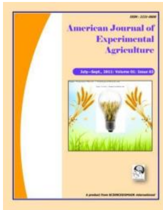

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Growth, Physiological, Yield and Quality Response in Chilli (*Capsicum annuum* L.) Under Wastewater Irrigation and Different Levels of Phosphorus

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Authors' contributions

This work was carried out in collaboration between all authors. Author SI designed the study, carried out the actual field work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript under the supervision of authors AI and AI while author SS together with author SI wrote the first draft, editing and formatting. The final draft was read and approved by all authors.

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ABSTRACT

Aims: India is one of the most densely populated, developing and industrially fast growing country of the earth that not only facing the problem of water scarcity, but also the mismanagement of tremendous amount of wastewater (WW) produced every day. Therefore a study was conducted in the Aligarh city of India on chilli (*Capsicum annuum* L.) cv. Pusa Sadabahar to observe the suitability of wastewater for irrigation along with different levels of phosphorus and how minimize the use of chemical fertilizers in agriculture by supplementing mineral nutrients through wastewater.
Study Design: Factorial randomized block design
Place and Duration of Study: The pot experiment was conducted during 2011-2012 in the net house of the Plant Physiology, Department of Botany, Aligarh Muslim University, Aligarh.
Methodology: Three levels of water, 50% wastewater, 100% wastewater and groundwater (GW) were used along with four basal doses of phosphorus at the rates of 0, 20, 40 and 60kgPha^{-1} with a uniform basal dose of nitrogen and potassium with the rates of 60kgNha^{-1} and 50kgKha^{-1}

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respectively. Fertilizers were applied one day before sowing. Both the waters and soil were analyzed for various physico-chemical characteristics.
Results: All the growth, physiological, yields as well as quality parameters were recorded at 60 days after sowing. Results revealed that wastewater irrigation significantly increases the growth, photosynthesis, yield and quality of the chilli. Lower dose of phosphorus fertilizer at the rate of 40kg ha^{-1} together with wastewater proved optimum and gave greater shoot and root length, shoot fresh and dry mass, leaf area, photosynthetic rate, chlorophyll content, nitrate raductase, carbonic anhydrase, ascorbic acid and leaf nitrogen content than control and even to higher nitrogen doses along with groundwater.
Conclusion: The wastewater proved an effective source of essential nutrients and even it could not be supplemented the whole nutrient requirement of the chilli but it can reduced the quantity of fertilizers because wastewater also a source of nutrients.

Keywords: Ascorbic acid; photosynthetic rate; yield, nitrogen.

1. INTRODUCTION

Water is undoubtedly a requirement for all of the living organisms in the biosphere [1]. Man has used the water systems for numerous purposes such as drinking, irrigation, fisheries, industrial processes, transportation and waste disposal, but due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants and also widened the gap between the supply and demand for water up to such alarming levels that in some parts of the world it posing a threat to human existence. Scientists around the globe are working on new ways of conserving water. On other hand the disposal of wastewater (WW) is a major problem faced by municipalities, particularly in the case of large metropolitan areas, with limited space for land based treatment and disposal. It is a suitable time, to refocus on one of the ways to recycle water-through the reuse of urban WW, for irrigation and other purposes [2] because the use of WW as a source of irrigation not only solves its disposal problem but also serve as a source of plant nutrients and organic matter. Its reuse can delivers positive benefits to the farming community [3]. However, WW may contain some undesirable constituents that may pose negative environmental effects and health risks. Thus, it can be considered as both a resource and a problem and there is urgent need for the WW management in a scientific manner. In many parts of the world, treated municipal WW and raw sewage WW and even industrial WW has been successfully used for the irrigation of various crops [4-5].

Nutrient supply in general considered to be most important limiting factor for growth and productivity [6]. Most of the plants require seventeen essential elements including Ni which was added more recently [7] for their normal growth and development. Of these N, P and K are the three major macronutrient effective in promoting the crop yields and required in large quantity, thus the maintenance of an adequate supply of nutrients to crop is one of the most basic and vital requirements for sustained crop growth and productivity. Agro ecosystems exports large quantities of nutrients in crop biomass and therefore, require large inputs regardless of internal cycling [8]. Use of inorganic and organic fertilizers has amused a great significance in recent years in vegetables production, for two reasons. Firstly, the need for continued increase production and per hectare yield of vegetables requires the increase amount of nutrients. Secondly, the results of a large number of experiments on inorganic and organic fertilizers conducted in several countries reveal that inorganic fertilizer alone cannot sustain the productivity of soils under highly intensive cropping systems [9]. Among nutrients nitrogen (N) increases the yield of chilli [10] and potassium (K) is reefed as quality element and known to improve colour and glossiness of chilli fruits. After nitrogen, phosphorus (P) is being considered second major element whose deficiency has become widespread in Indian soils [11]. P fertilizer is an expensive input and its use efficiency by crops may range from 10 - 25% [12]. Farmers mostly use single superphosphate as P source, which contains 16% P_2O_5 and 12% S [13]. Role of P in increasing tillering and growth is well recognized. P is an essential element and is involved in energy transfer through ATP. It is also involved in root development and in metabolic activities especially in synthesis of protein [14]. An adequate supply of P to the crop plants during their early growth period is very important for the initiation of leaves and florets primordial [15]. Application of P improves various growth parameters like, plant height, biomass and yield. Thus the main objective was to minimize the use of inorganic fertilizers and optimize the utilization of nutrients present in the WW and also safeguard the pollution of water and soil by using it in crop cultivation.

2. MATERIALS AND METHODS

Present study was aimed to evaluate the effect of WW along with different P levels on plant morphology, photosynthetic activity, yield and quality of the chilli. WW includes WW from the households and sewage together with the WW from local industries of lock and electroplating as well and was collected from the outside of Aligarh city nearly 5 km from the town where it was being used by local formers to irrigate crops especially vegetables. The experiment was carried out in the naturally illuminated net house of the department of Botany, Aligarh Muslim University, Aligarh, and each treatment was set simultaneously in triplicate using a factorial randomized design with four different P treatments at the rate of 0, 20, 40 and 60 $kgha^{-1}$ being watered by groundwater (GW) and wastewater (50%WW and 100%WW). A uniform basal dose of N at the rate of $60kgNha^{-1}$ and K at the rate of $50kgKha^{-1}$ was also applied along with different doses of P. The dilution of 50% WW was obtained by mixing the GW and WW in a 1:1 ratio. Many physio-chemical characteristics were analysed by adopting the procedures outlined in the standard methods [16] (Table 1). The soil for pot filling was collected from University Agriculture Farm and analysed for various physico-chemical properties by Ghosh et al [17] (Table 2). Microbiological analysis was also carried out and the mean values were obtained from three random samples (Table 3). Heavy metals (Cd, Pb, Ni and Cr) in the water and the soil samples were also analyzed using the atomic absorption spectrophotometer (AAP) (Table 1 and 2).

Table 1. Physico-chemical characteristics of groundwater (GW) and 100% wastewater (100%WW). All determinations in mg/l or as specified

Determinants	GW	WW	FAO* irrigation water quality
Colour	Colourless	Dark black	--
Odour	Odourless	Unpleasant	--
pH	7.3	7.9	6.5-8.4
EC (dS m^{-1})	0.75	1.42	0.25-3.0
TS	915	1627	--
TDS	549	1088	<2000
TSS	385	549	--
BOD	17.40	122.52	--
COD	55.22	152.44	--
NO_3-N	0.73	3.17	--
NH_4-N	0.00	5.24	<10
PO_4^{3-}	0.24	1.13	5.0
K^+	4.21	19.23	<2.0
Ca^{2+}	22.52	59.48	<400
Mg^{2+}	22.12	138.56	<61
CO_3^{2-}	49.48	138.44	--
HCO_3^-	59.56	89.24	<610
Cl^-	35.28	82.18	<350
Na^+	16.37	49.26	<460
Cd	ND	0.006	--
Cr	ND	0.004	--
Ni	ND	0.347	--
Pb	ND	0.031	--

**FAO- Food and Agricultural Organization, (ND)-Not detected*

Table 2. Physico-chemical characteristics of soil collected before sowing. All determinations in mg/l in 1:5 (soil-water extract) or as specified

Determinants	Soil
Taxture	**Sandy loam**
CEC (meq $100g^{-1}$)	2.25
pH	7.72
Organic carbon (%)	0.355
EC (µ mhos cm^{-1})	282.00
NO_3^{-1}-N(g kg^{-1} soil)	0.317
PO_4^{3-}	0.125
K^+	10.2
Ca^{2+}	27.15
Mg^{2+}	17.548
Cl^-	30.52
CO_3^{2-}	25.25
HCO_3^-	95.43
Na^+	13.98
SO_4^{3-}	17.05

The plants were sampled at 60 days after sowing (DAS) to make various observations. The plants were uprooted gently and washed under running tap water to remove adhering soil. The plant length and fresh mass of the whole plant were assessed. The samples were then dried in an oven at 80°C for 48h. The dehydrated samples were then weighed to recorded dry mass. Leaf area was measured using a portable leaf area meter (LA-21, Systronics, India). Nitrate reductase (NR) and carbonic anhydrase activities (CA) were determined in fresh leaves of plants by the method of Jaworski and Dwivedi and Randhawa [18-19] and Net photosynthetic rate (P_N) and Transpiration rate (E) were measured in upper most fully expended leaves on clear sunny days between11.00 and 12.30h, using a portable photosynthetic system (LICOR 6400, Lincoln, NE, USA). Leaf nitrogen was estimated by the method of Lindner [20] while the method of Mac-Kinney [21] was used to calculate the total chlorophyll content. Ascorbic acid was calculated by adopting the method of Sadasivan and Manickham [22]. At harvest, yield attributes including fruit length, fruit number and fruit fresh yield were measured.

2.1 Data Analysis

The data recorded from the experiment were subjected to two-way analysis of variance (ANOVA) using the SPSS (version 17.0) softwere package, and the mean were compared following the method given by [23]. An F test was applied to assess the significance of the data at 5% level of probability (P=0.05).

3. RESULTS AND DISCUSSION

Among the three water treatments, 100% WW proved more effective in enhancing the growth, enzymatic activity, photosynthesis, fruit yield and ascorbic acid content of fruit. Even 50% WW was also better when compared to GW, thus proving the utility of WW as a source of nutrients and irrigation water. The analysis of the WW has revealed that it was alkaline in nature with average EC, pH, TDS. The observed nutrients including some heavy metals also were within the permissible limits of [24] guideline for irrigation water quality except for Mg^{2+} and K^+ through all were higher than that of the gw (Table 1). WW obtained from urban source has a great potential for the reuse as a source of irrigation water and nutrient as well as a soil conditioning agent [25] because it contains considerable amount of nutrients essential for maintaining soil fertility and enhancing plant growth and yield. The pH (7.3-7.9) of the ww was within the range important for nutrient availability because it is the indicator of acidity and basicity of water. EC is widely used as a reliable indicator of the salinity of soils and its major effect on crop productivity is the inability of the plants to compete with ions present in the soil for water thus EC is the most useful and easily obtained property of the soil that influences the crop productivity [26]. The WW has considerably higher BOD and COD than GW, it determines the pollution power or strength of WW in terms of oxygen that micro-organism would require for complete stabilization. Chloride content of WW 82.18mg l^{-1} was comparatively low and may not cause toxicity problems. When nutrients are added though effluent and their accumulation are either equal or less than the demand of the crop, the build up in the soil is normally minimal. But there are various researchers that demonstrated that continuous irrigation with WW may lead the accumulation of nitrate in the soil [27] that leached to down layers and ultimately contaminated the GW.

As WW has beneficial effect on the physical, chemical and biological properties of soils [28-30], it could be used not only as a substitute of chemical fertilizer but also as the soil conditioner that would increase crop yield and enhance soil fertility and productivity [31-32]. It also increases the density of soil microorganisms including bacteria, fungi and actinomycetes that helps in nutrient availability of plants [33]. Concentrations of some essential inorganic ions were higher in WW than in GW. These could have played a beneficial role as these are essential for plants [34]. Dry matter accumulation (Fig. 1) was 16.18% higher in plants receiving WW than the plants irrigated with GW and among WW, 100%WW recorded much more dry matter because of the retention of the effluent components mainly due to the incorporation of the elements in the dry matter of the plants [35] leading to decreasing concentration in the surrounding GW. Although there is various studies, dilution has been described as an effective means to minimize toxicity [36-38], while in the present study source of irrigation is the urban WW which was sufficiently diluted by the mixing of household WW did not cause any toxicity problem. Use of urban WW without any dilution was also described by various researchers on different crops [39-42]. Among nutrients, comparatively higher phosphate (PO_4^{-3}) and N in water may be a cause of concern, N is the most important element, which is invariably required in large quantities and in WW it was present in both ionic forms (Table 1). Higher N and PO_4^{-3} in WW is very important for the plants because if it properly utilized by irrigation management can reduce the fertilizer demand and also contribute to solve the environmental degradation problem. In addition mineral nutrients WW also contains some heavy metals, which were within permissible limit of [24] guideline. 100% WW produced 11.63% and 13.46% higher shoot length and root length respectively over GW, while 14.38% (Fig. 1) increase was recorded by 100%WW in shoot fresh weight over GW. This enhanced vegetative growth under WW irrigation was due to the presence of both ionic forms of N in WW. WW contains considerable high amount of nitrate, which is the sole source of N supply and most important for better growth and yield of crop plants [43], because it involves in cell division and elongation and it is also an important structural constituent of many important metabolites amino acid, protein. Generally the highest growth and yield are obtained by the combined supply of both ions, but it may be noted that the form of N plays a key role in the cations-anion relationship in plants as about 70% of cations and anions taken up are represented by either NH_4^+ or NO_3^- [44]. A substantial increase of 18.82% in leaf area (Fig. 2), that might stimulated the photosynthetic rate (P_N) by influencing the light absorption within plant. The increase in leaf area brought by the N supply causing expansion of individual leaves has also been reported by [45-46] because N stimulated the cell division and cell expansion [47].

Increased N supply has also been found to enhance the activity of CA and RuBP carboxylase as reported by [48] and the stimulation in the activity of CA was also observed in this study (Fig. 3). Likewise vegetative growth WW also recorded higher values for the physiological parameters. It recorded an increase of 9.22% and 11.64% in photosynthetic rate and chlorophyll content over GW. Increase in total chlorophyll content may be due to higher availability of Mg^{2+} in WW as Mg^{2+} is the central atom of chlorophyll molecule which is required for the structural integrity of chloroplasts [49] and quantitative estimation of chlorophyll may be considered as an index of primary productivity. Like growth and physiological parameters WW application recorded an increase of 10.98, 8.75 and 6.60% in fruit length at I, II and III fruit pickings respectively over GW while an increase of 34.32, 16.66% and 11.92% were also registered in fruit number at I, II and III pickings respectively. Fruit yield recorded an increase of 35.57, 24.14 and 24.50 under WW at I, II and III pickings respectively (Figs. 4 & 5). This increase in yield under WW application may be explained on the basis of increase in leaf area by WW application. Increased leaf area might have allowed plants to trap more radiant energy required for enhanced photosynthetic activity which in turn might have increased the yield.

Table 3. Microbiological analysis of wastewater

Bacteria	Method used	Bacterial count
Total heterotrophic bacteria	Spread plate method	28.4×10^6 CFU $100ml^{-1}$
Coliforms	MPN method	1.9×10^3 $100ml^{-1}$
Faecal coliforms	MPN method	8.2×10^2 $100ml^{-1}$
Salmonella-Shigella sp	Spread plate method	1.3×10^2 $100ml^{-1}$

MPN= Most probable number, CFU= Colony forming unit

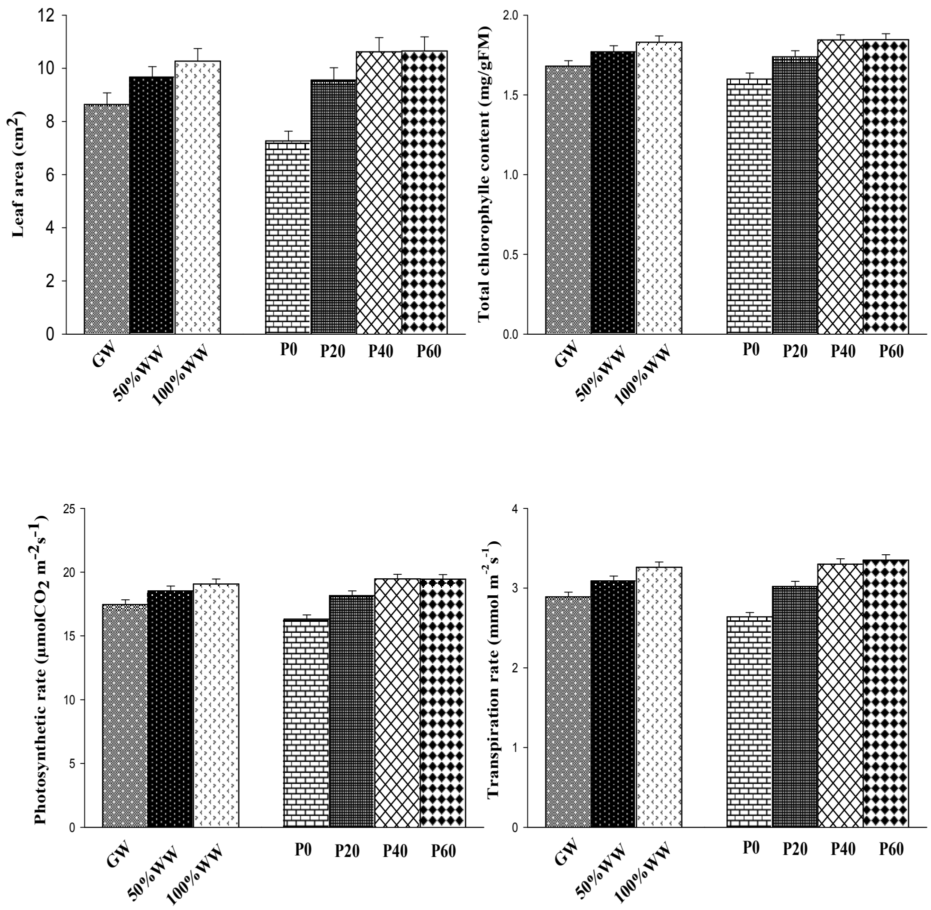

Fig. 1. Effect of groundwater and wastewater irrigation and different levels of phosphorus (P) on shoot length (cm), root length (cm), shoot fresh weight (gm) and shoot dry weight (gm) of chilli (*Capsicum annuum* L.) at 60 DAS. Values represent means of three replicates ± Standard Error (SE)

After N, P is a limiting nutrient for plant growth P inputs are also needed for crop production and in present study in addition of WW different levels of P with constant dose of N and K were also applied because nutrient supply only through WW is not sufficient for crop production and generally farmers use high levels of chemical fertilizers and pesticides, for higher production of vegetables [50]. Yield of the vegetables (tomato and cabbage) also increases with P application of soil [51-52]. P limits vegetable growth because of its highly immobile nature in soil and shallow rooting characteristics of vegetables. P directly enhanced metabolic processes and fruit set [53] and its availability at proper stage of plant growth also promoted flowering [54]. In short season crops, like vegetables growth responses to applied P may persist up to harvest. Thus irrigation through WW up to close to harvest could have ensured its availability and improved the growth and development which ultimately led to higher yield (Figs. 4&5). After N and P third essential nutrient K play significant role in stomatal opening and closing [55]. During photosynthetic phosphorylation guard cell produce abundant ATP under light condition that increases the uptake of K^+ with sufficient energy that create high turgor pressure that causes the opening of stomata [56], in addition it contribute up to 6% of plant dry weight [57] and also considered as a key factor for fruit quality [58].

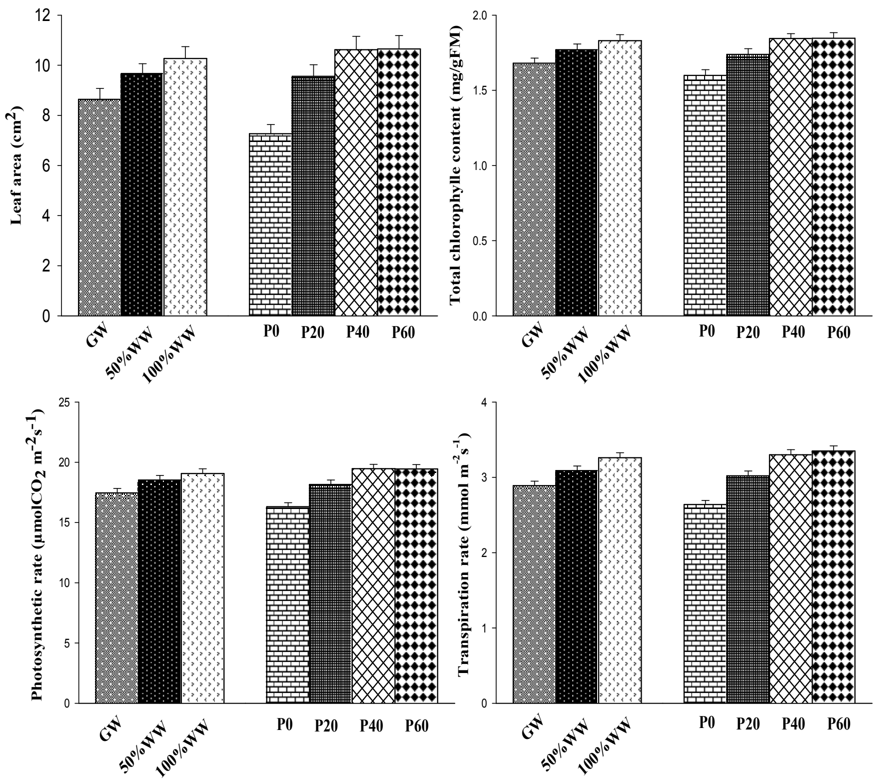

Fig. 2. Effect of groundwater and wastewater irrigation and different levels of phosphorus (N) on leaf area (cm2), chlorophyll content (mg/g FM), photosynthetic rate [µmol (CO2) m-2s-1] and transpiration rate [µmol m-2s-1] of chilli (*Capsicum annuum* L.) at 60 DAS. Values represent means of three replicates ± Standard Error (SE)

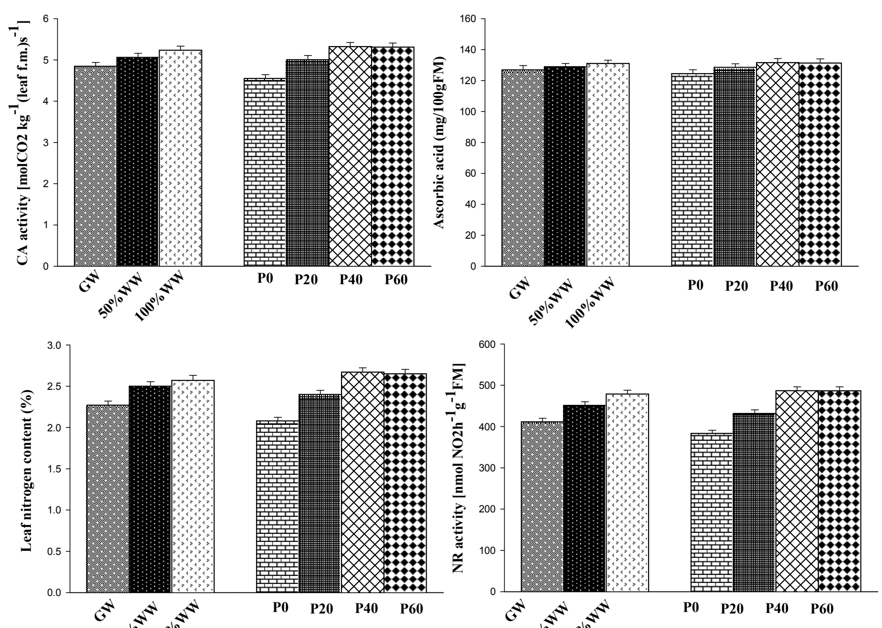

Fig. 3. Effect of groundwater and wastewater irrigation and different levels of phosphorus (P) on carbonic anhydrase activity [mol CO_2 kg^{-1} (leaf f.m.)s^{-1}], ascorbic acid content (mg/100g FM), leaf nitrogen content (%) and nitrate reductase activity NR [nmol NO_2 $g^{-1}s^{-1}$ (FM)] of chilli (*Capsicum annuum* L.) at 60 DAS. Values represent means of three replicates ± Standard Error (SE)

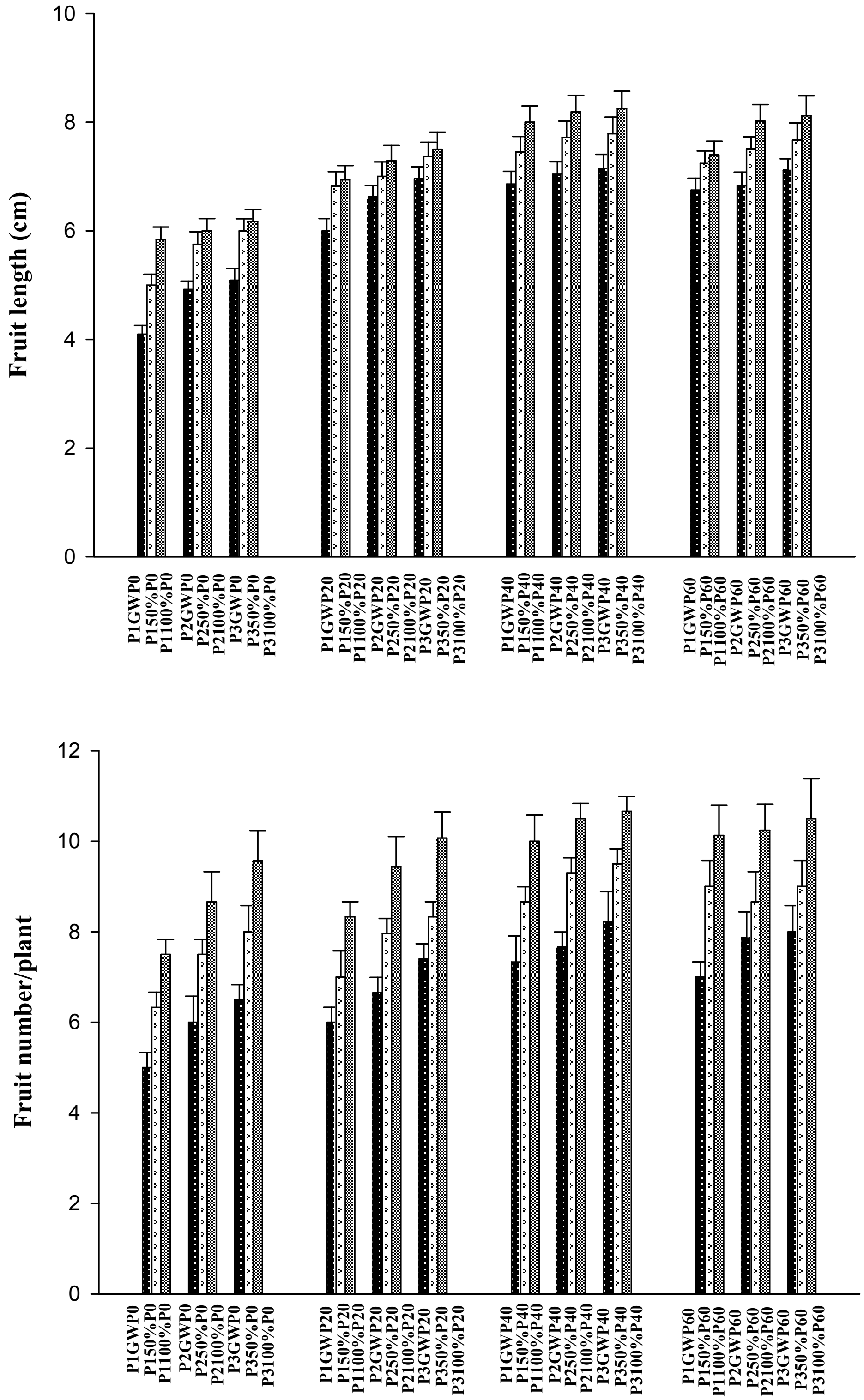

Fig. 4. Effect of groundwater and wastewater irrigation and different levels of phosphorus (N) on fruit length (cm) and fruit number plant-1 of chilli (*Capsicum annuum* L.) at I, II and III fruit pickings. Values represent means of three replicates ± Standard Error (SE)

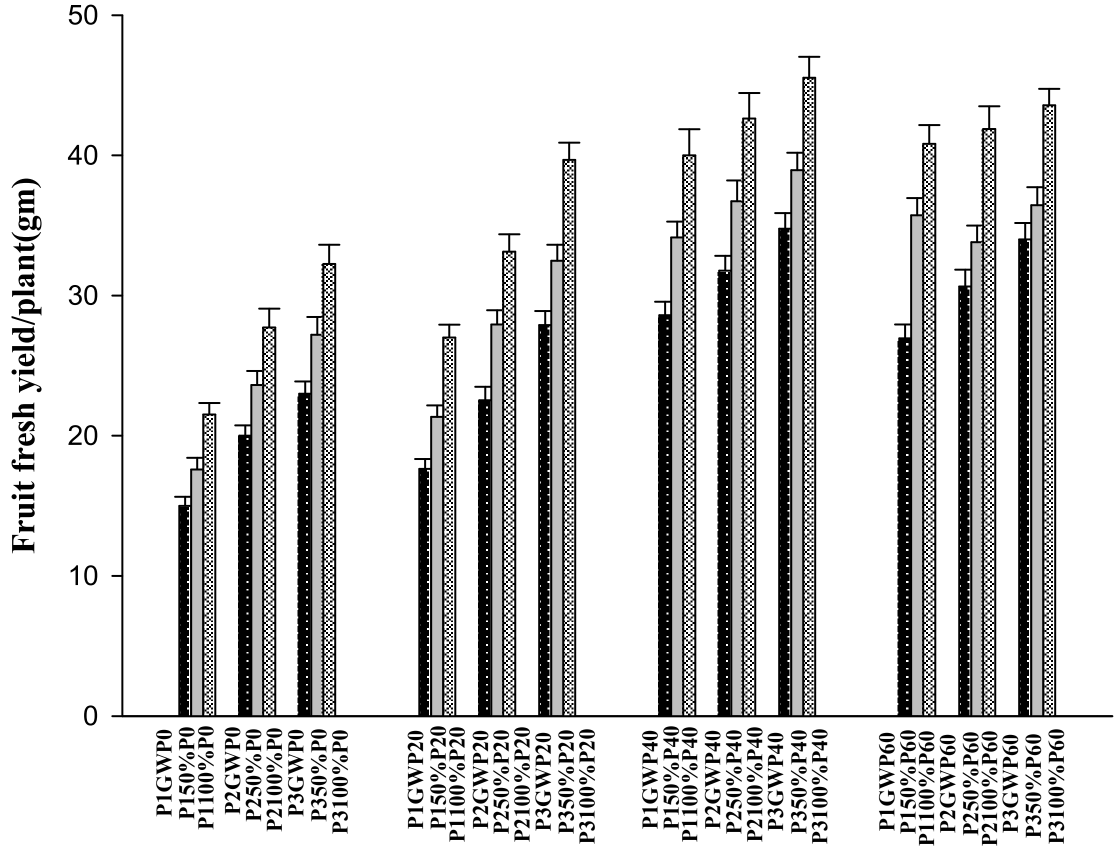

Fig. 5. Effect of groundwater and wastewater irrigation and different levels of phosphorus (N) on fruit fresh yield plant-1 of chilli (*Capsicum annuum* L.) at I, II and III fruit pickings. Values represent means of three replicates ± Standard Error (SE)

The presence of the other essential nutrient like calcium (Ca^{+2}) plays a key role in plant growth and fruit development and it is involved in many biochemical and physiological processes [59]. It is an essential component of the cell wall is involved in the cell division [60]. Sulphur (S^-) also played a vital role in plant metabolism as its deficiency is common [61], it is important to note here that the application of N in the form of urea is ineffective unless S is applied simultaneously and its deficiency decreases the leaf area [62] besides decreasing the chlorophyll content [63]. Chloride (Cl^-) is also one of the essential nutrient present in WW could played an important role in stomatal regulation while sodium (Na^+) is not essential and has been placed in the beneficial elements for plants, its presence may be stimulated the growth that is mainly caused by its effect on cell expansion and also on the water balance of plants. In the present experiment enhanced growth (Fig. 1), physiological (Figs. 2 & 3) and yield characteristics (Figs. 4 & 5) under P_{40} were also observed, while WW with a comparatively lower P dose (P_{20}) proved beneficial. Better performance of chilli crop under higher P dose was explained on the basis of vital role of P in metabolic processes and stimulation of various enzymes, which lead higher leaf area and number provide much surface area for photosynthesis and produce much more photosynthate that finally gave higher yield. WW with lower P dose proved beneficial because WW contains additional PO_4^{-3}.

4. CONCLUSION

Analysis of WW revealed its suitability for the irrigation as most of the values of the analyzed parameters were within permissible limits of FAO. Based on the findings of this experiment we can conclude that the urban WW proved beneficial for the crop growth and productivity because it is an effective source of essential nutrients. Even it could not be supplemented the whole nutrient requirement of the crop but can reduced the quantity of P. Although heavy metals were also detected in WW and their continuous use for irrigation may result in their buildup to phytotoxic level thus regular monitoring of WW is required for the safe irrigation and benefits. Thus WW reuse as a source of irrigation water and nutrient can effectively fill the increasing gap between water demand and water availability up to some extent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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