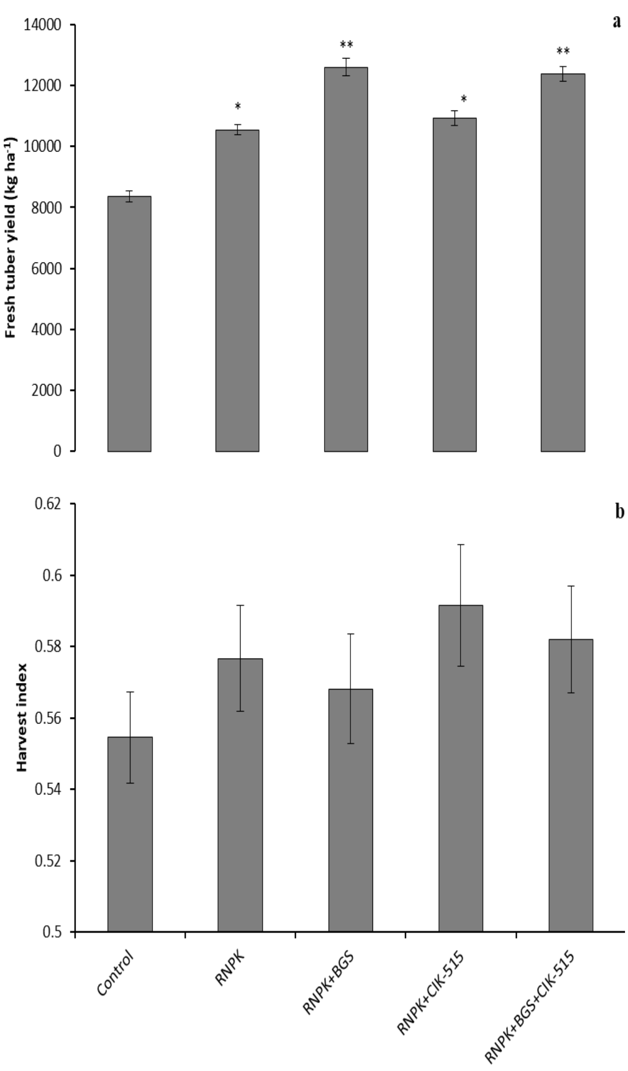

Fig. 3. Effects of BGS, CIK-515 and RNPK singly and in different combinations on tuber yield (a) and harvest index (b). Bars represent mean (±SE) and asterisk shows significant (p<0.05) differences from respective control* and RNPK**.

Nutrient concentration and nutrient use efficiency indices: Mineral nutrient (N, P) concentration increased in aerial and underground parts of potato plant by the application of different treatment combinations (Table 1). All the treatments showed significantly higher nutrient concentration (p<0.05) in potato plant in comparison to control. Integration of RNPK, BGS and CIK-515 enhanced maximum concentration of N and P in vine by 183 and 157%, respectively, as compared to control. Whereas, BGS performed better in the presence of RNPK by increasing N (68 and 80%) and P (64 and 71%) concentration in tuber and in potato plant (vine + tuber), respectively. Nutrient use efficiency indices (NUE) were also influenced by the inclusion of different treatments. NUE and agronomic nitrogen use efficiency (ANE) and physiological N use efficiency (PNE) have positive relation with tuber yield but in decreasing order (NUE, R^2 = 0.73 > ANE, R^2 = 0.58 > PNE, R^2 = 0.56), whereas, the results were completely opposite in case of P vs. tuber yield (PPE, R^2 = 0.72 > PUE, R^2 = 0.66 > APE, R^2 = 0.57). Above-mentioned results have shown a positive relationship between the nutrients (N, P) and tuber yield as both of the nutrients have improved NUE (Fig. 5a) and PPE (Fig. 5f), respectively. Similarly, these results have also shown relative contribution of each treatment in improving NUE indices and tuber yield and the results are thus indicating the supremacy of BGS either alone or in combination with CIK-515. However, application of BGS along with NPK fertilizers showed maximum improvement in these indices.

Discussion

Our results showed that potato plant growth has significantly improved upon application of BGS alone or in combination with CIK-515 inoculation (Fig. 1). Results from field experiments determined the significant effect of BGS and PGPRs on potato tuber production and, therefore, we further analyzed their sole and combined impacts on nutrients concentration, plant physical appearance in terms of vine and leave growth and productivity. Adequate supply of mineral nutrients has already been shown to stimulate different processes of plant growth development (Lima *et al*., 2010; Omondi *et al*., 2018) and, similarly, results from present study have shown that mineral nutrients have produced good effects on dry matter production (Fig. 2). Results of this study are in agreement of the previous findings conducted on cereal crops such as wheat and vegetable crops such as okra (Akanbi *et al*., 2010; Abubaker *et al*., 2012).

In addition, mineral nutrition have been reported to enhance fundamental processes of plant growth and development i.e. photosynthesis, flowering, fruiting and maturation (Kareem 2013), which may have enabled potato plants to produce good foliage and high yielding tubers (Figs. 2, 3). N and P are the most limiting nutrients for plant production, as addition of BGS increases N and P concentrations in soil and therefore, availability of these nutrients to potato plant that might be responsible for improved plant growth. Dry biomass of potato plant parts (vine and total plant) significantly increased in soil received RNPK fertilization along with BGS as compared to both control (without NPK) and RNPK treatments, which indicated beneficial effects of horizontal expansion approach of combined application of organic and inorganic fertilizers (Tables 1, 2).

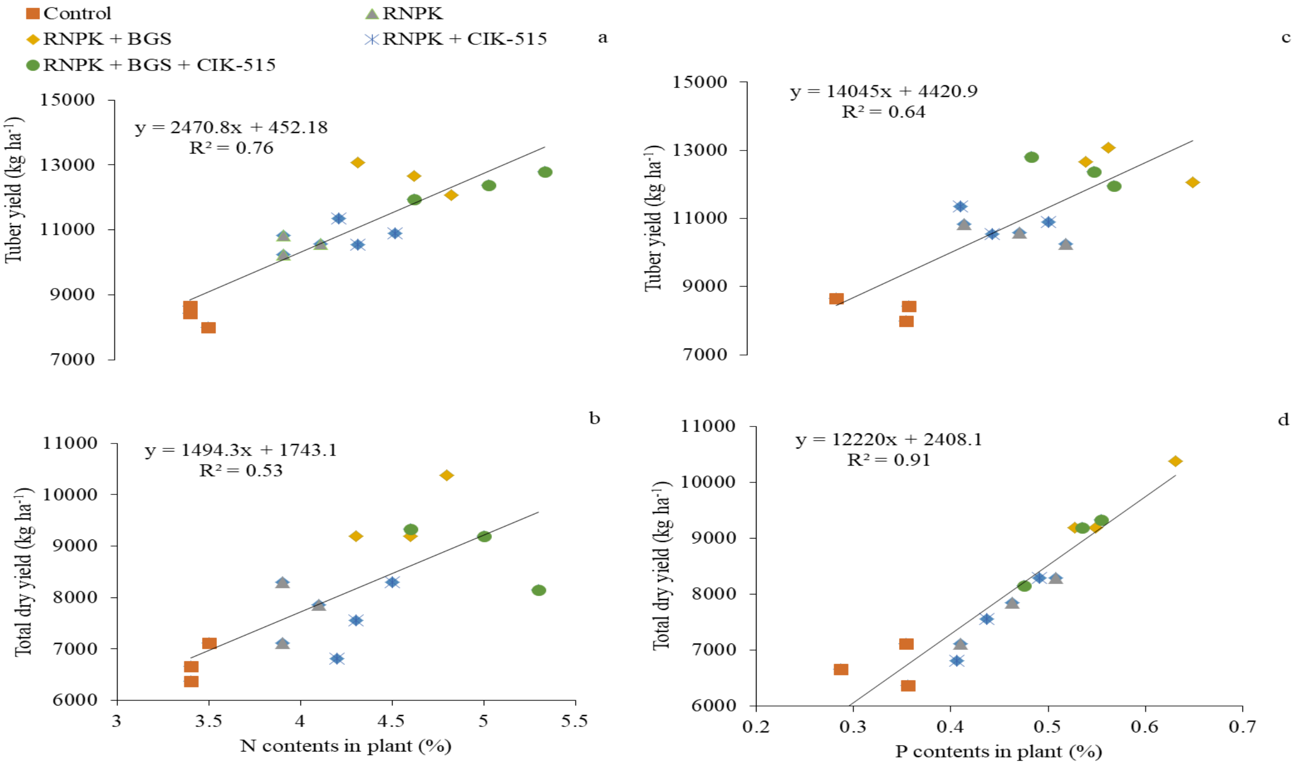

Fig. 4. Correlation of nitrogen and phosphorus contents in plant with fresh tuber yield (a, c), and total dry yield (b, d) as affected by the application of different treatments (n = 15).

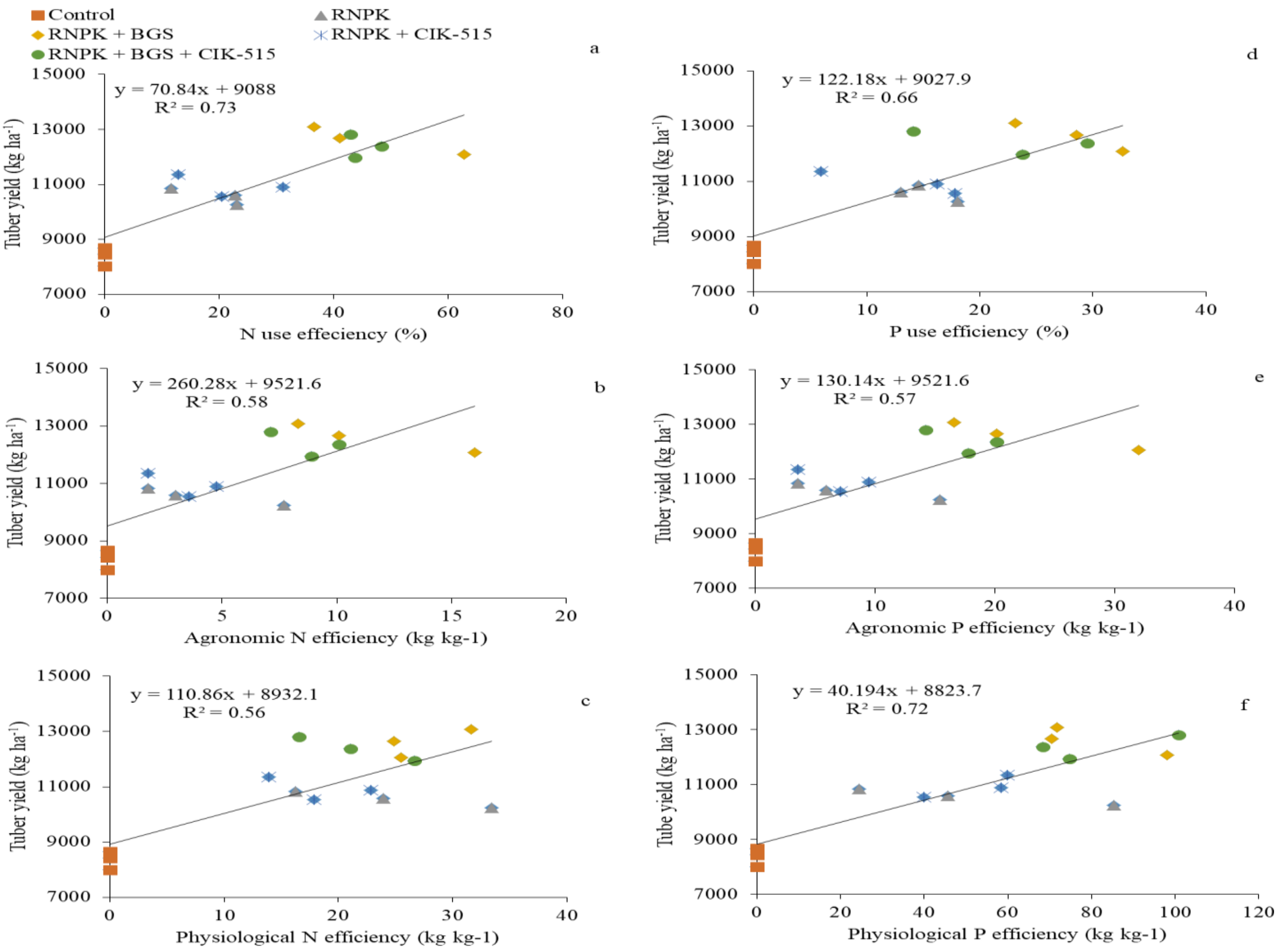

Fig. 5. Correlation of fresh tuber yield with nitrogen (N) or phosphorus (P) use efficiency (a, d), agronomic (b, e) and physiological N or P use efficiency (c, f) affected by the application of different treatments (n = 15).

Table 1. Effect of biogas slurry (BGS) and plant growth promoting *Bacillus* strain CIK-515 on nutrient concentrations and efficiency indices.

Treatments	Nitrogen concentration %			%	Nutrient efficiency indices kg kg^{-1}		
	Vine	Tuber	Plant	†NUE	‡NHI	¶ANE	¥PNE
Control†	1.10^{c}	2.33^{c}	3.43^{c}				
Recommended NPK	1.33^{bc}	2.63^{bc}	3.97^{bc}	19^{c}	0.68	4.15^{bc}	24.52
Recommended NPK + BGS	1.57^{ab}	3.00^{ab}	4.57^{ab}	47^{a}	0.69	11.46^{a}	27.34
Recommended NPK + PGPR	1.60^{ab}	2.73^{abc}	4.33^{ab}	21^{bc}	0.55	3.36^{bc}	18.22
Recommended NPK + BGS + PGPR	1.78^{a}	3.20^{a}	4.97^{a}	45^{ab}	0.64	8.69^{ab}	21.44
	Phosphorus concentration			**†PUE**	**‡PHI**	**¶APE**	**¥PPE**
Control	0.22^{b}	0.92^{b}	1.15^{b}				
Recommended NPK	0.28^{a}	1.14^{a}	1.43^{a}	15	0.85	8.30^{bc}	51.79
Recommended NPK + BGS	0.31^{a}	1.17^{a}	1.48^{a}	28	0.82	22.91^{a}	80.11
Recommended NPK + PGPR	0.29^{a}	1.16^{a}	1.44^{a}	13	0.76	6.72^{bc}	52.69
Recommended NPK + BGS + PGPR	0.32^{a}	1.18^{a}	1.50^{a}	23	0.76	17.38^{ab}	81.31

Means carrying similar letter(s) in a column do not differ significantly at $p>0.05$
†Control without NPK fertilizer; †N/P use efficiency; ‡N/P harvest index; ¶Agronomic N/P use efficiency; ¥Physiological N/P use efficiency

Though positive effect of combined application of chemical fertilizer along with organic amendments has previously been reported (Akanbi *et al*., 2010), however, the present study has shown that the duo effect may further be enhanced by co-inoculating with *Bacillus* strain CIK-515. This strain is known to produce growth hormone auxin (Ahmad *et al*., 2014a), which is responsible for production of higher plant biomass and yield. This strain also exhibiting catalase and exopolysaccharide activities (Ahmad *et al*., 2014a), which are helpful in nutrient and water acquisition to host plant. BGS is the major source of macro- and micro-nutrients which are plausible for inducing higher plant yield. For example, Abubaker *et al*., (2012) found that dry matter production of wheat increased in soil fertilized with BGS alone as well as combined with small amount of chemical fertilizers. Additionally, strong positive relation existed between total dry yield and P contents in plant ($R^2 = 0.91$) as compared to N contents in plant ($R^2 = 0.53$), which is an indication of triggering biochemical attribute of plant by exogenous P or other treatments (BGS, CIK-515) (Figs. 4b, 4d). A positive correlation of P contents in the youngest leaf blade with dry yield ($R^2 = 0.72$) was also reported by Omondi *et al*., (2018) in Cassava received NPK fertigation. Improvement of plant growth and tuber yield in this study might be due to increase in cell division, cell elongation and photosynthetic rate, which has been reported to be triggered in the presence of increased nutrient supply, especially N (Lima *et al*., 2010; Makino 2011; Omondi *et al*., 2018).

Harvest indices under different treatment combinations were increased but none of them showed significant impact as compared to control. Tuber yield might have been improved due to efficient uptake of N in the rhizosphere, decreased N losses from soil and higher assimilation of carbohydrates in tubers. This positive effect of BGS on plant attributes could be due to greater NH_4-N, which seems to be related with greater efficiency of applied N (Table 1, Fig. 5a). Sole application of organic fertilizer could not supply enough quantities of nutrient elements to the host crop, and therefore, it has been recommended integration of nutrients for getting higher crop production (Ahmad *et al*., 2014b). Relatively positive contribution of N input has also been confirmed from the higher level of correlation between N contents in plant and tuber yield ($R^2 = 0.76$, Fig. 4a) compared to the correlation exist between P contents in plant and tuber yield ($R^2 = 0.64$, Fig. 4c). These correlation results indicated that accumulation of essential nutrients in plant has increased with the application of exogenous application of BGS and CIK-515, alone or in combination, which could have been resulted into higher dry and tuber yield (Fig. 4).

Though, it is obvious that CIK-515 inoculation gave significant potato growth improvement, however, its efficiency found to be increased in the presence of BGS and their combination showed significant increase in economic yield of tuber in comparison to not only control but also from NPK fertilizer alone (Figs. 3 and 4). This enhancement may be due to ability of CIK-515 to solubilize inorganic soil P that could be available immediately to host plant for growth and development. Results showed that P concentration increases upon PGPR inoculation in the presence of BGS (Table 1) but these results are statistically at par with sole application of RNPK fertilizer and therefore, this explanation might not be necessarily applicable. Apart from this, inoculating CIK-515 are also known to produce auxin (Ahmad *et al*., 2014a) which may trigger cell division lead to greater biomass production, as the inoculated plants showed higher dry biomass, so that, this might be a possible reason for better plant health, which is translated into a greater agronomic N and P use efficiency indices (Table 1, Fig. 5). Inoculation of bacteria producing auxin has induced beneficial effects on maize, pea, potato biomass and yield (Ahmad *et al*., 2014a; Tahir *et al*., 2019; Bashir *et al*., 2020). These beneficial effects might be attributed to presence of BGS, as we have mentioned earlier that it contains substantial amounts of nutrients and hormones, which helps the plant to uptake essential nutrients required for plant homeostasis to grow and develop. Similar to our findings, Ahmad *et al*., (2014b) reported increased biomass and yield of maize upon inoculation of PGPR containing ACC-deaminase along with BGS.

Inoculation of beneficial PGPR along with BGS has proved to be an effective amendment to get higher production of crop not only in normal conditions (present study) but also equally good in harsh environment (Ahmad *et al*., 2014a, 2014b, 2015).

Different nutrient (N, P) use efficiency indices were influenced by the treatment combinations used in this study however recommended rates of NPK fertilizer along with BGS showed significant improvement in NUE, ANE and APE (Table 1). It implies that BGS is an important soil conditioner, which affects not only N concentration in potato plant but also translated it into agronomic produce (Figs. 1, 2, 3) by enhancing ANE and APE. It has been reported that integrated use of organic fertilizer (compost, farm yard manure, pig slurry) in combination with chemical fertilizer improved crop yield per unit fertilizer applied (Rahman *et al*., 2008; Akanbi *et al*., 2010; Akhtar *et al*., 2019). However, it needs careful manipulation of plant, soil, and chemical fertilizers (organic/inorganic). Results also confirmed that this combination (recommended NPK + BGS) improved potato productivity, total N and P concentrations, N and P use efficiency indices significantly (Table 1, Fig. 5). The applied treatments has produced NUE (R^2 = 0.73, Fig. 5a) > ANE (R^2 = 0.58, Fig. 5b) > PNE (R^2 = 0.56, Fig. 5c) whereas produced PPE (R^2 = 0.72, Fig. 5f) > PUE (R^2 = 0.66, Fig. 5d) > APE (R^2 = 0.57, Fig. 5c), which has translated into tuber yield. It has previously been reported that accumulation of macronutrients have influence on the allocation of photo-assimilates below ground plant parts, thus, the balanced accumulation of NPK could promote assimilation and conversion of nutrients into starch that accumulated in below ground plant parts (Omondi *et al*., 2018).

Additionally, distribution of N and P in different parts of potato (vine, tuber and total plant) plant was significantly influenced by the addition of BGS in the presence of NPK fertilizers (Table 1). Improvement of nutrient concentration in different plant parts could be due to the reason that BGS contained macro and micronutrients. Although, this study did not determine the impact of BGS on soil physical properties; however, its application brought changes in soil organic matter pool, nutrient concentrations, bulk density, water holding capacity and soil temperature, that has previously been reported as well (Akanbi *et al*., 2010), which ultimately would have increased NUE and crop yield. Another significant impact of BGS could be to stimulate diverse soil microbial community (Tuyishime *et al*., 2011) that has the ability to sustain N losses, enhance N mineralization and finally increase NUE of plant.

Conclusion

The results from this study has confirmed that co-inoculation of BGS and *Bacillus* strain CIK-515 in the presence of RNPK has shown productive combination for improving plant health, tuber yield and nutrient use efficiency indices, however, the effect has been seen significant even when used without CIK-515 as compared to control plants. Therefore, incorporation of BGS along with RNPK fertilizer and *Bacillus* strain CIK-515 could be manipulated in field for enhanced production of potato tuber yield through attenuation of basal soil nutrients. However, we suggest further studies to examine the effects of above combination on soil physical and microbial properties in relation to plant growth and physiology.

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