

IMPROVING THE PRODUCTIVITY OF CUCUMBER THROUGH COMBINED APPLICATION OF ORGANIC FERTILIZERS AND *Pseudomonas fluorescens*

Maqshoof Ahamd^{1,*}, Muhammad Sadam Hussain Zeshan¹, Muhammad Nasim², Zahir Ahmad Zahir³, Sajid Mahmood Nadeem⁴, Farheen Nazli² and Moazzam Jamil¹

¹Department of Soil Science, University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan; ²Pesticide Quality Control Laboratory, Bahawalpur-63100, Pakistan;

³Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan;

⁴University of Agriculture Faisalabad, Sub-campus Burewala, Vehari, Pakistan.

*Corresponding author's e-mail: maqshoof_ahmad@yahoo.com

Deficiency of macro and micronutrients in agricultural soils is major concern for crop production in Pakistan. The chemical fertilizers are being used to fulfill the crop requirements which are costly and their availability is also an issue for the farmers. The integrated use of organic fertilizer and microbial inoculants is an effective strategy for sustaining soil fertility, and has good impact on plant growth and soil health. A field experiment was conducted to evaluate different sources of organic manure and chemical fertilizer in combination with *Pseudomonas fluorescens* to enhance the production of cucumber (*Cucumis sativus* L.). The experiment was laid down in Randomized Complete Block Design (RCBD) with three replications. Results showed that the application of organic manure, chemical fertilizer and *P. fluorescens* separately and in combination significantly enhanced growth, yield and fruit quality of cucumber. However, the combined use of biogas slurry and *P. fluorescens* showed maximum improvement in shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and total yield of cucumber as compared to control (T₁). This treatment also improved the N, P, and K concentration in fruit of cucumber as compared to control. The results suggested that combined use of biogas slurry and *P. fluorescens* could effectively be used to improve the growth, yield and quality of cucumber. So, the integrated use of biogas slurry and *P. fluorescens* should be recommended to the farmers to enhance productivity of cucumber.

Keywords: cucumber, biogas slurry, *P. fluorescens*, yield.

INTRODUCTION

Integrated plant nutrient management system (IPNMS) is an approach to optimize the nutrient requirement of plants by managing soil on sustainable basis (Roy *et al.*, 2006). The IPNMS seeks for both, increase in agricultural production and sustaining the resources for the future generations. It is a strategy to utilize and manage the organic and inorganic plant nutrient sources for improving crop productivity and soil quality (Peter *et al.*, 2000; Rajamanickam *et al.*, 2011). Chemical fertilizers are considered as a compulsory component for crop production. The continuous and excess application not only reduces the profitability but deteriorates environment quality. Therefore, to get the maximum economic output, it is imperative to use organic nutrient sources in combination with chemical fertilizers.

The use organic nutrient sources (such as farm yard manure, poultry manure, etc.) in combination with chemical fertilizers is helpful for improving soil health and sustaining crop production and soil fertility (Timsina and Conner, 2001). It not only prevents the soil from deterioration but also maintains its fertility status (Rekhi *et al.*, 2000). Farm yard manure contains all major plant nutrients i.e. nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and all

the micronutrients but the quantity of these nutrients is not enough to fulfill the complete nutrient requirement of the crops. Due to smaller quantity of these nutrients, it is necessary to use farm yard manure along with other organic or chemical fertilizers (Dejene and Lemlem, 2012). Poultry manure contains large amount of micro and macronutrients. It adds organic matter in soil that improves soil structure, nutrient retention in soil, soil aeration, water holding capacity of soil and infiltration rate of water (Deksissa *et al.*, 2008). Biogas slurry is considered as a rich source of nutrients for plant growth and helps to improve the quality and yield of crops (Liu *et al.*, 2008). It contains the essential nutrients and also the organic matter contents and hence it is considered as soil amendment for the improvement of plant growth (Garg *et al.*, 2005).

In IPNMS, the use of plant growth promoting rhizobacteria (PGPR) to enhance crop productivity has gained significant importance. The inoculation of PGPR decreases use of chemical fertilizers by increasing their use efficiency. They promote plant growth by colonizing rhizosphere and play essential role in nutrient cycling thus help plants to grow efficiently under nutrient deficient conditions. The use of PGPR reduces agrochemical use and supports ecofriendly sustainable food production (Podile and Kishore, 2006). The

PGPR affect plant growth through various mechanisms such as nitrogen fixation, mineral solubilization and increasing the plant growth by changing the endogenous level of plant hormones (Ahmad *et al.*, 2011; Qasim *et al.*, 2014).

In Pakistan, the use of inputs, especially nutrients, is not much efficient due to poor management practices and the farmer cannot gain profit as per yield potential. The conditions are even worse for vegetables which require more fertilizer inputs (Tahir and Altaf, 2013). Cucumber is a major vegetable crop in Pakistan that is grown throughout the year as seasonal and off-season crop (Mukhtar *et al.*, 2013; Iqbal *et al.*, 2013). Improving IPNMS for vegetable production, by developing optimum management of organic, chemical and biofertilizers (PGPR), may help to enhance the use efficiency of inputs that not only increase the profitability of growers but also strengthen the economy of the country (Iqbal *et al.*, 2013). Keeping in view the above facts, a field study was conducted to evaluate the effect of chemical fertilizer in combination with organic manure and *Pseudomonas fluorescens* on the production of cucumber under field condition.

MATERIALS AND METHODS

Collection of PGPR strain: The PGPR strain *Pseudomonas fluorescens* (Nadeem *et al.*, 2009) was obtained from Soil Microbiology and Biochemistry Lab., Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad.

Preparation of inoculum: Inoculum was prepared by using DF minimal salt medium without agar (Dworkin and Foster, 1958) containing ACC as substrate (N source). Flask containing broth was inoculated with *Pseudomonas fluorescens* and incubated at 28 ± 1 °C for 72 hours under shaking (100 rpm). After incubation, the optical density was measured at 540 nm, and a uniform population ($OD_{540} = 0.45$; 10^7 – 10^8 cfu mL⁻¹) was achieved by dilution with sterilized water prior to seed inoculation.

Field study: A field study was conducted to evaluate the effect of different organic fertilizers and *P. fluorescens* in combination with chemical fertilizer on the production of cucumber in randomized complete block design (RCBD) with treatments as: T₁; Control (without NPK), T₂; Recommended dose of NPK from chemical sources, T₃; Farmyard manure @ 10 tons per hectare, T₄; Poultry manure @ 8 tons per hectare, T₅; Biogas slurry @ 600 kg per hectare, T₆; *P. fluorescens*, T₇; *P. fluorescens* T₈; Farmyard manure @ 10 tons per hectare + *P. fluorescens*, T₉; Poultry manure @ 8 tons per hectare + *P. fluorescens*, T₁₀; Biogas slurry @ 600 kg per hectare + *P. fluorescens*. In the treatments where organic sources of nutrients and *P. fluorescens* were used, the extra amount of NPK was met from chemical fertilizers.

Prior to sowing, soil samples were collected and analyzed for chemical characteristics according to standard protocol as described by Ryan *et al.* (2001). Soil organic matter contents were determined according to Moodie *et al.* (1959). The recommended dose of P and K, and 1/3rd dose of N were applied as basal dose, in the form of diammonium phosphate, sulfate of potash and urea, respectively. The remaining dose of N was applied in two splits with 15 days interval. Sources of organic fertilizers were applied at the time of seed bed preparation. Cucumber seeds were inoculated with *P. fluorescens* by using slurry prepared with sterilized peat, broth culture ($OD_{540} = 0.45$; 10^7 – 10^8 cfu mL⁻¹) of *P. fluorescens* and sterilized sugar solution (10%) in 5:4:1 ratio. Good quality irrigation water was used for irrigation along all the recommended agronomic practices. Mature plant samples were taken for growth, yield and quality parameters by following the standard protocols.

Table 1. Physical and chemical characteristics of the soil before sowing of crop

Parameter	Unit	Value
EC _e	dS m ⁻¹	1.3
Ph	---	7.6
Organic matter	%	0.57
Total N	%	0.082
Available P	ppm	3.0
Extractable K	ppm	88
Saturation percentage	%	33
Textural class	---	Sandy loam

Table 2. Chemical analysis of organic manures

Manures	N (%)	P (%)	K (%)	pH
Farm yard manure	0.52	0.96	1.05	8.3
Poultry manure	0.69	1.34	0.82	7.8
Biogas slurry	0.78	1.01	0.91	6.6

Fruit analysis: Nitrogen, phosphorus and potassium were determined after wet digestion. The plant samples were digested according to the method of Wolf (1982). For this purpose, 0.2 g dried and ground plant sample was taken in digestion tubes. After the addition of 4 mL of concentrated H₂SO₄ in each digestion tube, these were incubated over night at room temperature. Then, 2 mL of analytical grade H₂O₂ (35%) was poured down through the sides of the digestion tubes and was rotated. Tubes were ported in the digestion block and heated up to 350 °C until fumes were produced and continued heating for 20 minutes. Digestion tubes were removed and 2 mL H₂O₂ was added slowly and again the digestion tubes were placed back into digestion block until fumes were produced for 20 minutes. After the repetition of above step the plant material became colorless and was cooled. The volume of extract was made up to 50 mL with distilled water. After filtration, the aliquot was used to determine nitrogen, phosphorus and potassium as

described by Ryan *et al.* (2001) and Chapman and Prat (1961).

Statistical analysis: Analysis of variance techniques (ANOVA) were applied to analyze the data (Steel *et al.*, 1997) using randomized complete block design (RCBD), and means were compared using LSD test at 5 % probability level.

RESULTS

Results showed that application of farm yard manure, poultry manure and biogas slurry in combination with *Pseudomonas fluorescens* significantly improved root fresh weight as compared to control (Table 3). Maximum improvement in root fresh weight was observed in T₁₀ where biogas slurry was applied @ 600 kg ha⁻¹ in combination with biofertilizer. Results of this treatment were non-significant with T₈, T₉, T₃ and T₅ but significantly better than all other treatments. The separate use of these organic sources and biofertilizer also gave significantly better results as compared to control. The combined application of organic sources (farm yard manure, poultry manure and biogas slurry) and biofertilizer was also significantly better than control in improving the root dry weight of cucumber (Table 3). Maximum root dry weight (4.1g) was observed in T₁₀, where biogas slurry @ 600 kg ha⁻¹ was applied in combination with biofertilizer. Minimum root dry weight was observed in control.

The results showed that combined application of organic sources (farm yard manure, poultry manure and biogas slurry) and biofertilizer was significantly better in improving shoot fresh and dry weight of cucumber (Table 3).

Maximum improvement in shoot fresh weight (85%) was observed in T₁₀ where biogas slurry was applied in the presence of biofertilizer while maximum improvement in shoot dry weight (103%) as compared to control was observed in the T₂ where chemical fertilizer was applied.

Table 4. Effect of different sources of organic manures and chemical fertilizer in combination with *P. fluorescens* on N, P, K and protein contents in fruit of cucumber

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)
T1	0.76 c	0.45 f	0.83 c
T2	0.85 c	0.67 d-f	1.15 bc
T3	1.47 a-c	0.75 c-e	0.89 c
T4	2.22 a	1.11 a	1.67 ab
T5	1.87 ab	0.83 b-d	1.86 a
T6	0.99 bc	0.56ef	0.99 c
T7	1.68 a-c	0.76 c-e	1.55 ab
T8	1.75 a-c	1.02 ab	1.60 ab
T9	0.82 c	0.84 b-d	1.65 ab
T10	1.74 a-c	0.91 a-c	1.87 a
LSD	1.02	6.39	0.56

Bars sharing same letters are statistically at par at 5% level of probability (n=3)

T₁; Control (without NPK), T₂; Recommended dose of NPK from chemical sources, T₃; Farmyard manure @ 10 tons per hectare, T₄; Poultry manure @ 8 tons per hectare, T₅; Biogas slurry @ 600 kg per hectare, T₆; *P. fluorescens*, T₇; *P. fluorescens* T₈; Farmyard manure @ 10 tons per hectare + *P. fluorescens*, T₉; Poultry manure @ 8 tons per hectare + *P. fluorescens*, T₁₀; Biogas slurry @ 600 kg per hectare + *P. fluorescens*.

Table 3. Effect of different sources of organic manures and chemical fertilizer in combination with *P. fluorescens* on root and dry weight, and shoot fresh and dry weight of cucumber

Treatments	Roots fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	No. of internodes plant ⁻¹
T1	5.30 e	0.82 e	103.30 cd	5.89 c	13.6 e
T2	6.86 e	1.30 de	143.00 bc	12.00 a	18.6 cd
T3	27.20 a-c	1.96 c	133.00 c	10.50 ab	19.3 b-d
T4	22.70 cd	1.98 cd	79.30 d	6.97 c	21.3 a-d
T5	25.60 a-d	2.61 b	131.60 c	6.63 c	23.3 a
T6	21.30 d	1.62 cd	114.30 cd	6.00 c	17.6 d
T7	24.30 b-d	1.86 cd	183.30 ab	9.57 b	19.3 b-d
T8	28.10 ab	1.98 bc	142.00 bc	7.00 c	22.3 a-c
T9	26.10 a-c	1.68 cd	127.60 cd	7.10 c	23.0 ab
T10	29.50 a	4.10 a	191.60 a	11.46 ab	23.3 ab
LSD	4.71	0.19	0.15	2.32	3.75

Bars sharing same letters are statistically at par at 5% level of probability (n=3);

T₁; Control (without NPK), T₂; Recommended dose of NPK from chemical sources, T₃; Farmyard manure @ 10 tons per hectare, T₄; Poultry manure @ 8 tons per hectare, T₅; Biogas slurry @ 600 kg per hectare, T₆; *P. fluorescens*, T₇; *P. fluorescens* T₈; Farmyard manure @ 10 tons per hectare + *P. fluorescens*, T₉; Poultry manure @ 8 tons per hectare + *P. fluorescens*, T₁₀; Biogas slurry @ 600 kg per hectare + *P. fluorescens*.

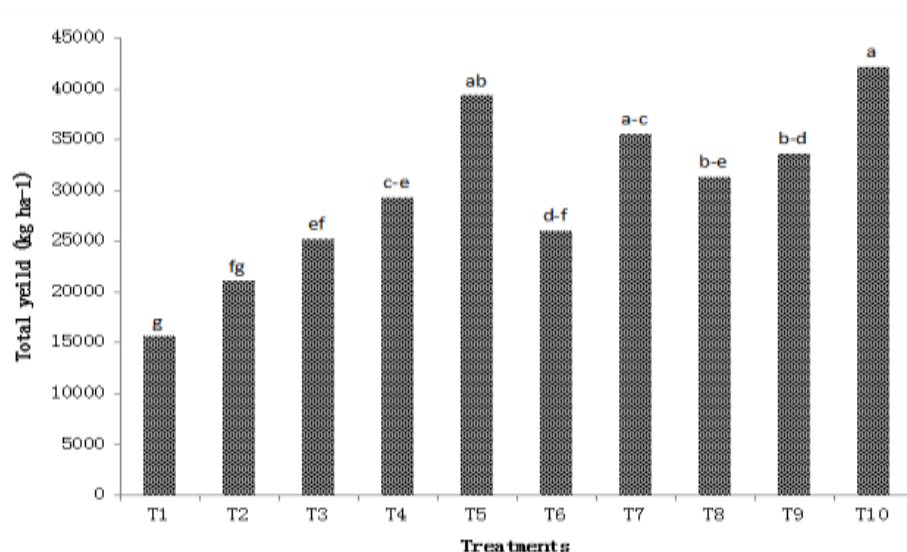


Figure 1. Effect of different sources of organic manures and chemical fertilizer in combination with *P. fluorescens* on total yield of cucumber.

Bars sharing same letters are statistically at par at 5% level of probability (n=3);

T₁; Control (without NPK), T₂; Recommended dose of NPK from chemical sources, T₃; Farmyard manure @ 10 tons per hectare, T₄; Poultry manure @ 8 tons per hectare, T₅; Biogas slurry @ 600 kg per hectare, T₆; *P. fluorescens*, T₇; *P. fluorescens* T₈; Farmyard manure @ 10 tons per hectare + *P. fluorescens*, T₉; Poultry manure @ 8 tons per hectare + *P. fluorescens*, T₁₀; Biogas slurry @ 600 kg per hectare + *P. fluorescens*.

Data also showed that separate application of organic sources and biofertilizer in presence of chemical fertilizer was significantly better than control in improving the number of

internodes plant⁻¹ (Table 3). However combined application was more effective and maximum number of internodes (23 nodes plant⁻¹) was observed in T₅. These results were non-significant with T₁₀, T₉, T₈, and T₄ while significantly better than control and chemical fertilizer (T₂). The nutritional quality of cucumber was also improved by the application of farm yard manure, poultry manure and biogas slurry separately and in combination with *P. fluorescens* (Table 4). Data revealed that maximum nitrogen (2.22%) and phosphorus (1.11%) contents were observed in T₄, where poultry manure was applied in the presence of chemical fertilizer. The maximum potassium contents (1.87%) were observed in T₁₀, where *P. fluorescens* was applied in combination with biogas slurry.

Data regarding total yield (Figure 1) showed that, the use of *P. fluorescens* and organic manures in the presence of chemical fertilizer significantly improved total yield as compared to unfertilized control. However, higher yield was observed when organic manures and *P. fluorescens* were applied in combination. Maximum total yield of cucumber ((42185 kg ha⁻¹) was observed in T₁₀ followed by T₅ (39325 kg ha⁻¹).

DISCUSSION

In present study, the evaluation of farm yard manure, poultry manure and biogas slurry was in combination with *Pseudomonas fluorescens* was carried out to observe improve the productivity of cucumber. The results from present study revealed that the combined use of organic and chemical fertilizers significantly enhanced growth, yield and fruit quality of cucumber as compared to chemical fertilizer and un-fertilized control. The integrated plant nutrient management (IPNM) for sustainable crop production targets to use mineral and organic sources of nutrients in combination. The IPNM also ensures the soil and environmental health in addition to improvement in crop productivity and quality (Mehdi *et al.*, 2007; Mahmoud *et al.*, 2009). The use of plant growth promoting rhizobacteria (PGPR) in combination with different organic sources and chemical fertilizer can be more effective in enhancing crop productivity and quality, in addition to improvement in physical and chemical conditions of soil. Results of our studies are in line with the findings of Ahmad *et al.* (2014) who reported that the combined use of organic and mineral fertilizers along with PGPR resulted in significant improvement in growth (54%) and yield (43%) as well as soil properties. This may be attributed due to the slow release of nutrients due to mineralization, retaining optimum

nutrients in solution, and increase in microbial activity (Bationo *et al.*, 2004). It has been documented that application of *P. fluorescens* having the ability to produce plant hormones results in enhanced root growth and nutrient use efficiency (Khakipour *et al.*, 2008). The improved root development and efficient uptake of nutrients by plants leads to increase in plant height and shoot biomass (Zahir *et al.*, 2007; Akhtar *et al.*, 2009).

The integrated use of organic fertilizers and *P. fluorescens* also improved the nutritional quality of cucumber fruit but with different degree of efficacy. The results are in line with previous findings as the integrated use of microbial and organic fertilizers resulted in significant increase in nitrogen, phosphorus and proteins contents of fruit (Esitken *et al.*, 2010; Sarwar *et al.*, 2014; Abou-El-Hassan *et al.*, 2014). This may be attributed to a number of plant growth promoting characteristics of *P. fluorescens* in addition to phosphate solubilizing ability (Nadeem *et al.*, 2009). The improved nutritional quality of fruit due to the integrated use of organic and microbial fertilizers has also been reported in the previous studies (Kumar *et al.*, 2011; Iqbal *et al.*, 2013). This may also be attributed to multifarious growth promoting traits of PGPR, in addition to beneficial effects of organic sources of nutrients (including poultry manure, farmyard manure and biogas slurry), which results in improved nutritional status of soil and physico-chemical properties including most imperative the water holding capacity (Ahmad *et al.*, 2014).

The combined use of organic manures (poultry manure, farmyard manure and biogas slurry) and chemical fertilizers along with *P. fluorescens* enhanced the crop yield due to increased soil fertility and availability of nutrients in soil solution (Abdel-Mouty *et al.*, 2001). This outcome could be because of the beneficial effects of *P. fluorescens* and increased bacterial activity (due to organic manures) that enhanced the increase in supply of mineral nutrients and increased syntheses of hormones (Saharan and Nehra, 2011). The increased root growth caused an improvement in nutrient uptake leading to improved shoot growth and yield of cucumber.

Conclusion: It is concluded from the present study that the combined use of biogas slurry and *Pseudomonas fluorescens* is significantly better than sole use of use of farmyard manure, poultry manure, and biogas slurry or chemical fertilizers. It may also be preferred over the use of farmyard manure and poultry manure in combination with *P. fluorescens*. Thus, the combined use of biogas slurry and *P. fluorescens* along with chemical fertilizers should be recommended to farmers for improving productivity of cucumber.

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