CHAPTER V. DISCUSSION

The critical appraisal of the results described in Chapter IV clearly indicated the better performance of the crop chickpea receiving wastewater as a source of irrigation and also showed the possible beneficial role of the nutrients present in it. Comparatively higher salt contents in the wastewater may be a cause of concern (Chapter II) however, if properly utilized by irrigation management it can lower the fertilizer consumption thereby lessening the degradation of water and soil.

5.1 Nutrients present in the wastewater and their role (Experiments I-IV)

The wastewater contained considerable amount of nutrients which are considered essential for maintaining the soil fertility as well as for enhancing the plant growth and productivity. Among them, nitrogen for example, the single most important element limiting plant growth and invariably required in large quantities was present in both ionic forms (Table 3) and thus deserves special consideration. As vegetative growth includes formation of new leaves, stems and roots, the involvement of N through protein metabolism controls them. This was also clearly indicated by the observed enhanced growth (Table 6, 7, 10a, 17, 18, 21a, 28, 29, 29, 32a, 39, 40 and 43a) and N content in the leaves (Table 11b, 22b, 33b and 44b) under the wastewater irrigation. Suitability of NH$_4^+$ or NO$_3^-$ for the growth and development of plants depends upon many factors (Kirkby, 1981). However, normally the highest growth rate and plant yield are obtained by combined supply of both and therefore, in the present study, the improvement in growth could be due to the cumulative effect of ammonium as well as nitrate ions together. It may also be pointed out that the form of nitrogen plays a key role in the cation-anion relationship in plants as about 70% of cations and anions taken up are represented by either NH$_4^+$ or NO$_3^-$ (Vanlauwe et al., 2002). Thus, in principle, NH$_4^+$ fed plants are characterized by high cation-anion uptake ratio and in contrast, NO$_3^-$ fed plants by a high anion-cation uptake ratio. This may explain that the optimal growth for most plant species is usually obtained with mixed supply of NH$_4^+$ and NO$_3^-$. It is noteworthy that applied NH$_4^+$-N is toxic for some higher plants including bean and pea (Maynard and Barker, 1969). However, in presence of NO$_3^-$-N, it has been reported to benefit sunflower (Weissman, 1964) and wheat (Cox and Reisenavar, 1973). Thus the observed nutritional superiority of the
wastewater (containing both ammonium-N and nitrate-N) for growth of chickpea was not exceptional and possibly explains the reason of better performance of the crop grown under the wastewater irrigation (Experiments I-IV). A substantial increase in dry matter of the test plants in all the four experiments was also observed (Table 7b, 18b, 29b and 40b) because of the increased leaf area and expansion (Table 10a, 21a, 32a and 43a) which might have influenced the light absorption within a plant causing stimulation of \( P_N \) (Table 13b, 24b, 35b and 46b), thereby optimizing the \( \text{CO}_2 \) assimilation and photosynthetic production. The increase in the leaf area brought about by N supply causing the expansion of the individual leaves was also reported by Taylor et al. (1993) and Gastal and Lemaire (2002) which may be through its effect on cell division and cell expansion (Lemaire, 2001). Similarly increased N supply has also been found to enhance the activities of CA and RuBP carboxylase as reported by Terashima and Evans (1988) and the stimulation in the activity of CA as observed in the present study (Table 10b, 21b, 32b and 43b).

Another essential nutrient, P when supplied in limiting amounts to sugarbeet, it has much greater impact on growth than on photosynthesis (Rao et al., 1986; 1987a, b; Rao and Terry, 1989). During the present study (Experiments I-IV), better growth of plants was observed receiving wastewater having 1.26, 1.13 and 1.59 mg P/l (Table 3) in addition to other nutrients, and it was also comparatively richer than the ground water. The observation of the improved performance of the crop receiving the wastewater was therefore, understandable. Although it further needs emphasis that application of phosphorus has its limitations as P applied to the soil is very rapidly changed to less soluble form and therefore, becomes less and less available with time. Admittedly in short season crops, like some vegetables, growth responses to applied P may persist up to harvest. On the contrary, comparatively long season crops, like corn and chickpea, may show only slow growth responses and much lesser effect at seed formation and maturity. Therefore, regular supply of the wastewater as explained in Chapter II up to close to harvest could have ensured its availability and thus might have improved the growth and development which ultimately led to higher seed productivity (Table 16, 27, 38 and 49).
Next to nitrogen and phosphorus, potassium is the third important macro nutrient required in the largest amount by the plants. Its requirement for optimal plant growth is in the range of 2-5% of the plant dry weight of the vegetative parts and in the present study it varied from 3.44 to 4.41% (Table 12b, 23b, 34b and 45b). It is known to play a significant role in stomatal opening and closing (Fisher and Hsiao, 1968) and under light conditions the guard cells produce abundant ATP in photosynthetic phosphorylation, thus supporting the active K⁺ uptake with sufficient energy (Humble and Hsiao, 1970) and the resulting high turgor pressure thus causes the opening of the stomata. The diffusion of CO₂ into the stomata is followed by its transport into the chloroplasts where it is reduced by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). It is this supply of CO₂ which catalyses reversible dehydration of HCO₃⁻ to CO₂ in close proximity to the CO₂ fixing enzyme. The functioning of this entire mechanism is likely to be improved by enhancement of the activity of both CA which was also observed in this study (Table 10b, 21b, 32b and 43b) and RuBPCO studied elsewhere. It is also well known that N is fully utilized for crop production only when K is adequate (Mengel and Kirkby, 1982) and the presence of K in wastewater was nearly double the amount present in the ground water (Table 3) and therefore, the crop under study was benefitted not only due its own physiological role (Wolf et al., 1976) but also by enhancing the effect of N. This was also strengthened by the presence of higher N and K contents in the leaves of the plants receiving the wastewater (Table 11b, 12b, 22b, 23b, 33b, 34b, 44b and 45b).

In addition to these three major macronutrients explained above, presence of other essential nutrient like sulphur could have also played a vital role in plant metabolism as its deficiency is common (Murphy and Boggan, 1988). It may be pointed out that the application of nitrogen in the form of urea is ineffective unless sulphur is applied simultaneously and its deficiency reduces the leaf area (Wang et al., 1976) besides decreasing the chlorophyll contents (Dietz, 1989). In legumes during early development of sulphur deficiency, nitrogenase activity in root nodules is much more depressed than photosynthesis (DeBoer and Duke, 1982). Moreover in sulphur deficient plants not only the protein content decreases but also the sulphur content in the proteins indicating that the proteins with lower proportions of
methionine and cysteine but higher proportion of other amino acids such as arginine and aspartate are synthesized. This decrease in the sulphur rich proteins is not confined to wheat grains but can also be found in other cereals and legumes (Randall and Wrigley, 1986) and the lower sulphur content of the proteins influences the nutritional quality considerably (Arora and Luchra, 1970). Although in the present study, quality of the protein was not worked out but the total protein was significantly enhanced in the wastewater fed plants (Table 16, 27, 38 and 49). Similarly, the presence of $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ (Table 3) could have further added the benefits as $\text{Ca}^{2+}$ being an essential component of cell wall is involved in the cell division (Schmit., 1981) while $\text{Mg}^{2+}$ is a central atom of chlorophyll and is required for structural integrity of chloroplast (Moorby and Besford, 1983) on which the rate of photosynthesis is directly dependent and thus rate of photosynthesis is lower in $\text{Mg}^{2+}$ deficient plants (Forster, 1980). It may be pointed out that the chlorophyll contents and the photosynthetic rate was enhanced in plants grown under wastewater (Table 13, 24, 35 and 46) indicating the possible involvement of $\text{Mg}^{2+}$ in addition to other nutrients. Similarly, the presence of $\text{Cl}^-$, one of the essential micronutrients, could have played an important role in stomatal regulation and its impairment in palm trees was considered to be the major factor responsible for growth depression (Braconnier and d’Auzak, 1990) and reduction in the leaf surface area and there by the plant dry weight. Although $\text{Na}^+$ is not essential and has been placed in the category of beneficial elements for plants but its presence (Table 3) may be responsible for growth stimulation which is caused mainly by its effect on cell expansion and also on water balance of plants. It can replace $\text{K}^+$ in its contribution to solute potential in vacuoles and consequently in generation of turgor and cell expansion. It may also surpass potassium in this respect since it accumulates preferentially in vacuoles (Nunes et al., 1984). The observed enhanced growth and photosynthetic capacity ultimately led to increase in 100 seed weight (Table 16c, 27c, 38c and 49c) because of the ensured supply and availability of above mentioned nutrients might have played a cumulative role in enhancing the metabolic activities and finally the seed yield (Table 16d, 27d, 38d and 49d) and protein (Table 16e, 27e, 38e and 49e).
It is also important to point out here that the characteristics of wastewater used for irrigation varied with and among the years of this study. In general, it was alkaline with basic pH ranging from 7.8 to 8.3. The average EC, pH, TDS and the observed nutrients including some heavy metals also were within the permissible limits of FAO guidelines for irrigation water quality except for $K^+$ (Ayers and Wescot, 1994). The major effect of EC and TDS on crop productivity is the inability of the plants to compete with ions present in the soil for water while the chloride content of 114.23 mg l$^{-1}$ was also comparatively low and may not cause toxicity problems. When nutrients are added through effluents and they are either equal or less than the demand of the crop, the build up in the soil is normally minimal. There are reports where it has been shown that the continuous use of effluents may result in the accumulation of nitrate in the soil (Vazquez-Montial et al., 1996) and is subject to move to down layers (Cameron et al., 1995) which may ultimately result in the contamination of ground water. Similarly NPK fertilizers added at the rate higher than the rate of crop removal accumulate in the soil therefore, a balance between the wastewater and fertilizer dose has to be identified. Although in various studies, dilution has been described as an effective means to minimize the toxicity (Chapter II) and under current experimental conditions since the source of irrigation was the urban sewage wastewater which was sufficiently diluted automatically due to the mixing of the household wastewater to an extent that when analyzed for various physicochemical characteristics was found suitable for irrigation and no further dilution was required.

Therefore, among the three water treatments, it may be pointed out that 100%WW proved more effective (Experiments I-IV) and even 50%WW was better than ground water (Table I) probably because of the higher nutrient content than GW. However, the wastewater together with comparatively higher doses of NPK fertilizers, as pointed out earlier in Chapter IV, significantly decreased the yield while the lower doses in combination with it promoted the crop growth as well as the seed yield. Thus nutrient management through the wastewater and fertilizers yielded better results in terms of productivity and at the same time excessive use of fertilizers can be minimized.
Besides the nutrient content of the wastewater, the presence of some heavy metals can be a cause of concern indicating the possibility of their accumulation in plants (Table 4a). Although these concentrations remained well within the limits permissible (Pendias and Pendias, 1984) but regular monitoring must be maintained for safe use of wastewater in crop cultivation as the continuous application may lead to their buildup (Aziz et al., 1993ab, 1994, 1999). In addition to this, the microbial examination of wastewater revealed the presence of some pathogenic and non pathogenic bacteria which can be a cause of concern however, in our case since the crop is not eaten raw therefore, the chances of harm are comparatively negligible to consumers although the growers must be warned to take precautions while applying the wastewater to their crops in the fields.

5.2 Nitrogenous fertilizer (Experiment I)

Plant growth is the expression of interplay between meristematic activities and metabolic processes leading to an increase in biomass (Moorby and Besford, 1983). In addition to its role in cell division and expansion (Gardner et al., 1985), nitrogen is also essential for a number of biologically important molecules. Therefore, the requirement for N (and the other essential nutrients) during the vegetative growth is determined primarily by the rate of CO₂ assimilation and if it is high, the required nutrients must be correspondingly at their optimums.

Data pertaining to growth such as plant height (Table 6a), plant fresh and dry matter accumulation (Table 7), leaf area (Table 10a), dry mass of nodules (Table 9a) have shown a positive trend with increase in N dose up to 30 kg ha⁻¹. Thus growth and yield parameters were significantly enhanced by this dose which was also optimum for leaf area, NRA, chlorophyll content (Table 10a, 11a and 13a) and the photosynthetic rate (Table 13b) which finally led to more pods and the heavier seeds (Table 16). Nitrogen up to 30 kg ha⁻¹ was also suggested by Sharma et al., 1989 for chickpea. On the contrary, application of 45 kg ha⁻¹ resulted in decreased growth and seed yield therefore, proved toxic. It may be pointed out that toxicity due to N, when applied in excess in the form of urea is known to appear at two stages of plant growth. The first at seedling stage, due to accumulation of NH₄⁺ after hydrolysis of urea and the second may be due to accumulation of NO₂ under certain conditions damaging the
young plants (Court et al., 1964). In case of legumes due to Rhizobial activities and biological nitrogen fixation, host plants grow well in soil even with low N doses and no benefit from this association may occur if high levels of fertilizer N are given (Ozanne, 1980). It is noteworthy to mention here that when interactions with wastewater were observed still lower nitrogen dose (N₁₅) proved optimum. It was not surprising as the wastewater had sufficient N in the form of NH₄⁺ and NO₃⁻ ions as also discussed earlier (Table 3) and the crop tested was nitrogen fixing one. In addition some nitrogen was also available from the soil as well (Table 5). It may also be pointed out that N₁₅ in combination with wastewater (100%WWxN₁₅) was at par with N₃₀ when given with ground water (GWxN₃₀) thus N₁₅ when given with wastewater proved economically and environmentally viable dose however, the same dose with GW was inadequate where almost all the growth, physiological and yield parameters registered lower values.

Nitrogen at lower rate in combination with wastewater (100%WWxN₁₅) also stimulated nodulation while the higher rate (45 kg ha⁻¹) proved excessive under both waters and the effect was more adverse under the wastewater (Table 8 and 9a). These reports are in tune with the findings of Harper and Gibson (1984); Becana and Sprent (1987) and Macduff et al. (1996), where high levels of nitrate in soils impaired nodulation and depressed N₂ fixation. It has been established that nodulation and N₂ fixation activity in both legumes and actinorhizal plants are strongly inhibited by high levels of N (Streeter, 1988). As the symbiotic N₂ fixation is an adaptive mechanism, excess of the combined nitrogen in the rooting medium not only inhibits new nodule formation, but also limits the functional span of the already existing nodules. It may be pointed out that nodules function under a paradoxical situation. In order to fix nitrogen, bacteroids inside the nodules must be supplied with oxygen at a sufficiently faster rate to meet their respiratory requirement for efficient ATP production, but at the same time, level of free oxygen inside the nodule must be kept low to protect the enzyme ‘nitrogense’ which is highly oxygen labile. It needs further emphasis that leghemoglobin content facilitates oxygen diffusion inside dense nodular tissues maintaining a constant supply at low tensions of free oxygen. In the present study the leghemoglobin content was also significantly decreased under the influence of higher
nitrogen dose especially in combination with the wastewater (Table 9b) thereby affecting nodule function. The inhibitory effect of exogenous nitrate on N\textsubscript{2} fixation has variously been attributed to a direct competition between nitrate reductase and nitrogenase for reducing power (Stephens and Neyra, 1983; Vassileva and Ignatov, 1996) or to the fact that nitrite a by product of NR inhibits the function of leghemoglobin (Becana and Sprent, 1987) and in our experiment a significant reduction in the later was observed with the higher nitrogen rate (N\textsubscript{45}) while the maximum was observed under the lower nitrogen rate (N\textsubscript{15}) and wastewater (Table 11a). There were reports where chickpeas were found capable of fixing all the N they need (Mahler \textit{et al.}, 1988) while others have also reported that the crop does not fix adequate N for maximum growth even when vigorous nodules are present (Beck \textit{et al.}, 1991). Therefore, the supply of optimum N was essential as it can serve as a starter dose.

Due to the availability of photosynthetic reductant, leaf mesophyll cells are the main sites of nitrate reduction. This is initiated by NAD/NADP dependent NR enzyme, which catalyses the reduction of nitrate to nitrite in cytosol. Nitrite is then transported to chloroplast, where it is further reduced into ammonium ions. Being the first irreversible and often rate determining step of the N assimilatory pathway, nitrate reduction has been a favorite step for physiological approach to optimize N fertilizer use. Nitrate reductase levels have been shown to fluctuate in response to changes in environmental conditions, including availability of N (Afridi and Hewitt, 1964; Beevers and Hageman, 1972). Enzymes are therefore, sensitive to nutrient levels as indicated in the present study where NR activity was decreased with comparatively higher N dose (Table 11a) and improved under the optimum dose. Similar observations were made in trifoliate leaves of \textit{Phaseolus lunatus} at different canopy positions by Wallace (1986) and Andrews \textit{et al.} (1990).

There are numerous studies suggesting that photosynthetic capacities of higher plants change dramatically in response to different N supply (Sims \textit{et al.}, 1998; Bondada and Syvertsen, 2003; Marchese \textit{et al.}, 2005) as may be observed in this study (Table 13b, 14 and 15). The stimulation of plant photosynthesis due to N may have positive effect on plant growth (Table 6, 7 and 10a). It may be pointed out that
about 70% of N in plant leaves exists in chloroplast and most of it is used to synthesize photosynthetic apparatus. Thus its supply is extremely important for the performance of photosynthetic apparatus (Huber et al., 1989; Uprety and Mahalaxmi, 2000). During this experiment also, best photosynthetic activity with maximum P, g, and WUE was recorded either under N30 alone or N15 with 100% WW (Table 13b, 14 and 15). Similarly, total dry matter (Table 7b) was increased as a result of increased leaf area (Table 10a) which provided larger area for the interception of solar radiation and thus higher photosynthetic rate (Table 13b) thereby giving more pods plant⁻¹, higher seed weight and seed yield compared to control (Table 16 and Fig. 3a). The linear regression obtained between leaf area and photosynthetic rate further buttresses this view (Fig. 2). More importantly protein content was also increased with 15kg N ha⁻¹ plus wastewater (Table 16, 27, 38 and 49). Therefore it can be argued that the integrated nutrient management through (i) regular supply of N present in wastewater, (ii) inherent N₂ fixation capability of the crop, (iii) optimum nitrogen dose and (iv) available N of soil (Table 5) might together have proved beneficial for higher productivity and protein content which was aptly demonstrated in Table 50 wherein an increase in NPK contents and NR activity in leaves were positively correlated with protein content.

5.3 Phosphatic fertilizer (Experiment II)

It is also indispensable for plant growth and development and its deficiency retards plant growth, cell and leaf expansion (Marschner, 1986). It has many roles in cell division, stimulation of early root growth, hastening plant maturity and fruiting and seed production.

Application of P thus promoted growth and the effect was significant nearly at all sampling stages being more pronounced when given with wastewater. Phosphorus at 40 kg ha⁻¹ proving optimum for shoot and root length (Table 17) and plant fresh and dry mass (Table 18). The leaf area was also increased under this treatment (Table 21a) and it was at par with 60 kg ha⁻¹ thereby showing the luxury consumption of phosphates. The beneficial effect of phosphorus on the leaf area has also been reported by Rao and Subramanian (1990) in cowpea and Reddy et al. (1991)
Fig. 2. Linear regression between leaf area and net photosynthetic rate of Experiment I (a), Experiment II (b), Experiment III (c) and Experiment IV (d).
Table 50. Correlation coefficient (r) values of NR activity and leaf N, P, K contents with protein content at three stages in Experiments I-IV.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Protein content</th>
<th>Experiments</th>
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<tr>
<td></td>
<td>Stages</td>
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<tr>
<td></td>
<td>Vegetative</td>
<td>Flowering</td>
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<tr>
<td>NRA activity</td>
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<td>0.907**</td>
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<td></td>
<td>0.850**</td>
<td>0.783**</td>
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<td></td>
<td>0.897**</td>
<td>0.873**</td>
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<tr>
<td></td>
<td>0.939**</td>
<td>0.960**</td>
</tr>
<tr>
<td>N</td>
<td>0.970**</td>
<td>0.489&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>0.951**</td>
<td>0.964**</td>
</tr>
<tr>
<td>K</td>
<td>0.939**</td>
<td>0.928**</td>
</tr>
<tr>
<td></td>
<td>0.916**</td>
<td>0.965**</td>
</tr>
<tr>
<td>NR activity</td>
<td>0.769**</td>
<td>0.734**</td>
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<tr>
<td></td>
<td>0.874**</td>
<td>0.898**</td>
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<td></td>
<td>0.821**</td>
<td>0.805**</td>
</tr>
<tr>
<td></td>
<td>0.809**</td>
<td>0.811**</td>
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<tr>
<td>NR activity</td>
<td>0.867**</td>
<td>0.882**</td>
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<td></td>
<td>0.895**</td>
<td>0.889**</td>
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<td></td>
<td>0.880**</td>
<td>0.930**</td>
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Chapter V
in ground nut. The importance of leaf area in terms of leaf area index as determinant of radiation interception has been recognized in field crops (Kumar et al., 1997) and is also known to improve the rate of assimilate production per unit leaf area as reported by Jacob and Lawlor (1991) and as also observed under the present study (Fig. 2b). On the contrary, the most striking effects of phosphorus deficiency are reduction in leaf expansion and leaf surface area (Freedan et al., 1989) and also the number of leaves (Lynch et al., 1991) as may be observed under P20 which proved as deficient dose (Table 21a) and which is common when the concentration of an essential element is low and often results in limiting the yield and although under moderate or slight deficiencies symptoms may not be visible, but yields may still be reduced. While under optimum level, plant dry matter was increased (Table 18b) and in combination with wastewater accumulated more dry mass indicating the possible role of P given as fertilizer and nutrients present in the wastewater which was evident from higher NPK content in the leaves (Table 22b and 23). Similar increases in the dry matter accumulation due to P fertilizer has also been reported in chickpea by Raju and Verma (1984) and Khokar and Warsi (1987) who have observed that legumes show an evident preference of phosphatic fertilizers. It may be pointed out that the differences in the dry matter among different P fertilizer doses were probably caused due to the differences in the leaf area (Table 21a) which enabled the plants to produce more photosynthates because of higher photosynthetic activity (Table 13a and Fig. 2b) leading to more pods (Table 27b) and higher seed weight (Table 27c). In addition, the chlorophyll contents (Table 24a) were also improved and there are reports wherein insufficient P has been shown to limit chlorophyll and protein content (Usuda, 1995; Xu et al., 2007).

In contrast to the direct role of nitrogen in NR activity, P in leaf tissues is known to be responsible for phosphorylation and release of photosynthates from chloroplast and oxidation of these sugars to produce more reducing power for nitrate metabolism (Kow et al., 1982). In case of photosynthetic activity, deficiency of P was found to decrease it in spinach (Bottrill et al., 1970), tobacco (Kakie, 1970) and subterranean clover (Bauma, 1967) which was also reflected under (P20) the deficient dose of phosphorus (Table 23a). This inhibition of photosynthesis by P limitation has
often been explained by depressing Calvin cycle activity in particular and also by decreasing the amount and activity of rubisco (Heldt et al., 1977; Lauer et al., 1989; Stark et al., 2000). Similarly, the decline in the stomatal conductance (Table 25a) leading to decrease in the photosynthetic rate (Table 24b) has also been reported by Xu et al. (2007).

Legumes also require phosphorus for nitrogen fixation and if its availability is limited, growth and nitrogen fixation are adversely affected (Prasad and Sanoria, 1981). Thus in Experiments II and IV, the nodule number (Table 19a) and their fresh and dry mass (Table 19b and 20a) were increased with increasing P levels up to P₄₀ as P₆₀ could not increase it further while the wastewater with P₄₀ improved it. Robson et al. (1981) had concluded that P nutrition increased symbiotic dinitrogen fixation in subterranean clover (Trifolium subterraneum L.) by stimulating host plant growth rather than by exerting specific effects on Rhizobial growth or on nodule formation and function. Under this study similar observations were made where shoot growth (Table 17a) and root growth (Table 17b) were enhanced significantly under P₄₀. There are instances where, it has been observed that the dinitrogen fixation by Rhizobium is enhanced if the plants are better supplied with P along with K (Gukova and Tjulina, 1968, Wu et al., 1969, Mengel et al., 1974) and was also the case in this experiment where P doses were supplemented with K (Table 1).

The increase in plant N concentration in response to increased P supply has been noted for several leguminous species (Andrews and Robins, 1969; Israel, 1987, 1993). Consequently, application of P also proved valuable for seed protein content (Table 27e) due to its assured availability and utilization by carbon skeletons for amino acid synthesis as well as for energy rich ATP. Being a part of the protein molecules enhanced N levels due to the application of P (Table 22b) might have triggered and maintained the conversion of various organic acids (produced from carbohydrates during respiration) into amino acids. The strong corelation coefficient values of seed protein content with leaf NPK contents and NR activity at various stages of growth (Table 50) further strengthen our view. Beneficial effects of phosphorus application on seed yield and quality compared to control have also been reported in cluster bean by Gura et al. (1996) and Bhadoria et al. (1997), on LAI,
biological index and seed yield on *Pisum sativum* by Yamane and Skjelvag (2003) and in legumes by a number of authors including Berg and Lynd, 1985; French, 1990 and Bolland *et al.*, 2001. Phosphorus fertilization also increased the number of pods and 100 seed weight (Table 27b, c) which may be attributed to the increase in the number of branches and reproductive nodes and this proposition was substantiated by a strong positive correlation between seed yield and number of pods per plant \( r = 950** \).

### 5.4 Potassic fertilizers (Experiment III)

In the present experiment also the enhanced growth characteristics (Table 28, 29 and 32a) and nodulation (Table 30 and 31a) under \( K_{40} \) were observed while with wastewater comparatively lower dose (\( K_{20} \)) proved beneficial as the later beside containing \( K \) was also rich in several other essential nutrients. The observed higher leaf area (Table 32a) due to \( K \) may be ascribed to its role in augmenting the cell size (Mengel and Arneke, 1982). It is more likely that the presence of \( K \) is essential for attaining full activity of enzymes which have an impact on numerous physiological processes. Some of them are of major relevance for the plant growth and production. Besides this there are several plant nutrition studies where in \( K \) has been identified as the only monovalent cation essential for all higher plants playing a major role in various physiological and biochemical processes including the photosynthesis (Marschner, 1971). Thus the observed enhancement of the photosynthetic activity measured in terms of photosynthetic rate (Table 35b) and stomatal conductance (Table 36a) besides other is justified in the light of the pioneering work of Fisher (1968) and Fisher and Hsiao (1969), that the mechanism of stomatal opening and closure is known to depend on \( K^+ \) fluxes in the guard cells. Thus, a considerable decrease in the photosynthetic rate (Table 35b) and stomatal conductance (Table 36a) was observed in the plants grown without potassium (\( K_0 \)).

Similarly the role of potassium in many physiological processes and in activation of number of enzymes (Mengel and Kirkby, 1980) leading to better development of sink was aptly demonstrated by higher number of pods plant\(^{-1}\) (Table 38b) and increased 100 seed weight (Table 38c) and even higher protein content (Table 38e). Though the treatment \( K_{40} \) and \( K_{60} \) were similar in their effect for
various growth and physiological parameters and even for seed yield and 100 seed weight (Table 38) but the quality parameter i.e. seed protein content (Fig. 3c) was significantly more under the higher potassium dose (K$_{60}$). It may be of interest to point out that K is often described as the quality element and the observed increase in the protein content with this dose is thus not surprising, as K is known to play key role in the transport of essential ingredients in protein to the site of the protein synthesis. Several studies have demonstrated the stimulation of NO$_3^-$ uptake and transport when K is the accompanying cation compared with other cations (Blevins et al., 1978a, 1978b; Minotti et al., 1968) which could have encouraged the protein synthesis.

5.5 NPK fertilizers (Experiment IV)

The optimum doses of NPK obtained in earlier three experiments were applied together in this experiment and it was observed that in general, lower doses with wastewater were equivalent and in some cases even better to the ground water in combination with comparatively higher fertilizer doses. The influence that one element makes on the other is important in plant nutrition and these may occur in soil, in plant or in both which has been aptly explained by Russell (1973) “if two factors are limiting or nearly limiting growth, adding only one of them will have little effect on growth whilst adding both together will have a considerable effect. Two such factors are said to have a large positive interaction in such circumstances for the response of the crop to both together is larger than the sum of their responses to each separately”. It may be pertinent to point out here that there have been studies where it has been shown that the ionic interactions that exist in plant nutrition may be both synergistic and antagonistic (Coic and Lesaint, 1971; Epstein, 1972; Mengel, 1973; Mengel and Kirkby, 1982). The present data revealed that there was a synergistic effect of N, P and K when applied at their optimums and therefore, significant increases in growth, physiological and yield parameters were observed under the treatment N$_{30}$P$_{60}$K$_{40}$ which also proved better for protein content. Although N$_{15}$P$_{60}$K$_{20}$ proved optimum for protein content but seed yield was decreased which was obvious because of the dilution factor. However, together with the wastewater the fertilizer combination of N$_{15}$P$_{40}$K$_{40}$ and N$_{15}$P$_{60}$K$_{20}$ were equally effective for seed yield as well as for the protein content. Thus, confirming the possible involvement of the nutrients
present in wastewater in supplementing them. It may be pointed out that these three nutrients at their optimum levels increased the root length and also enhanced the nutrient absorption capacity of the crop (Table 44b and 45). Thus synergistic interplay of the three nutrients which are known to accelerate root proliferation (Salisbury and Ross, 1992), thereby extracting more nutrients in the root zone lead to the development of larger canopies (Table 43a) and greater dry matter accumulation (Table 40b) and higher NPK contents (Table 33b and 34). Similar positive interactions between N and P were also noted by Russell (1973) and between N and K by Murphy (1980).

Similarly, the improvement in nodule number (Table 41a), their fresh and dry mass (Table 41b and 42a) was not unexpected as it is well known that N₂ fixation by Rhizobium is enhanced in host plants well supplied with P and K (Gukova and Tjulina, 1968; Wu et al., 1969; Mengel et al., 1974). This was further strengthened by the positive correlations of NPK contents with seed protein content (Table 50). While the increased height (Table 28a) and leaf area (Table 32a) would provide better orientation of leaves which would help in harvesting the required quantity of solar energy leading to enhanced dry matter production (Table 40b), number of pods (Table 49b) and 100 seed weight (Table 49c). Therefore, this whole sequence of events led to increased yield attributes thus resulted in maximizing the seed yield (Table 49d and Fig. 3d). This was also reflected by the positive correlation coefficient values of seed yield with pod number (r = 0.971) and 100 seed weight (r = 0.949).

5.6 Plant growth pattern (Experiments I-IV)

Apart from the effect of the nutrients on growth, yield and quality performance of the crop, the growth pattern at different stages were also taken into consideration. Among the parameters studied, shoot fresh and dry mass and shoot length increased progressively up to the fruiting stage (Fig. 4a and b) which is a common phenomenon in various cereals and pulses. While nodule number, their fresh and dry mass and leghemoglobin content were increased from vegetative to flowering stage only and thereafter it declined towards the fruiting (Fig. 4c, d, e and f). This may possibly because of the fact that initially the competition for photosynthates was
Fig. 3. Seed yield and protein content of chickpea as affected by nitrogen (a), phosphorus (b), potassium (c) and NPK (d) along with water treatments.
confined to roots, nodules and aerial vegetative organs but when flowering and fruiting started, the resulting new sinks due to their high demand for photosynthates may have created shortage for the nodules, leading to their senescence or degradation (Fig. 4c, e and f). Similar observations were made for chlorophyll content (Table 13a, 24a, 35a and 46a), CA activity (Table 10b, 21b, 32b and 43b) and photosynthetic characters (Table 13b, 14, 15, 24b, 25, 26, 35b, 36, 37, 46b, 47 and 48). The decrease in the photosynthetic characters may be ascribed to the fact that old, senescent leaves eventually become yellow because of the chlorophyll breakdown and loss of functional chloroplast. In addition the transport of some nutrients from old to the young leaves to maintain their nutrient level may lead to their senescence (Greenway and Gunn, 1966) and hence the effective leaf area decreased (Table 10a, 21a, 32a and 43a) and thereby declining photosynthetic rate (Table 13b, 24b, 35b, 46b) towards the fruiting stage. Contrary to the above mentioned parameters, NPK contents in leaves decreased with growth (Fig. 4g and h) and the possible reasons may be ascribed to the “dilution with growth effect” due to which even higher quantities of the nutrients appeared to be less when expressed on per unit basis (Moorby and Besford, 1983). Similar decrease in nutrient concentrations with age was also observed by Gomide et al. (1969); Rhykerd and Overdahl (1972) and by Moorby (1977). Besides, the translocation of nutrients towards the seeds during their formation may also deplete the nutrient contents of leaves at later stages. It may be pointed out that among the three nutrients, K was accumulated more followed by N and P. In this context, it may be added that in higher plants, K is the most abundant cation (Huber, 1985) and is also absorbed at the higher rate by plants (Mengel and Kirkby, 1982). It may also be pointed out that in natural ecosystems P is usually the life limiting element due to its low availability. In addition, soils have low total and low plant available phosphate supplies because mineral phosphate forms are not readily soluble and unfortunately, most soluble phosphates become fixed before plants can absorb them. Tisdale et al. (1995) and Roy et al. (2006) also reported comparatively less concentration of P than N and K in plants.
Fig. 4. Growth pattern of different parameters at three stages of growth: continuous increase (a, b), continuous decrease (g, h) and first increase then decrease (c, d, e, f).
5.7 Conclusions

- The wastewater proved an effective source of essential nutrients and even if it could not supplement the whole nutrient requirement of the crop, it reduced the quantity of fertilizer doses and thus acted not only as a source of irrigation water but of nutrients also.

- Although in several studies, carried out in the past, dilution has also been shown as the effective measure to minimize the negative effects of the wastewater irrigation due to toxicity of some salts and heavy metals but under current experimental conditions as the source of the wastewater was the urban sewage which was already sufficiently diluted to an extent that it proved beneficial for the crop and was better compared to GW.

- The wastewater was also analyzed for various physicochemical characteristics that were within the permissible limits of irrigation water quality (Ayers and Wescot, 1994).

- The texture of the soil was sandy loam and also contained some essential nutrients, like N, P, K, Mg, Ca, Cl. The pH was alkaline which is considered suitable for availability of most macro nutrients.

- The presence of some pathogenic bacteria, like coliforms, salmonella and shigella may be a cause of concern therefore, the farmers have to be informed to take due care while irrigating the fields and selecting the crop to be grown.

- Four heavy metals Cd, Ni, Cr and Pb were also undertaken for the analysis because they are the main constituents of local lock and electroplating industrial effluents and when analyzed in the wastewater except Ni, the rest of the three were also within the permissible limits. (Gupta et al., 2000).

- Nitrogen at 30 kg ha$^{-1}$ proved optimum while 45 kg N ha$^{-1}$ was toxic and the effect was more pronounced when it was given with the wastewater. Thus 100%WW$\times$N$_{15}$ proved optimum and effectively increased the growth and physiological parameters including the seed yield and protein content.

- Phosphorus at the rate of 40 kg ha$^{-1}$ proved optimum while 60 kg P ha$^{-1}$ was luxuriously consumed and 100%WW$\times$P$_{40}$ proving most suitable combination for both the yield and protein content.
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- Potassium at 40 kg ha$^{-1}$ proved optimum while 60 kg K ha$^{-1}$ was at luxury consumption and 20 kg K ha$^{-1}$ was deficient when applied without wastewater. However, the protein content was maximally enhanced under $K_{60}$. Therefore, if the protein enhancement is the prime objective then the crop is to be supplemented with higher potassium dose. Among interactions like nitrogen, potassium at the rate of 20 kg ha$^{-1}$ together with the wastewater proved to be the most effective combination showing the fertilizer saving of 20 kg K ha$^{-1}$.
- Finally the two combinations, $N_{15}P_{60}K_{20}$ and $N_{15}P_{40}K_{40}$ together with wastewater were equally effective in enhancing the seed yield and protein content.
- Shoot length, plant fresh and dry mass increased with the age while leaf number, nodule number their fresh mass and dry mass, chlorophyll contents, NR activity and CA increased up to flowering and thereafter decreased while others like NPK contents decreased with aging.

5.8 Proposal for future studies

The observations recorded meticulously in the pot experiments and spread over three years have in no doubt established the suitability and utility of urban wastewater for growing chickpea. The study also served the proposed objective of lowering the optimum doses of fertilizers especially in case of N and K. However, before recommending the doses to the local farmers for adopting in the fields regular monitoring of the heavy metal, salt accumulation and microbial contamination needs to be carried out. Therefore, these concerns should be essential components of any management of wastewater irrigation. In addition, an attempt may be made to record the following observations that could not be undertaken due to limited facilities and time:

- Estimation of some more heavy metals and their uptake and concentration in plant parts especially in seeds which is the edible part.
- Nitrogenase activity for assessing the $N_2$ fixation ability of chickpea.
- Protein quality.
- Experiments also needs to be carried out under field conditions.