Discussion
## Contents

### Discussion

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Discussion

5.1 Varietal response

It is always the effort of agricultural scientists that the genetic potential of crop plants must be optimally exploited through the management of agricultural practices. One such effort has been made in the present study where the wastewater, fly ash and NPK fertilizers, combining the three different sources of nutrients, were interacted with a popular locally grown nitrogen fixing crop. Expectedly, this accounted for the differences in productivity of simultaneously grown two varieties of the same crop. The pooled data of Experiments I-VI confirmed the assumption that nutrients present in wastewater and fly ash generated from the common source and applied NPK could be profitably utilized as the two varieties performed better but responded differently. It may be noted that the wastewater along with FA20, N15, P30 and K30 significantly improved the performance of PDM-11 (Experiments I, III, V) due to its inherent genetic make up for efficient and effective utilization of nutrient resources in comparison with PDM-54 (Experiments II, IV, VI) which gave the maximum seed yield under the wastewater, FA20, N15, P45, K30 (Fig. 3).

It is known that any crop and even among the species, the magnitude of differences varies under the same climate, soil and even the same management practices. Naturally, the better performance of PDM-11 was due to its enhanced nodulation, root length, root fresh weight (Tables 15, 16, 18, 33, 34, 36, 51, 52 and 54) and leaf area resulting into higher dry matter accumulation (Tables 12, 14, 17, 30, 32, 35, 48, 50 and 53). This variety also responded better under wastewater, fly ash, nitrogen (Experiments I-II), phosphorus (Experiments III-IV) as well as potassium (Experiments V-VI) doses in seed protein (Fig. 4). The pooled analysis revealed that the two varieties also differed in leaf NRA, chlorophyll content and photosynthetic rate under the treatments applied in six experiments. Similarly the differences in the N, P and K status of the two varieties reflected their differential efficiency to absorb and utilize these nutrients (Tables 22-24, 40-42 and 58-60) which was reflected in seed yield and seed protein where the doses of P and K proved more effective in PDM-11 than PDM-54. Such
Fig. 3. Seed yield of Experiments I (a), II (b), III (c), IV (d), V (e) and VI (f) in green gram (Vigna radiata L.).
Fig. 4. Total seed protein contents of Experiments I (a), II (b), III (c), IV (d), V (e) and VI (f) in green gram (Vigna radiata L.).
varietal differences in nutrients effect and requirement, therefore, signify the importance of such trials.

There can be wide differences between some species while in others the differences may be small and this is the one reason why some species can grow on lower nutrients supply while other on comparatively higher level as noted in case of phosphorus. In this context the conclusion drawn by Glass and Perley (1980) may be mentioned wherein it was reported that ion absorption by plants is under genetic control and that considerable differences exist both between and within genera. It has been shown that the crop cultivars responding better to soil adaptability, nutrition and water generally produce higher yield as noted in PDM 11. In the opinion of Yoshida (1972) varieties differ in their response to changes in their environment. Therefore, the crop cultivars have a characteristic; one could label it as “yield elasticity”. That is, yield may be enhanced as required inputs are increased and maintained in proper balance.

5.2 Wastewater

As explained in Chapter IV, the wastewater proved beneficial, when compared with the ground water, for most of the parameters including the import at economic one, the seed yield (Figs. 3a, b, c and d). It was apparently due to the presence of some essential nutrients specially N, P and K in addition to Ca, Mg, S and Cl (Table 9) and their availability to roots because of daily watering of pots after seedling emergence. Presence of higher NPK contents in the leaves of wastewater treated plants (Tables 22-24 and 40-42) and linear regression between leaf NPK, chlorophyll content and photosynthetic rate (Figs. 5-12) also confirmed this observation (Experiments I-IV).

The role of these nutrients is well established as nitrogen is involved in cell division, expansion (Gardner et al., 1985); phosphorus in energy transfer, nucleic acids, cell membranes, phosphoproteins (Hewitt, 1963) and nodulation (Andrew, 1977); potassium in photosynthesis, leaf area and cofactor of many enzymes (Mengel and Kirkby, 1996) and Mg in chlorophyll and middle lamella in addition to be an essential element for various enzymatic reactions. Similarly, the presence of S in some amino acids and hence in proteins along with N (Deckard et al., 1973) and Mg (Gardner et al.,
Fig. 5. Linear regression between total chlorophyll contents and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment I.
Fig. 6. Linear regression between photosynthetic rate and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment I.
Fig. 7. Linear regression between total chlorophyll contents and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment II.
Fig. 8. Linear regression between photosynthetic rate and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment II.
Fig. 9. Linear regression between total chlorophyll contents and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment III.
Fig. 10. Linear regression between photosynthetic rate and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment III.
Fig. 11. Linear regression between total chlorophyll contents and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment IV.
Fig. 12. Linear regression between photosynthetic rate and NPK content at vegetative (A), flowering (B) and fruiting (C) stages of Experiment IV.
Therefore, the effective absorption and subsequent utilization of these nutrients may be responsible for the increase in growth and yield parameters, including seed yield. In this context, references may be made of Ahmad et al. (1990), Joshi and Billore (1998), Choudhary and Khanif (2001) and Olness et al. (2001) who have observed similar effect while working on crops like rice, soybean and sorghum grown with N, P, S, Mg, Ca, Mn, Zn and V alone or in combination. Since the wastewater contained good amount of nitrate nitrogen which might have stimulated the NRA (Tables 9, 19, 37 and 55), an important enzyme of the nitrate assimilation pathway, leading to protein synthesis. It was further confirmed in regression studies wherein a positive relation was observed between N content, NRA and protein content (Figs. 13-18).

5.3 Fly ash

Most of the observed parameters exhibited significant increase under 20% fly ash. Although 40% fly ash was also effective in enhancing the leaf area, nodule number, NRA, photosynthetic rate, 1000 seed weight and seed yield to some extent, therefore, even this dose may not be treated as toxic. The beneficial effect of the coal fly ash in soil fertility and crop productivity was also reported by Kaakinen et al. (1975), Klein et al. (1975), Plank et al. (1975), Hill and Lamp (1980), Matte and Kene (1995), Patil et al. (1996) and Sugawe et al. (1997). Because the fly ash can be a source of K (Martens et al., 1970), Zn (Schnappinger et al., 1975), S (Elseewi et al., 1978), Mg (Hill and Lamp, 1980), Cu (Wallace et al., 1980), Mo (Cary et al., 1983), B (Wallace and Wallace, 1986) and P (Srivastava et al., 1995) however, significantly it lacks nitrogen (Khan and Khan, 1996; Sahu and Dwivedi, 1999). Therefore, the crop plants due to additional nutrients of fly ash and alteration in physico-chemical characteristics of soil were probably able to extract them as a result of its application, thereby improving the crop performance (Experiments I-II). These observations were further strengthened by the work of Mishra and Shukla (1986) who have reported that the flyash contained the three textural size particles of silt, sand and clay at 65, 25 and 10% respectively in addition to N, P, K and Ca as macro
Fig. 13. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment I.
Fig. 14. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment II.
Fig. 15. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment III.
Fig. 16. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment IV.
Fig. 17. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment V.
Fig. 18. Linear regression between nitrate reductase activity (NRA) (A) and total seed protein contents (B) with nitrogen content at vegetative, flowering and fruiting stages of growth of Experiment VI.
and B, Cu, Mn and Zn as micronutrients. More importantly, these nutrients were in concentration than normally found in soil, except nitrogen.

The protein content was also increased under the application of the fly ash and due to the combined effect of nitrogen applied as fertilizer and wastewater having both forms of nitrogen along with the doses of fly ash. Bhaisare et al. (2000) in green gram and Sriramachandrasekharan (2001) also recorded similar observations in groundnut.

The comparative decline in various parameters above 20% fly ash may be because of some excessive amounts of soluble salts like sulphate, chloride, carbonate and bicarbonate (Table 8) in addition to some toxic compounds, like dibenzofuran and dibenzo-p-dioxine mixtures (Sawyer et al., 1983) and heavy metals Ni, Cd, Cr, Pb (Wadge and Hutton, 1987), although not observed in this study. Similarly, there were reports wherein phytotoxicity of B (Adriano et al., 1978; Singh and Yunus, 2000) was also responsible for adverse effect. However, in our case the results clearly demonstrated the economical gains from the addition of fly ash in the soil admittedly within a limited supply.

5.4 Fertilizer
5.4.1 Nitrogen

Among nitrogen levels, comparatively lower dose, N<sub>15</sub> proved optimum for most of the growth and yield parameters of the two varieties (Experiments I and II). The optimum fertilizer dose, therefore, need to be decided individually for each area or type of soil, crop and their varieties. In the present study, well developed root system (Tables 15 and 16) might have enabled efficient absorption of nitrogen required by the plant when the concentration of the nutrient was adequate in the soil. This is also confirmed by the correlation studies where N content in leaves had strong positive correlation with root fresh weight at vegetative (r = 0.952 and 0.995), flowering (r = 0.962 and 0.999) and fruiting (r = 0.992 and 0.994) stages in Experiment I and II respectively. It may further be highlighted that the supply of this essential element affected the growth in general and leaf number (Table 13) and leaf area (Table 14) in particular. In this study, nitrogen present in the wastewater in the form of NO<sub>3</sub> and NH<sub>4</sub> has also
contributed towards this enhanced growth. Therefore, it was not surprising that $N_{15}$ proved as effective as $N_{20}$, which was at luxury consumption especially in seed yield thereby proving wasteful. $N_{15}$ also proved optimum for nodulation while $N_{20}$ suppressed it as Wilson and Hallsworth (1965) observed that increased supply of combined nitrogen reduced the nodulation as well as haem content of the nodules. Similarly, detrimental effect of higher N dose on nodulation was also reported by Koike et al. (1997) and Krugova (1997).

Application of nitrogen also resulted in higher N uptake by roots as indicated by its higher concentration in leaves (Table 22) as the crop was fertilized with ammonical forms of N (urea) which is readily converted to $\text{NO}_3^-$, normally available form of N in soil. While the increase in leaf P and K content may be due to the synergistic interplay of these nutrients which are known to accelerate root proliferation, thus, extracting more nutrients present near the root zone leading to higher dry matter (Table 12 and 17). Such positive interactions among the nutrients are common, as pointed out by Russell (1973) between N and P while between N and K by Murphy (1980). It may also be noted that N as an essential macronutrient has a distinction being absorbed by the plants as cation as well as anion. This keep N in a unique relationship of both anion-cation as well as cation-cation interaction.

Seed yield was also enhanced under this dose as a result of cumulative enhancement of various growth and physiological parameters which finally lead to more pods and seeds (Table 25) and also the heavier seeds (Table 26) leading to higher seed yield (Table 27) in both varieties. Plants grown without nitrogen were poorer in nodulation as some starter dose was supposed to be needed even for the leguminous plants. Nitrate reductase levels have been shown to fluctuate in response to changes of environmental conditions, including nitrogen (Beavers and Hageman, 1972). The higher leaf nitrogen level (Table 22) might be responsible for higher NRA (Table 19) as it is an inducer as well as stabilizer of nitrate reductase (Hewitt and Afridi, 1959). This was also proved by the strong positive correlations between N content of leaves and NRA. Their ‘r’ values were 0.981, 0.955,
0.964 (Experiment -I) and 0.995, 0.997 and 0.986 (Experiment -II) at vegetative, flowering and fruiting respectively. The seed protein content was also enhanced, as the optimum dose was positively responsible for the conversion of organic acids into amino acids. As pointed out by Pretty (1980) some quality factors in a few grasses were related to the effective utilization of nitrogen and the conversion of its compounds into true proteins.

5.4.2 Phosphorus

Among phosphorus doses tested, P$_{30}$ proved optimum for most of the parameters, including seed yield in PDM-11 (Experiment -III). This might be due to increased meristematic activity of treated plants, thereby increasing the leaf number (Table 31) and leaf area (Table 32) which were ultimately responsible for increased dry weight (Tables 30 and 35) leading to higher seed yield (Table 45) on account of total response of the plant. The role of P in increasing cell size and leaf area has also been reported by Rao and Subramanian (1990) in cowpea and by Reddy et al. (1991) in groundnut and on dry matter accumulation by Nandal et al. (1987), Reddy et al. (1990), Balachandran and Sasidhar (1991), Mahalle et al. (1994), Das et al. (1999), Chowdhury et al. (2000) and Ram and Dixit (2001) in green gram. In this context, mention may be made of Bunting and Drennan (1966) who have emphasised that “the vegetative stage may have an important and direct effect on seed production”. Similar views were also expressed by Yoshida (1972) and Moorby and Besford (1983). Contrary to PDM-11, variety PDM-54 performed better under P$_{45}$ as this variety recorded higher NPK content at this level (Tables 40-42) thereby increasing the chlorophyll content (Table 38) and photosynthetic rate (Table 39) which ultimately lead to more seed yield (Table 45). In this context, reference may be made of Milthorpe and Moorby (1979) who were of the opinion that “there is usually a positive relationship between the supply of mineral nutrients and the rate of photosynthesis, which is exerted through effects on the internal and stomatal conductances". It is pertinent to note that P is known to facilitate the partitioning of photosynthates between source and sink (Giaquinta and Quebedeaux, 1980) leading to enhanced 1000 seed weight (Table 44) and seed yield.

The observed nodulation due to P application was due to its role in proliferation of roots, which could have provided larger surface area for...
bacterial infection. This assumption was supported by the significant higher root fresh weight of phosphorus treated plants (Table 34). In the opinion of Diener (1950), phosphorus stimulates nodulation through its effect on rhizobia. It also enhanced the leaf NPK contents and observations of enhanced N concentration due to P fertilization in tropical legumes are not uncommon (Shaw et al., 1966; Andrew and Robins, 1969b; Dradu, 1974). It may be attributed that the higher nodulation might have increased the accumulation of N in the leaf through more efficient dinitrogen fixation and then P and K accumulation indirectly leading to the enhanced growth performance of the crop. It may be pointed out that legumes show an evident preference for phosphorus fertilizers (Raju and Verma, 1984) in comparison with nitrogenous fertilizers, which is generally compensated through N2 fixation. While phosphorus is often limiting due to its low availability compared with potassium, which is easily recycled from organic residues in addition to its easy availability through fertilizers. Therefore, the quantity of P fertilizer to be applied to such crops becomes critically important as the available P often becomes limited.

Phosphorus (Experiments III and IV) also proved beneficial for seed protein (Table 45) possibly due to its continuous requirement in amino acid synthesis as well as that of energy rich ATP for protein synthesis. Further, the added N through fertilizer and wastewater might have triggered the conversion of some organic acids into amino acids and K added through flyash and wastewater on the other hand might have activated the enzymes involved in protein synthesis (Evans and Sorger, 1966; Tamhane et al., 1970).

5.4.3 Potassium

It was optimum when applied @ 30 kg ha⁻¹ (Experiments V-VI) and the lower levels 10 and 20 kg ha⁻¹ proved deficient for most of the parameters, except seed protein which was optimum under K20 while the higher level, given @ 40 kg ha⁻¹ exhibited the luxury consumption. The optimum dose was responsible for maximum leaf expansion (Table 50) thus providing larger surface area for photosynthesis and partitioning towards root development (Tables 51-53) and nodulation (Table 54). Thus, it could increase the N fixation by increasing nodulation, as observed in the present study, or it
could also increase the nodule productivity (moles of N₂ fixed per unit time per unit mass of nodule) as reported by some other workers. Various studies on forage, grain and vegetable legumes have strongly suggested that under most conditions, legumes show maximum nodulation and their growth under optimum potassium supply. Mention may be made of Jones et al. (1977) who observed increased number of nodules in soybean with increase in K nutrition while *Rhizobium* along with K was effective in increasing production and N₂ fixation by improving both nodule size and number.

It is to be noted that effect of K on photosynthetic rate varied with the growth period. K₃₀ was optimum at early stage while at later stages K₉₀ was needed by the plants for maintaining higher photosynthetic rate. Wolf et al. (1976), while studying alfalfa photosynthetic efficiency, reported that K increased leaf area and photosynthesis on per plant basis. It was also responsible for transport of photosynthates to the nodules and increase in root growth. This naturally resulted in greater consumption of nutrients, which ultimately lead to further absorption and requirement of this nutrient.

Seed protein content improved due to the addition of K, with K₃₀ proving optimum. This could be explained as this nutrient can activate the enzymes involving in protein synthesis (Tamhane et al., 1970). Crops with high protein contents have high harvest index for K and thus they mobilize K more efficiently into the developing seed. Therefore, such high protein crops remove sufficient quantities of K from the soil even when it was at comparatively low level. Usherwood (1985), while reviewing the work on the improvement in yield and quality of corn, soybean, wheat and legumes by the application of potassium, reported its beneficial effect along with that of P and N on essential amino acids. It may be pointed out that P or NP alone were less effective in comparison with the treatment where K was also included which highlighted its role (Keeney, 1969). Similarly, Mengel et al. (1981) also reported an indirect role of K in grain protein formation of wheat. They were of the opinion that amino acids were translocated from vegetative parts to the developing grains, which favoured enhanced de-novo synthesis of amino acids.

### 5.5 Growth stages

In all the six experiments, shoot length, shoot fresh weight, shoot dry
weight, root length, root fresh weight and root dry weight increased progressively up to the fruiting stage. This is a common phenomenon among most flowering plants. Contrary to these growth parameters, nodule number increased only up to the flowering stage and then decreased. It was due to the initial competition for photosynthates, which was confined to roots, nodules and aerial vegetative organs only up to the flowering. However, when fruit setting started, comparatively more products of photosynthesis moved towards the sink thus, creating a shortage for nodules and thus resulting in their degeneration. Similarly, NRA decreased also after flowering. This could be ascribed to the observation that the total nitrate reducing capacity of the plants, according to Campbell (1999), is not only dependent on the availability of the substrate in the cytoplasm and the level of functional nitrate reductase and the activity level, but also on the relation of nitrate reductase with the overall state of plant metabolism where co-ordination is operated through sensors and/or signal transducers.

Contrary to these parameters, chlorophyll content and the rate of photosynthesis decreased towards the maturity which might be due to the leaf senescence because old leaves were unable to photosynthesize due to chlorophyll breakdown and loss of functional chloroplasts. Giaquinta (1978) also reported the similar results on sugarbeet.

Similarly leaf NPK content decreased with increase in growth and age of the plants. The observed decrease may be due to the exponential increase in growth (weight and volume) of plants due to which an increase in nutrient concentration appear to be less when expressed on per unit basis (Moorby and Besford, 1983). On similar lines, decline in leaf P concentration with growth was also observed by Gomide et al. (1969) in six tropical grasses when observed over intervals of 4, 12 and 36 weeks. They were also of the opinion that this decline was due to the “dilution with growth” effect because of a higher rate of dry matter accumulation than absorption of nutrients and/or redistribution to younger plant parts. Similarly, Rhykerd and Overdahl (1972) observed a rapid decline in leaf K concentration with maturity in forage legume herbage. In addition, the translocation of nutrients to sinks during their formation and subsequent development could be considered reasonably responsible for nutrient depletion in leaves at the
later stages of growth. It was also noted that leaves accumulated more amount of K contents (Tables 24, 42 and 60) followed by N (Tables 22, 40 and 58) and P (Tables 23, 41 and 59). In this context it may be pointed out that for higher plants, K is the only essential monovalent cation among the essential macronutrients and it is the most abundant cation in plant tissues (Huber, 1985) due to its higher rate of uptake by plants (Mengel and Kirkby, 1982).

5.6 Conclusion

Keeping the results recorded in view, the following points emerge:

1. The analysis of the wastewater revealed its suitability for irrigation as the values for the analysed parameters were within the permissible limits of the Indian Standards for Irrigation Water (IS: 3307-1965).

2. As the wastewater proved beneficial for growth, yield and quality of the crop tested, it may be recommended for irrigation.

3. In experiments I and II, 20% fly ash was most effective and even 40% was not toxic as the latter also enhanced some of the parameters, including seed yield in comparison with the no fly ash control.

4. Nodulation, NRA and photosynthetic rate also improved due to the application of wastewater and fly ash.

5. Since, the fly ash was deficient in N, leguminous plants, which have the ability to fix atmospheric nitrogen are suited for cultivation as observed in the present study.

6. Among the nitrogen doses, N15 proved optimum, while N10 deficient and N20 at luxury consumption especially for seed yield, however N30 was as effective as N15 in case of protein.

7. Of phosphorus doses, P30 was the optimum for seed yield and quality while P15 was deficient and P45 was luxury for variety PDM-11 (Experiment III). In case of PDM-54 (Experiment IV), P45 proved optimum for seed yield.

8. Nodulation increased with increasing levels of phosphorus.

9. Among potassium doses, K10 and K20 (Experiments V-VI) proved deficient for most of the parameters, while K30 and K40 were optimum and at luxury consumption respectively. However, K20 proved optimum for seed protein while K40 for photosynthesis.
10. Among the three major nutrients, K accumulated more in leaves, followed by N and P.

11. It was noted that shoot length, shoot fresh and dry weight, leaf number, leaf area, root length, root fresh and dry weight increased with increasing age of the plants.

12. Contrary to the above observations, photosynthetic rate, chlorophyll and leaf NPK content decreased with increasing age of the plants while nodule number and NRA increased up to flowering stage only and decreased at fruiting.

13. Both PDM-11 and PDM-54 varieties grew well under TPPW irrigation but the former performed comparatively better and may, therefore, be recommended to the local farmers for the cultivation under the wastewater irrigation along with 20% fly ash, 15 kg N, 30 kg P and 30 kg K ha⁻¹.

14. Finally, the better performance of this crop confirmed the suitability of the wastewater as a source of irrigation as well as nutrients (Table 9) and the fly ash (Table 8) as a supplement of some nutrients needed by the plants. Thus, the wastewater and fly ash, by all means the waste products of the thermal power plant, may therefore be utilized profitably for agricultural purpose where such waste resources are easily and freely available particularly in the areas closer to the thermal power plants.

5.7 Proposal for the future studies

The observations recorded during the three years have helped to some extent in observing the utility of the wastewater and coal fly ash in crop cultivation and in determining the optimum doses of N, P and K for obtaining optimum yield of green gram. However in our opinion, the study has some shortcomings, which may be undertaken on the following lines in future studies:

1. The experiments may be repeated in the farmers' field near the leachate reservoir of the thermal power plant.

2. Some of the important heavy metals commonly present in the wastewater and fly ash may be estimated specially in the seeds.
3. The acetylene reduction assay (nitrogenase activity) for nitrogen fixation ability of the legumes under wastewater and fly ash application may be another important area of study.

4. Microbiological and mycorrhizal studies of the wastewater and soil may also be undertaken.