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Combined Effect of Animal Manures and Di-Ammonium Phosphate (DAP) on Growth, Physiology, Root Nodulation and Yield of Chickpea

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Abstract: The key plant nutrients determine the crop's nutritional status and vigor, while their deficiency impairs the plant growth resulting in absolute failure of crop productivity. Phosphorus is the second major essential nutrient for the growth and establishment of crops. Drastic variations in climatic conditions across the world and low soil fertility, especially phosphorous (P) fixation in alkaline calcareous soils of arid climate have become serious issues threatening the productivity of crops. In this regard, the application of different organic fertilizers in combination with the lower dose of chemical fertilizers has been proved as an effective strategy to improve plant growth and yield. Thus, a pot experiment was conducted in order to evaluate the combined effects of animal manures (normal and processed) along with the recommended and half of the chemical fertilizer di-ammonium phosphate (DAP) on the growth, physiology, nodulation, and yield of chickpea. Results show that the animal manure (normal and processed) with half or recommended dose of DAP significantly improved the growth, yield, and physiological attributes of the chickpea. However, the combined application of normal animal manure with half DAP showed the highest results among all other treatments. It increased the contents of leghaemoglobin, P, K, primary branches, pods, and seed weight up to 113.7%, 97.8%, 80.6%, 78.5%, 119%, and 145% over control, respectively. This treatment also increased protein contents, nodule count, and nodule weight up to 78.38%, 147%, and 93.59% than the control, respectively. Maximum chlorophyll b contents (0.78 μg/mL) were obtained with the application of a recommended dose of DAP alone. These indigenous manure applications provide a novel value addition that is critical to boosting crop yield and agricultural sustainability.

Keywords: crop production; nutrient use efficiency; phosphorous; manures; soil nutrients

1. Introduction

With the advent of the 21st century, agricultural production is more efficient than ever before, due to the intensive farming systems, but at the expense of environmental deterioration. In this agricultural farming system, the immense use of fertilizer and pesticides, retard the agroecosystem health and ability to supply goods and services. Nutrient and toxic chemical contamination via runoff and leaching into surface and groundwater is becoming a rising concern, resulting in eutrophication and loss of soil quality [1,2]. However, the agricultural resources are being rapidly degraded indicating the unsustainable current farming practices and soil fertility is also depleting [3]. The world still faces the challenges of food security and food safety. Poor management practices, low soil quality, and fertility particularly are some of the major causes of a decline in the overall productivity of the crops [4]. Food legumes are one of the most important dietary components of the world's population, owing to their high nutritional content. Apart from being a component of the diet, legumes are also important in improving the nutritional status of the soils [5–8], reporting that legumes acquire more phosphorus as compared to non-leguminous crops as nodules are the main sink for phosphorus. Moreover, nodulation and symbiotic associations are also affected by the amount of P contents [9,10]. Therefore, understanding P utilization in legumes under the intensive farming system is pivotal for developing more phosphorus efficient and judicious farming system.

Some of the discerning characteristics of the P are high fixation, slow diffusion, and low availability, which implies that P is a major limiting factor for normal plant growth in arid climates [11]. The soil P cycle is majorly governed by anthropogenic activities and agricultural practices [12]. P available fraction in the soil is rarely over 10 μM [13] which is very much inferior to the plant tissue's concentration that is 5–20 mM [14]. Since P has low mobility and concentration in the soil, there is a need for increased application of chemical fertilizer to meet the P requirements of the plant, so applying chemical P fertilizer is an effective way for the sagacious use of chemical P fertilizers [15]. However, the P fertilizers are being used inefficiently, worldwide, only 1/5 of mined P become available due to high losses from field-to-fork, so a multi-criteria-based approach should be adopted for a sustainable agriculture system. Organic matter sustains soil health, is a substrate to support microbial decomposition, makes mineral nutrients available to the crop, improves soil structure, increases water holding capacity, reduces soil-borne pathogens, and minimizes heavy metals toxicity [1]. The long-term organic amendments to the soil increase the P availability to plants, improve soil quality, and enhance enzymatic activity in the soil which regulates biogeochemical nutrient cycle [16]. These amendments influence the precipitation and sorption mechanism of the P, which facilitates more availability to P to the plants than from synthetic fertilizers [17]. Though the organic amendments are not rich in P by themselves yet, they release certain types of humified materials such as organic acids alkaline phosphatase (ALP), which effectively influence the P availability and mobility in soil. The use of organic additions in conjunction with inorganic or chemical fertilizers promotes ALP activity, increases soil organic C substrate, and maintains soil aggregate stability for microbial biodiversity, resulting in more plant-available P in the soil [18]. Continuous application of manures in alkaline/calcareous soils for a longer period increases the P availability [19]. A large amount of the P in the form of nucleic acid and phospholipids has also been reported, which through the process of mineralization can be released, increasing the concentration of soil P [20]. Manures can greatly change the availability mechanisms of soil P by improving soil characteristics and by lowering the soil pH. However, there will always be space to investigate manure-induced transformation and P availability mechanisms [11].

The present study was conducted to investigate the impact of different P-fertilizing regimes, to monitor the nutrients dynamic in legume crop (chickpea), including the conventional chemical fertilizer (DAP), organic fertilizers (normal or processed animal manure), and a combination of both chemical and organic fertilizers with or without reducing mineral P input on the growth, physiology, root nodulation, and yield of chickpea. We proposed how various fertilization strategies could alter phosphorus availability, sustain ecosystem health, and improve soil quality. The partial substitution of chemical fertilizer by processed animal manure would be capable to use the phosphorus reserve wisely. So, this study aimed to investigate how much increases in the P availability and the high yield could be attained in an alkaline calcareous soil on a sustainable basis.

2. Materials and Methods

2.1. Site Description and Treatment Plan

A pot experiment was carried out at the wire house of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad, Pakistan (UAF) to evaluate the combined effect of normal and processed animal manure with di-ammonium phosphate (DAP) fertilizer (full and half dose) on growth, yield, and nodulation of chickpea. The experiment was arranged in a completely randomized design (CRD) with six replicates and a desi variety of chickpea Bittal 2016 was grown as a test crop. The experiment comprised nine treatments, i.e., control, recommended DAP (56 kg ha^{-1}), normal manure (20 tones ha^{-1}), processed animal manure (20 tones ha^{-1}), half DAP (28 kg ha^{-1}), and a combination of all treatments. Both nitrogen and potassium fertilizer were applied at the rate of 20 kg ha^{-1} at the time of sowing to all the pots.

Pots were filled with 8 kg (2 mm sieved) soil taken from the field area of ISES, UAF, and a sub-sample of sieved soil was collected for the analysis of soil basic physiochemical characteristics (Table 1). The samples were analyzed for pH [21] using Hanna portable pH meter, electrical conductivity (EC) using EC meter model Jenway 4510, saturation percentage (SP) following gravimetric method, available P fraction was determined by taking 5 g of soil, extracted using 0.5 M of sodium bicarbonate extractant solution having pH of 8.5, the color-developing reagent containing ascorbic acid solution of ammonium heptamolybdate and antimony potassium tartrate in sulfuric acid was added in the prepared sample. This sample was proceeded for absorption at 882 nm wavelength through [22] using model UV-visible spectrophotometer (T-60), soil texture class [23], the total amount of $CaCO_3$, extractable potassium [24] with a flame photometer (EI 392), and for total nitrogen macro Kjeldahl method was followed for the distillation of (NH_4) into 4% boric acid (H_3BO_3) following [25]. All reagents and chemical were analytical grade, provided by Sigma-Aldrich, USA and Merk, Germany.

Table 1. Characteristics of soil, fresh and processed animal manure used for experiment.

Parameters	Soil	Normal Manure	Processed Manure
pH	8.09 ± 1.00	7.8 ± 1.02	6.9 ± 0.92
EC * (dS m^{-1})	1.76 ± 0.56	1.80 ± 0.60	1.75 ± 0.67
Cation Exchange Capacity (c mol_c kg^{-1})	11.2 ± 1.40	43.60 ± 1.90	96.34 ± 2.01
Carbon (g kg^{-1})	22.67 ± 1.98	405± 3.38	265 ± 2.19
Calcium Carbonate (%)	3.0 ± 1.60	-	-
Soil textural Class	Sandy Clay Loam	-	-
Saturation percentage (%)	34.6 ± 2.34	-	-
Total Nitrogen (%)	0.36 ± 0.04	1.62 ± 1.05	2.90 ± 0.99
Olsen P (mg kg^{-1})	4.34 ± 0.18	5.4 ± 0.92	6.8 ± 0.95
Extractable K (mg kg^{-1})	113 ± 2.23	68 ± 2.89	87.04 ± 2.01

* EC: electrical conductivity. The values are mean (n = 3).

Five healthy seeds were sown in each pot and after germination three plants per pot were maintained. Pots were irrigated with good quality canal water when required.

2.2. Collection and Preparation of Normal and Processed Animal Manure

Fresh animal manure was collected from the University of Agriculture animal farms. For processed acidified manure (AM), manure was collected from the livestock section, Faculty of Animal Husbandry, University of Agriculture Faisalabad, Pakistan, and sun-dried to remove extra moisture. After drying, molasses (0.1% w/w) and inoculum (1% v/w) of cellulase producing *Bacillus* sp. were mixed. The mixture was composted by running locally fabricated mechanical composter for three weeks [26]. The moisture contents of the compost were maintained at 50–55% (v/w) during the composting till maturity of the product (dark humus like material). The non-processed and processed AM were analyzed for physico-chemical properties as mentioned in (Table 1).

2.3. Growth and Yield Attributes of Chickpea

Plant growth parameters, i.e., shoot and root length were measured by using a meter rod. The dry (dried at 65 °C in forced air oven) biomass of the shoot and root was recorded using electronic weighing balance. Yield parameters, i.e., the number of primary branches, total numbers of pods, 100 seed weight, nodulation count, and weight were recorded at the vegetative stage after harvest of 3 repeats [27].

2.4. Physiological Parameters of Chickpea

Plant physiological parameters, i.e., chlorophyll a, b, and carotenoids were measured using the chemical method identified by Sumanta et al. [28]. Fresh leaf samples (0.5 g) were homogenized with 10 mL methanol, centrifuged, and analyzed on a spectrophotometer at a wavelength of 665.2, 652.4, and 470 nm.

The following Equation was used to calculate the concentration of chlorophyll a, b and carotenoids.

$$\text{Chlorophyll a} = 16.72 * \text{A } (665.2) - 9.16 * \text{A } (652.4)$$

$$\text{Chlorophyll b} = 34.09 * \text{A } (652.4) - 15.28 * \text{A } (665.2)$$

$$\text{Carotenoids (Cx + c)} = [1000 * \text{A } (470) - 1.63 * \text{Chl-a} - 104.96 * \text{Chl-b}]/221$$

while A = Absorbance.

For determination of leghaemoglobin concentration, nodules were isolated, and the 200 mg crushed in sodium phosphate buffer, filtered, and the reddish-brown filtrate was centrifuged. The supernatant was collected and diluted up to 6 mL using the sodium phosphate buffer. An equal volume of pyridine was also added into the solution mixture of nodules. The color becomes greenish yellow indicating the presence of ferric hemochrome. The solution was divided into two equal parts and very few crystals of sodium dithionate were added and stirred in the absence of air. Reading was noted at 556 nm using a spectrophotometer. To the other part, potassium hexaferrocynate was added for the oxidation of hemochrome. Absorbance was noted at 539 nm using a spectrophotometer [29].

For calculation of leghaemoglobin, the following formula was used:

$$\text{Leghemoglobin concentration (mM)} = \text{A (556 nm)} - \text{A (539 nm)} \times 2 * \text{D}/23.4$$

where A is absorbance and D is the initial dilution.

The method was followed for measuring protein contents [30]. A fresh leaf was extracted in the Bradford reagent. The mixture was incubated for 20 min and after that a spectrophotometer reading was recorded at 595 nm. Bovine serum albumin standard curve was used for the calculation.

2.5. Phosphorous and Potassium Determination in Plant Tissues

The method was followed for the digestion of plant samples (including root and shoot) [31]. Ground plant samples (0.5 g) were taken in a Pyrex glass flask and H_2SO_4 (5 mL) was poured into it and was left overnight. The next day H_2O_2 (2 mL) was added to it and heated on to a hot plate until a colorless solution obtained. The obtained solution was filtered, and volume was made up to the mark and then stored in the bottle for P and K determination.

Digested plant material (5 mL) was taken into the volumetric flask, and along with this color developing ammonium-vanadomolybdate reagent was added. The sample solution was allowed to stand for 30 min for color development. Using blanks, standards, and samples, absorption was recorded using a UV-Visible spectrophotometer (T60) at the wavelength of 410 nm [32].

EI-392 Flame photometer (FPM) was used for the determination of K from the already digested root and shoot samples (digestion is same as for phosphorus analysis). Firstly, the KCl standards were prepared and run on the flame photometer, after that the root and shoot samples were analyzed on the FPM, and the concentration of K in the plant samples was calculated [33]. The plant total phosphorus uptake and phosphorus use efficiency were calculated by following formula [34]:

$$\text{Total P-uptake} = \text{P-uptake (Grain)} + \text{P-uptake (Shoot)} + \text{P-uptake (Root)}$$

$$\text{PU} = \frac{\text{Total plant biomass (oven dried)}}{100} \times \text{P}(\%)$$

where PU = phosphorus uptake.

$$\text{PUE}(\%) = \frac{(\text{Total P uptake by fertilized plant} - \text{Total P uptake by unfertilized plant})}{\text{Amount of fertilizer applied}} \times 100$$

where PUE = phosphorus use efficiency.

2.6. Statistical Analysis

The data were statistically analyzed, and mean values were compared using analysis of variance (ANOVA) at the probability level of 0.05. Tukey's HSD test was applied to check the significant difference among treatments using statistical software, Statistix v. 8.1 [35].

3. Results

3.1. Growth Parameters of Chickpea

The combination of organic manures (normal and processed) with di-ammonium phosphate (DAP) has a significant effect on the growth attributes such as shoot and root length, fresh and dry biomass of the plants, along with the number of days to flower initiation and pods formation. The results indicate that a combination of normal animal manure (NM) and half DAP increased the shoot and root length up to 48.2% and 81.69%, while the combination of NM with recommended DAP 37.70% and 74.35 and processed manure (PM) with half DAP 27.32% and 61.53%. In the same manner, normal manure and half DAP significantly enhanced the dry weight of the shoot and root upto 33% and 260.8% over control treatment, while this treatment required 22.5% and 14.7% less number of days to flower and pod initiation followed by PM plus half DAP 17.81% and 3.2%, respectively (Figure 1).

3.2. Yield Parameters of Chickpea

The combined application of normal manure with a half dose of the recommended DAP showed the statistically significant results and this treatment increased the number of primary branches 78.5%, the number of pods 119%, and 100 seed weight to 145% mark up, while through the sole application of the recommended DAP improved primary branches 55.32%, pods 102.35% and seed weight to 139.70%. The nodule fresh weight and nodule count increases up to 93.59% and 147% through the combined application of NM and half DAP followed by the sole normal manure to 69% and 52.63% than the control respectively (Figure 2).

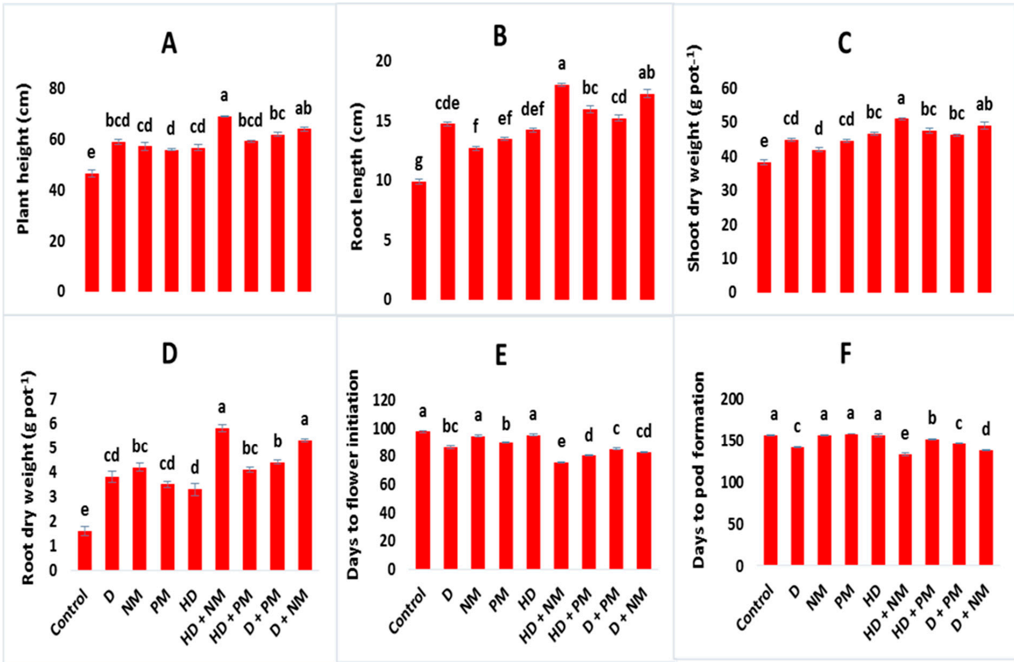

Figure 1. Effect of animal manures with DAP fertilizer on plant growth and root–shoot biomass of chickpea (**A**) plant height, (**B**) root length, (**C**) shoot dry weight, (**D**) root dry weight, (**E**) days to flower initiation and (**F**) days to pod formation of chickpea D: Recommended DAP; NM: Normal manure; PM: Processed manure; HD: Half of the recommended dose of DAP. Figure bars are showing the mean values ($n = 3$). All means followed by different letters have statistically significant differences according to Tukey's HSD test at probability level of 0.05.

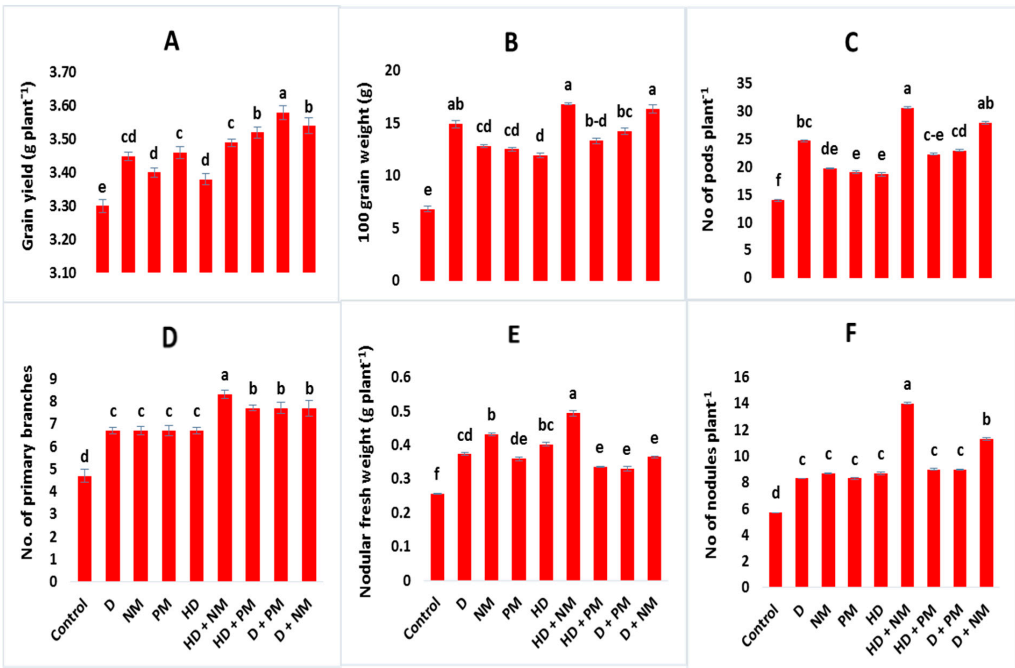

Figure 2. Effect of animal manures with DAP fertilizer on nodulation and yield attributes of chickpea (**A**) grain yield, (**B**) 100 grain weight, (**C**) No. of pods per plant, (**D**) No. of primary branches, (**E**) Nodular fresh weight and (**F**) No. of nodules per plant of chickpea. D: Recommended DAP; NM: Normal manure; PM: Processed manure; HD: Half of the recommended dose of DAP. Figure bars are showing the mean values ($n = 3$). All means followed by different letters have statistically significant differences according to Tukey's HSD test at probability level of 0.05.

3.3. Physiological Attributes of Chickpea

The addition of organic manures (normal and processed) in combination with the half and full dose of DAP significantly improved the physiological parameters of chickpea such as chlorophyll a and b, carotenoids, protein, and leghaemoglobin contents over the control. The integrated application of normal animal manure with half DAP improved the physiological attributes such as chlorophyll a (3.6 μg mL^{-1}), carotenoids (1.5 μg mL^{-1}), and leghaemoglobin contents (112.5 mM). The combination of the recommended DAP and NM increases the chlorophyll a by 94.12%, sole DAP improved chlorophyll b content about 47.17%, DAP plus PM develops 160% more carotenoids and leghaemoglobin increased 54.08% by sole NM application over control. Protein contents were found to be 78.38% more in the treatment having half DAP and normal manure compared to the control and followed by half DAP plus PM was 68.42% (Figure 3). It was also noted that chlorophyll b value (0.78 μg mL^{-1}) was at its maximum in the treatment where only the recommended dose of DAP was applied.

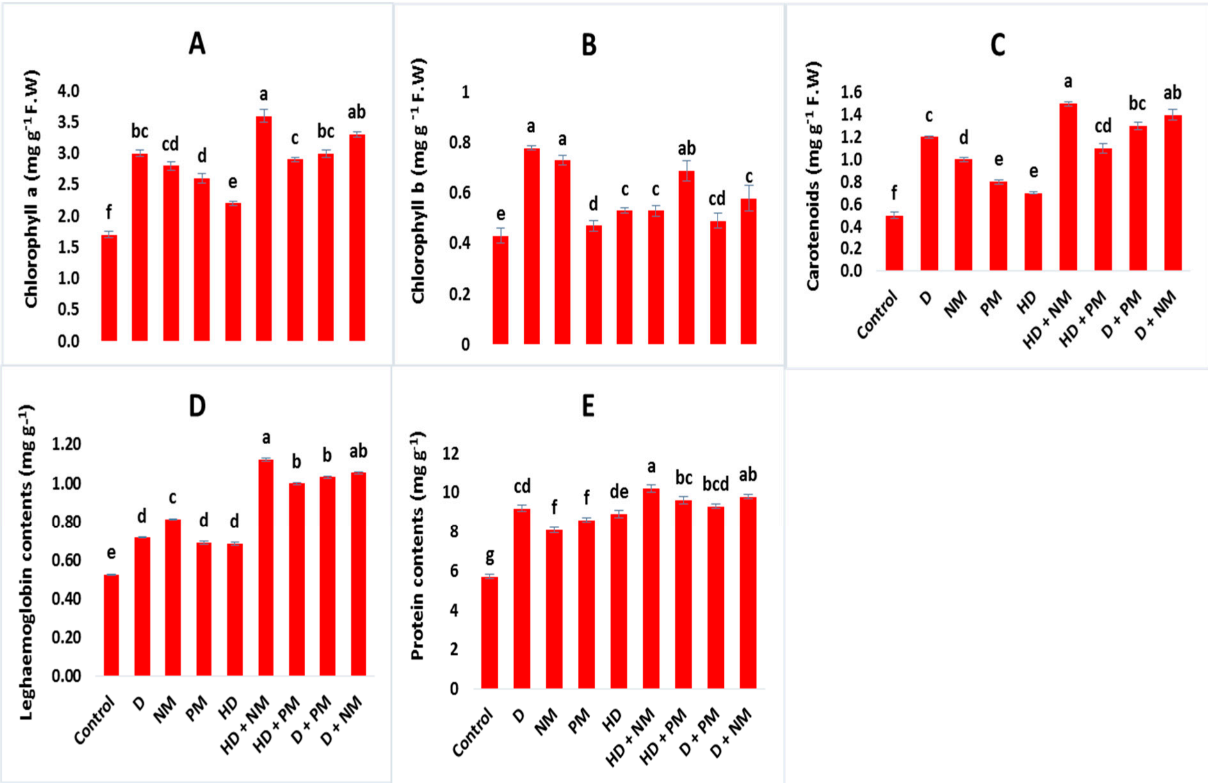

Figure 3. Effect of animal manures with DAP fertilizer on physiological attributes of chickpea (**A**) chlorophyll a, (**B**) chlorophyll b, (**C**) carotenoid contents, (**D**) leghaemoglobin contents and (**E**) protein contents of chickpea. While D: Recommended DAP; NM: Normal manure; PM: Processed manure; HD: Half of the recommended dose of DAP. Figure bars are showing the mean values (n = 3). All means followed by different letters have statistically significant differences according to Tukey's HSD test at probability level of 0.05.

3.4. Mineral Concentrations

Total phosphorous (P) and potassium (K) content in the plant shoot and root were also significantly enhanced where organic amendments were applied. The phosphorous content in the shoot and root by normal manure with half of the recommended dose of DAP was observed to be 97.7% and 139%, followed by NM and DAP where they were 93.33% and 100%, respectively over the control treatment. In the same context, potassium content in the shoot and root increased up to 80.5% and 123%, with normal manure with half DAP. While 69.23% and 118.18% were through the combined application of NM with DAP over the control treatment (Figure 4).

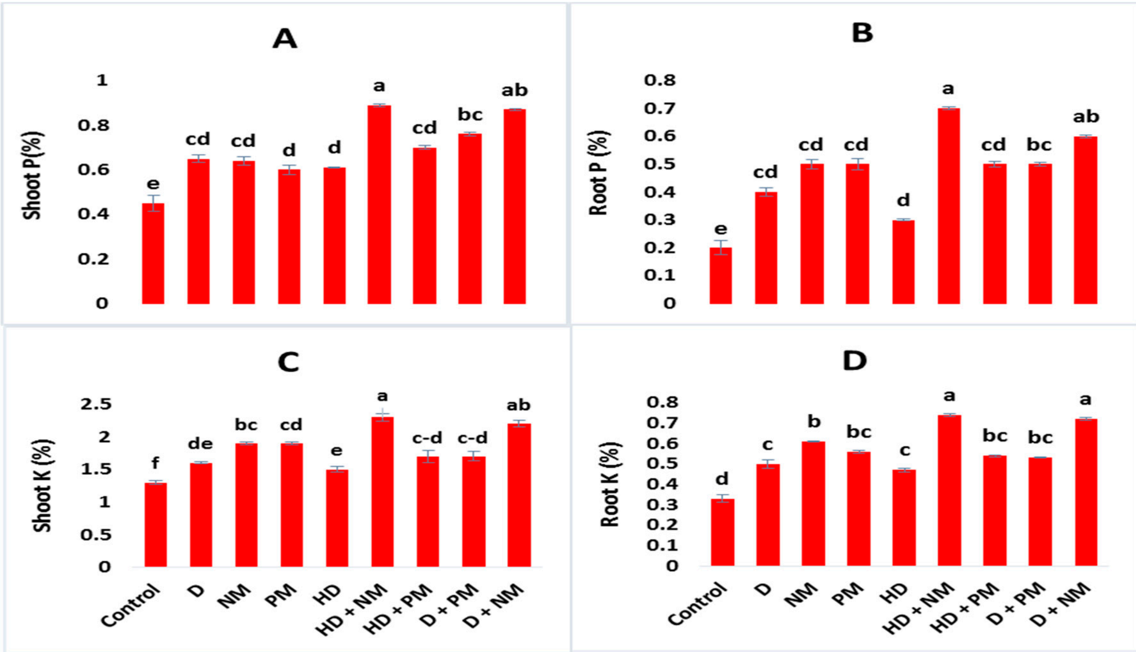

Figure 4. Effect of animal manures with DAP fertilizer plant minerals contents of chickpea (**A**) shoot phosphorus contents, (**B**) root phosphorus content, (**C**) shoot potassium content and (**D**) root potassium content of chickpea D: Recommended DAP; NM: Normal manure; PM: Processed manure; HD: Half of the recommended dose of DAP. Figure bars are showing the mean values (n = 3). All means followed by different letters have statistically significant differences according to Tukey's HSD test at probability level of 0.05.

3.5. P Uptake and P Use Effeciency

The leading treatment, normal manure applied with a half DAP, brought about a 179% and 215% increase in P uptake and P use efficiency of chickpea, respectively, and followed by recommended DAP plus NM was 47.56% and 68%, then recommended DAP plus PM showed 21.5% increase in P uptake and 51.45% in PUE (Figure 5).

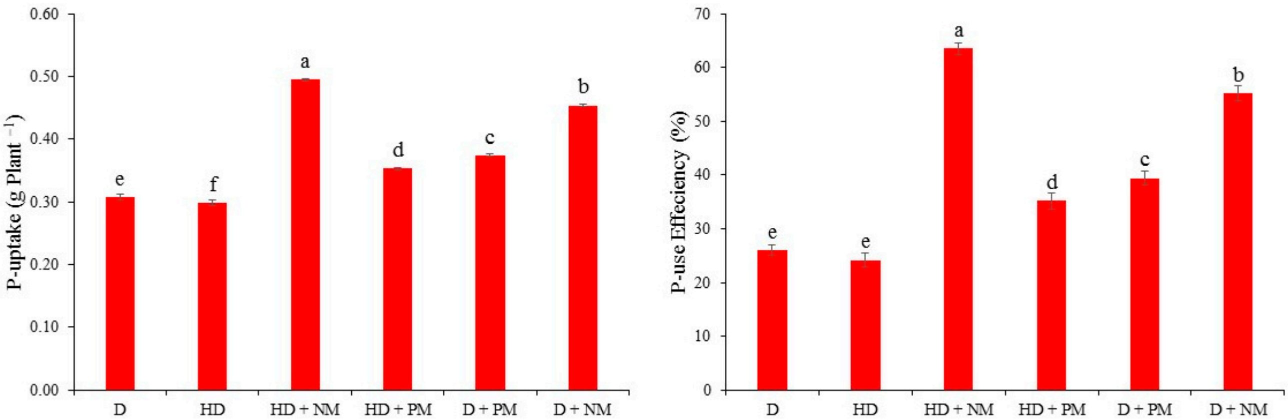

Figure 5. Effect of animal manures with DAP fertilizer on plant phosphorus contents uptake (%) and phosphorus use efficiency (%). D: Recommended DAP; HD: Half of the recommended dose of DAP; NM: Normal manure; PM: Processed manure. Figure bars are showing the mean values (n = 3). All means followed by different letters have statistically significant differences according to Tukey's HSD test at probability level of 0.05.

4. Discussion

The world population is increasing at an alarming rate over the past couple of centuries, growing from 1 billion in 1800 to 7.875 billion in 2021 [36]. However, agricultural land, soil fertility, and food production are not increasing according to world population demand, due to various climatic factors as the inefficient use of fertilizers and their related losses. In an arid climate, phosphorus (P) fixation with the alkaline compounds of calcium (Ca) is a common problem of low soil fertility [37,38]. Almost 80% of the applied P is unattainable to plants just because it gets fixed, while its demand is continually increasing globally by 3–4% per annum [39,40]. Therefore, there is a need to adopt those strategies that are efficient for sustainable agriculture and P utilization. One possible way around this is the utilization of organic amendments that improve the efficacy of the chemical P fertilizers.

In the present study, the combination of the animal manures and di-ammonium phosphate (DAP) has improved the growth and yield parameters of the chickpea plant (Figures 1 and 2) which is an indication that the amalgamated use of organic and inorganic sources is providing the sufficient P to the chickpea plant than any of the single sources. An increase in plant growth and physiological attributes was observed which could most probably be associated with enhanced soil characteristics and nutrient availability by both organic and chemical amendment [41]. Enhanced soil characteristics and efficient nutrient uptake have increased the rate of cell division, expansion, and elongation [42,43]. The increased root length was due to the use of organic manure with chemical fertilizers providing better aeration and low bulk density of the surface soil, thus helping better root proliferation [44,45]. The lower growth response in the treatments where only the organic and inorganic fertilizers were involved suggested sole sources were unable to supply the sufficient P and the organic amendment alone slowly released the nutrient which is not sufficient to meet the crop requirements and lower growth [46]. Normal manure with a half dose of DAP significantly improved the shoot dry weight and number of pods, which is synonymous with the findings of [47]. The increase in yield, since the combination of organic manure and mineral fertilizer provides the nutrients to the plant at a rapid pace, helps the plants in building up the mass, resulting in increased yield and these results are also in line with the findings of [48–51]. It was noted that the combination of animal manure with a half dose of DAP also furnished a positive impact on the nodulation of chickpea (Figure 2F), organic manure a medium for microbial growth, providing better aeration and nutrient availability [52]. Poor nodule growth in the plants has low phosphorus (P) because [53] suggested microbes do not have enough P to form the nodule). Thus, based on these facts we proposed that the application of animal manure with DAP provided sufficient P to the microbial community that ultimately led to better nodulation. This fact is well supported by the findings of [54,55] that 25% of P is transferred towards nodules.

The amalgamated use of animal manure and DAP fertilizer also boosted the physiological parameters of the chickpea such as chlorophyll a, b, carotenoids, and protein content (Figure 3). The increase in the chlorophyll and protein contents resulted in a higher photosynthetic rate [42,56]. Further, this composite source of AM and DAP (100:50 ratio) improved not only chemical constituents (N and K) of the chickpea grain and straw, but also yielded grains with better nutritional quality by improving nutrient use efficiencies, especially PUE [34]. The application of organic amendments provided carbon substrate, essential prerequisites for the growth of microbes. It has a vital role for microbial respiration, it boosts up the microbial population, (N-fixing bacteria) increasing nodulation and P mineralization, and this high P availability contributes to a higher crop yield [57,58]. The other reason associated might be that animal manure contained higher organic matter, increased soil moisture retention, and improved the dissolution of nutrients, particularly phosphorus, ultimately leading to the increased yield parameters [59]. The results of increased yield and number of pods with the amalgamated use of organic and inorganic sources were also reported by the scientists [60–62].

Organic amendments such as animal manure amalgamated with the chemical P fertilizer play a key role in efficient nutrient uptake and sustaining soil health. It reduces the

P fixation in the soil, increasing the water holding capacity in the soil and the diffusion shell of the P [63]. The results of our study revealed that the treatment where the combination of normal manure and DAP was used had significant nutrient uptake and increased the total mineral concentration P and K in the root and shoot as well (Figure 4). It indicates that the amalgamated use of animal manure and DAP fertilizer exhibited the higher potential to supply nutrients to the chickpea and increase their availability for a longer time. The increased nutrient availability (N, P, K) to the plants, decomposition of organic material released H+ ions, increased the substitution reaction and enhanced the nutrient availability. The presence of organic matter in soil have also increased the solubility of soil K as reported by [64–68].

5. Conclusions

This study concludes that the sole application of manures, DAP, and their combinations significantly improved growth, yield, physiology, and quality of the chickpea crop. However, the combined application of normal animal manure with a half dose of DAP proved the most effective treatment in promoting growth, physiology, and yield of chickpea. It also increased nutrient concentrations of K and P in plants. Our findings suggest that the combined use of normal animal manure with a half dose of DAP is a more efficient strategy in promoting plant growth, physiology, and productivity of chickpea as well as restoring soil fertility and health. It can serve as a better alternative to the individual application of inorganic fertilizers.

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