Nutrient uptake and pharmaceutical compounds of *Aloe vera* as influenced by integration of inorganic fertilizer and poultry manure in soil

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**A B S T R A C T**

*Aloe vera* had been used for numerous medical and cosmetic applications since ancient times. The study aimed to investigate the integrated effects of inorganic fertilizer (IF) and poultry manure (PM) on the nutritional and pharmaceutical constituents of *A. vera*. Eighteen month old *A. vera* seedlings were used following completely randomized design with three replications. Six combinations of IF [Nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) at the rate of 150, 80, 120 and 30 kg ha$^{-1}$, respectively] and PM (at the rate of 5 t ha$^{-1}$) were considered viz., IF00PM00 (IF 0%, PM 0%), IF100PM00(IF 100%, PM 0%), IF75PM25 (IF 75%, PM 25%), IF50PM50 (IF 50%, PM 50%), IF25PM75 (IF 25%, PM 75%) and IF00PM100 (IF 0%, PM 100%) as treatments. Different treatment combinations of IF and PM exerted significant influence on the nutritional and pharmaceutical contents of *A. vera*. Concentrations and uptake of the concerned nutrients were gradually increased with the increased levels of PM except NPKS which were highest in sole application of IF. The aloin concentration of leaf was gradually increased with the increased level of PM and by 42.44% over control. The highest chlorophyll, total phenolic and flavonoid concentrations were found in the plants receiving the treatment IF25PM75 except protein content which was obtained from IF100PM00. Significant and positive relationships between N and S with P concentrations and P and S with K concentrations of *A. vera* leaf were noticed. Aloin, total phenolic and flavonoid concentrations were significantly and positively correlated with Mg, Fe and Mn concentrations of *A. vera* leaf. Farmers may be advised to cultivate *A. vera* applying 75% PM at the rate of 5 t ha$^{-1}$ along with 25% IF (N, P, K and S at the rate of 150, 80, 120 and 30 kg ha$^{-1}$, respectively) for obtaining better quality leaf in terms of nutrients and pharmaceutical compounds under the agro-climatic conditions of the study area.

**1. Introduction**

The increasing use of chemical and synthetic drugs in the last half-century has resulted to the development of resistance infectious diseases. This has led to increased emphasis on the use of plants as a source of medicines for a wide range of human diseases [1]. According to World Health Organization (WHO) data, about 80% of the world population utilizes traditional medicines derived from plant extracts for health care due to the effectiveness of the treatment in most cases and their relative safety as well as their low cost. Approximately 25% of the world pharmaceutical products use raw materials derived from plants [2]. This proves that medicinal plants have become an important source of material for modern herbal medicine.

*A. vera* (syn.: *Aloe barbadensis* Miller) is a perennial *liliaceous* plant belonged to the family Xanthorrhoeaceae having green, tapering, spiny, marginated and dagger shaped fleshy leaves filled with a clear viscous gel features [3, 4, 5, 6]. The plant's leaves contain four kinds of vitamins, minerals and six type of enzymes, fat, carbohydrates, proteins and 18 essential amino acids. Besides, *A. Vera* is furnished with calcium (Ca), sodium (Na), potassium (K), manganese (Mn), magnesium (Mg), copper (Cu), zinc (Zn), chromium (Cr) and antioxidant selenium [7, 8, 9]. They also contain secondary metabolites: alkaloids, aloins, lectins, lignin,

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saponins, tannins, phenolic and glucosamin [10, 11, 12, 13, 14, 15]. All these active substances contribute to the effectiveness of aloe leaves by synergistic action. A. vera possesses a wide variety of biological and physiological activities in cosmetology and medicine, healing ability of skin burns and cutaneous injuries, antitumor, anti-diabetic, anti-fungal, anti-bacterial, anti-inflammation, anti-AIDS and anti-cancer [16, 17, 18, 19, 20].

Poultry manure has a better effect on soil conservation than any other animal and livestock wastes. It contains two to three times more N, P, and K as other farm manures and acts as an important soil conditioner, animal and livestock wastes. It contains two to three times more N, P, and K and S at the rate of 150, 80, 120, 40 kg ha⁻¹ were applied. PM contained 16.86, 2.20, 1.72, 0.42, 0.27, 0.21 and 0.20% of N, P₂O₅, K₂O, Mg, Ca, S, and Zn, respectively.

2. Materials and methods

A pot experiment was conducted during October, 2017 to June, 2018 in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh. The experimental setup was done following Completely Randomized Design (CRD) with three replications comprising of six treatments viz., IF₀₀PM₀₀, IF₂₅PM₇₅, IF₅₀PM₂₅, IF₅₀PM₇₅, IF₂₅PM₇₅ and IF₀₀PM₁₀₀. Inorganic fertilizer (IF) consisting of K₂SO₄ and NH₄NO₃. The use of potassium (K) and phosphorus (P) in combination with inorganic fertilizers is considered a good management practice because it stimulates microbial activity and increases soil fertility and quality through subsequent mineralization of plant nutrients [25]. However, traditional organic input methods such as the use of crop residue and animal manure are generally of low effectiveness [26] and are usually unable to satisfy the nutrient demands of cultivated crops. An alternative practical strategy is to co-apply available plant residues with readily available inorganic fertilizers. In some instances, this approach can overcome organic substrate limitations while reducing the rate of application of readily available nutrient sources. This practice has been suggested as a promising strategy to enhance agricultural productivity [27].

A. vera comes from the Arab Peninsula but grows wild in tropical, semi-tropical, and arid habitats across the world [28]. In Bangladesh, it is grown in many places, but not in a wide range. Fertility management in the A. vera field may be one of the techniques for boosting up the yield. The quality and quantity of phytochemicals are dependent on the relative composition of the mineral constituents, which is greatly influenced by the agro-climatic condition as well as nutrient management [29, 30]. In another investigation it was determined that the nutrients have a positive influence on the rate of the active substances like aloin of A. vera [31]. As A. vera is a succulent plant and thus it is more responsive to nutrients [4]. Fertilization with inorganic nutrients especially nitrogen (N) enhances the physical growth and biochemical contents of A. vera [32, 33]. Constant use of inorganic fertilizer renders unbalancing of nutrients in soil which has deleterious effects on crop yield as well as soil health. This is first time we are reporting the medicinal compounds of A. vera under integrated fertilizer management. To the best of our knowledge, no detailed research report has yet been published on the conjoint application of poultry manure (PM) and inorganic fertilizer (IF) on nutritional and pharmaceutical contents of A. vera in Bangladesh. Keeping this in view, the present study was conducted to investigate the integrated effects of PM in combination with IF at different percentages on the nutritional and pharmaceutical constituents of A. vera.

2.1. Preparation of A. vera leaf for chemical analyses

For preparing the extraction, the fresh leaf was chopped, washed, and cut from the middle. The gel was separated by scraping it with a spoon. Then the leaf gel was sun dried for 2 days. Sun dried leaf gel was oven dried at 70 °C for 48 h and ground, preserved in polythene bag and kept in desiccators.

2.2. Preparation of leaf gel extract for nutrient analyses

Requisite quantity of powdered A. vera leaf gel was weighed accurately and taken for extraction. For the determination of mineral nutrients, exactly 0.5 g of gel powder were taken into a 250 mL conical flask and 10 mL of di-acid mixture (HNO₃:HClO₄ = 2:1) was added to it. Then, they were placed on sand bath (180 °C) until the solid particles disappeared and milky dense white fumes were evolved from the flask. Then they were cooled at room temperature (25 °C), washed with distilled water and filtered into 100 mL volumetric flasks through Whatman No. 42 filter paper making the volume up to the mark with distilled water following wet oxidation method. The extracts were used for the determination of P, K, S, Ca, Mg, Fe, Mn, and Zn.

2.2.1. Determination of N by Kjeldahl method

Total N of the A. vera leaf gel was determined by Kjeldahl method [36]. Powdered gel samples were digested with conc. H₂SO₄ in presence of K₂SO₄ catalyst mixture (K₂SO₄:CuSO₄.5H₂O:Se = 10:1:0.1). Nitrogen in the digest was collected by distillation with 40% NaOH. The distilled ammonia was trapped in 4% H₃BO₃ solution and 4 drops of mixed indicator (Methyl red and Bromocresol green) solution. Finally the distillate was treated with standard H₂SO₄ (0.005M) until the color changed from green to pink. The amount of N was calculated using the following formula:

\[ \text{Total N (\%)} = \frac{(T-B)\times M\times 1.45}{S} \]

Where, T = Sample titration, mL standard H₂SO₄; B = Blank titration, mL standard H₂SO₄; M = Molarity of H₂SO₄ and S = Sample weight (g).

2.2.2. Determination of P by ascorbic acid blue color method

Total P of the A. vera leaf gel extract was determined colorimetrically using molybdylate blue ascorbic acid method [37]. Ascorbic acid was used as a reducing agent to develop blue colour and was measured at the wave length of 660 nm with the help of a spectrophotometer (Model: TG-60 U, UK).

2.2.3. Determination of K by flame photometric method

The concentration of total K in the A. vera leaf gel extract was determined with the help of a flame photometer (Model Number: Genway PPFT) [38].

2.2.4. Determination of S by turbidimetric method

The concentration of total S in the A. vera leaf gel extract was determined by turbidimetric method [35] with the help of a spectrophotometer (Model: TG-60 U, UK). Turbidity was developed by using barium chloride (BaCl₂.2H₂O) and measured at the wave length of 425nm.
2.2.5. Determination of Ca and Mg by complexometric titration method

The concentrations of total Ca and Mg in the A. vera leaf gel extract were determined by complexometric method of titration using Na₂-EDETA as a complexing agent where calgon and eriochrome black T were used as indicators at pH 12 (Using 10% NaOH solution) and 10 (Using NH₃-NH₄ buffer solution), respectively. 10 mL leaf gel extract was taken in a 250 mL conical flask and 50 mL of distilled water was mixed. Then, desirable amounts of buffer and indicator solutions were added. It was then titrated with standard Na₂-EDETA (0.02M) solution until the colour of the solution changed from pink to sharp blue (end point). The titration was repeated at least three times along with the blank.

2.2.6. Determination of Fe, Zn and Mn

Total Fe, Zn and Mn concentrations in the A. vera leaf gel extract were determined by atomic absorption spectrophotometer (Model UNICAM 969, England) [40].

2.2.7. Nutrient uptake was calculated using the following formula

Nutrient uptake (g plant⁻¹) = [(Nutrient content (%)/100) X dry weight (g plant⁻¹)] [41], [42].

2.2.8. Computation of protein content

Protein content was computed by multiplying N content of A. vera leaf gel by a conversion factor of 5.85 [43].

2.2.9. Determination of chlorophyll concentration

Chlorophyll concentration of A. vera leaf was determined following Arnon method [44]. Five hundred milligrams of fresh leaf was treated with 15 mL of 80% acetone and centrifuged at 2500 rpm for 10 min. One mL aliquot of the extract and 9 mL of 80% acetone were transferred to a cuvette and the absorbance was read at 645 and 663 nm for chlorophyll a and b, respectively with the help of a spectrophotometer (Model: TG-60 U, UK).

2.2.10. Determination of total phenolic concentration

Total phenolic concentration of A. vera leaf gel extract were determined by using the Folin-Ciocalteu's assay using gallic acid as standard [45]. In the procedure, 0.5 mL of plant extracts were mixed with 1.5 mL Folin-Ciocalteu's reagent (FCR) diluted 1:10 v/v than after 5 min 1.5 mL of 7% Na₂CO₃ solution was added. The final volume of the tubes was made up to 10 mL with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer (Model: TG-60 U, UK). All the chemical analyses were done in the laboratories of the department of Agricultural Chemistry, Biochemistry, Professor Muhammed Hussain Central Laboratory (PMHCL), BAU, Mymensingh and SRDI regional laboratory, Dhaka. All the data were analyzed for ANOVA with the help of a computer package program of MSTAT (Mathematical and Statistical Calculation). A one way ANOVA was made by F variance test. The pair comparisons were performed by LSD (Least Significant Difference) test and DMRT (Duncan’s Multiple Range Test) at 5% and 1% level of probability [48].

3. Results and discussion

3.1. Nutrient concentrations and their uptake by A. vera

3.1.1. N concentration and uptake

N concentration of the A. vera leaf gel was significantly affected by different combinations of IF and PM (Table 1). The sole application of inorganic fertilizers increased N concentration by 2.71% which was statistically identical with the IF25PM25 treated plants but significantly different from other treatments. The lowest N concentration was obtained from the plants receiving no fertilizer. The N uptake by A. vera leaf gel varied from 3.10-11.20 g plant⁻¹. The highest uptake (11.20 g plant⁻¹) was found in sole application of IF which was identical with the 25% IF and 75% PM treated plant and the lowest uptake of 3.10 g plant⁻¹ was observed in control treatment.

| IF and PM levels | Nitrogen (N) | Phosphorus (P) | Potassium (K) | Sulphur (S) |
|------------------|-------------|----------------|---------------|-------------|
|                  | Conc. (%)   | Uptake (g plant⁻¹) | Conc. (%) | Uptake (g plant⁻¹) | Conc. (%) | Uptake (g plant⁻¹) |
| IF0PM0           | 1.87 ± 0.11d | 3.10 ± 0.3d      | 0.13 ± 0.03d | 0.21 ± 0.01d | 1.02 ± 0.06e | 1.7 ± 0.18e     | 0.17 ± 0.01c | 0.4 ± 0.29c |
| IF50PM50         | 2.71 ± 0.14a | 11.20 ± 0.8a    | 0.34 ± 0.04a | 1.43 ± 0.28a | 1.88 ± 0.09a | 7.81 ± 1.09a   | 0.24 ± 0.01a | 1.88 ± 0.98a |
| IF0PM25          | 2.14 ± 0.16c | 7.24 ± 0.2b     | 0.31 ± 0.02b | 1.08 ± 0.22b | 1.82 ± 0.07a | 6.18 ± 0.76bc  | 0.22 ± 0.01ab | 1.53 ± 0.77bc |
| IF25PM50         | 2.38 ± 0.14b | 7.55 ± 0.2b     | 0.22 ± 0.02c | 0.99 ± 0.14b | 1.61 ± 0.06b | 5.13 ± 0.52c   | 0.23 ± 0.04ab | 1.39 ± 0.74c |
| IF25PM75         | 2.58 ± 0.03ab| 10.87 ± 0.7a    | 0.32 ± 0.01a | 0.91 ± 0.06b | 1.48 ± 0.09c | 6.21 ± 0.18b   | 0.22 ± 0.01ab | 1.84 ± 0.91ab |
| IF0PM100         | 2.08 ± 0.09cd| 5.72 ± 0.9c     | 0.21 ± 0.01c | 0.56 ± 0.08c | 1.17 ± 0.05d | 3.20 ± 0.28d   | 0.19 ± 0.02bc | 0.92 ± 0.54d |
| CV%              | 5.28        | 8.03            | 5.20         | 18.47       | 4.80         | 11.94          | 9.11         | 13.09       |
| LSD0.05          | 0.22**      | 1.09**          | 0.02**       | 0.28**      | 0.13**       | 1.07**         | 0.03**       | 0.16**      |

IF = Inorganic fertilizers; PM = Poultry manure; NS = not significant. Means within the same column followed by the different letter(s) were significantly different according to DMRT (**P < 0.01; *P < 0.05), Values are mean ± SD; LSD = Least significant difference; CV = Coefficient of variance.
The combination of manure and mineral N fertilizers was reported to improve total organic N, the microbial biomass N, the labile N, the inorganic N including NH4-N and NO3-N concentrations, the net ammonification, nitrification and N mineralization [49] through the comparatively prolonged N supply and uptake by plants [50]. This would explain why N concentration and uptake was increased by combinations of IF and PM than sole PM treatment. These results were in line with [51] and [52] who reported that the combination of organic and inorganic fertilizers increased N uptake. The application of N fertilizer and manure significantly influenced the N uptake by rice plant [53].

3.1.3. K concentration and uptake

K concentration and uptake by A. vera leaf gel were significantly affected by different treatments of IF and PM (Table 1). The highest K concentration (1.88%) was obtained from IF50PM50 which was statistically identical with the K concentration of the leaves of A. vera plant fertilized with IF50PM25 but significantly different from other treatments. The minimum K concentration was recorded in case of zero fertilizer. The uptake of K varied from 0.61-1.23 g plant⁻¹. The uptake of K was highest when the plant was treated with IF100PM00 which was identical with IF25PM75. This could be due to the highest leaf P concentration and dry leaf weight harvested from these treatments as nutrient uptake was calculated from their concentrations and corresponding dry leaf weight. The lowest P uptake as expected was observed in the control treatment.

The interaction between organic and conventional farming showed an increasing effect on P uptake by potato tuber and haulm [54]. The present study coincided with the findings of [55, 56] who reported application of inorganic and organic fertilizers facilitated the maximum uptake of N, P and K. Ghosh et al., [57] and Shah et al., [58] support the results of the study that the combined application of organic and inorganic fertilizers renders highest P uptake by plants. Maximum NPK uptake by rice under FYM + NPK treatment compared to control was also reported [59].

3.1.4. S concentration and uptake

Different combinations of IF and PM brought a significant influence on the S concentration and uptake by A. vera leaf (Table 1). The highest S concentration (1.32%) was obtained from IF50PM50 which was statistically identical with the S concentration of the leaves of A. vera plant fertilized with all other treatments except sole application of PM. The lowest S concentration was obtained from the plants receiving no fertilizer. Sulphur uptake varied from 0.40-1.88 g plant⁻¹. The uptake of S as expected was maximum in 100% IF followed by 75% PM and 25% IF. The lowest S uptake was observed in the control treatment. Increased uptake of NPK and S by tomato plants was observed by [63] applying poultry litter. The results are in accordance with the findings of [64] who reported sole application of chemical fertilizer and combination of 75% chemical fertilizer and vermicompost at the rate of 7.5 t ha⁻¹ resulted in maximum S uptake by stevia.

3.1.5. Ca concentration and uptake

Concentration and uptake of Ca by A. vera leaf gel showed a significant variation for treatment combinations of IF and PM (Table 2). The highest Ca concentration (0.25%) was recorded in 100% poultry manure treated plant and the lowest (0.13%) was recorded in case of zero fertilizer treatment which was statistically similar with plants treated with 100% IF and 75% of IF with or without PM. The Ca uptake by A. vera ranged from 0.21-1.03 g plant⁻¹. Maximum uptake was noticed as expected in 75% PM treated plants which was statistically dissimilar with other treatments. Ca uptake of other treatment combinations except control was statistically identical. The results of our study was supported by the findings of [55] who reported that Ca uptake by squash fruit was significantly enhanced with the addition of farm yard manure.

3.1.6. Mg concentration and uptake

The results presented in Table 2 indicate that there were significant variation in Mg concentration and uptake by A. vera due to the effect of various treatments. The highest Mg concentration (0.41%) was determined from 75% PM treated plant which was statistically similar with sole application of PM and dissimilar with others. The second highest Mg concentration was from 100%, 75% and 50% of IF treated plants with or without PM. The lowest concentration (0.21%) was obtained from control. Magnesium uptake by A. vera was in the range from 0.36 to 1.5 g plant⁻¹. Maximum Mg uptake (1.5 g plant⁻¹) by A. vera leaf gel was found where 75% poultry manure and 25% IF was used followed by 100% PM treated plant. The lowest Mg uptake was calculated from no fertilizer treated plant. The result of the present study was congruent with the results of [55] in potato due to the application of poultry manure and [57] in NERICA 10 due to the combined application of IF and cow dung.

3.1.7. Fe concentration and uptake

Various treatment combinations of IF and PM significantly differentiated the concentration and uptake of Fe by A. vera (Table 2). The concentration ranged from 119 to 331 μg g⁻¹ due to different treatments. The highest Fe concentration (331 μg g⁻¹) was recorded in the 100% PM treated plant which was statistically different from other treatments. Lowest Fe concentration (55.81 μg g⁻¹) was found where no fertilizer was used. Maximum Fe uptake (105 mg plant⁻¹) was observed in 75% PM and 25% IF treated plants which was statistically dissimilar to all other treatments. The second highest Fe uptake (89 mg plant⁻¹) was found in the sole application of PM. The lowest Fe uptake (20 mg plant⁻¹) was found in the control.

Fe concentrations were gradually increased with the increased percentages of PM though uptake did not follow the same trend. Prasad et al., [66] reported that addition of poultry manure alone or in combination within organic fertilizers increased the uptake of Zn and Fe by wheat and rice. Faiyard et al., [67] recorded an increase in N, P, K, Fe, Mn and Cu concentrations in Faba beans due to the application of poultry manure and [68, 69] Application of organic manure along with inorganic fertilizer enhances the microbial activity [70], nutrient use efficiency [71] and the availability of the native nutrients and thus higher uptake of nutrients [72].

3.1.8. Zn concentration and uptake

Zn concentration of A. vera leaf was not significantly affected by different combinations of IF and PM though the uptake was significant (Table 2). It might be due to the fact that dry weight of A. vera leaf of the treatments was significantly different. However, the highest Zn concentration was from 100%, 75% and 50% of IF treated plants with or without PM. The lowest concentration (0.21%) was obtained from control was statistically identical. The results of our study was supported by the findings of [55] who reported that Ca uptake by squash fruit was significantly enhanced with the addition of farm yard manure.
concentration (80 μg g⁻¹) was obtained from IF0PM100 and the lowest from plant receiving no fertilizers. The highest Zn uptake (34 mg plant⁻¹) was found in 75% of PM along with 25% of IF which was identical with the 100% IF treated plant and the lowest uptake of 12 mg plant⁻¹ was observed in the control treatment. Ayeni et al., [73] showed that poultry manure increased the uptake of N, P, K, Ca, Mg, Mn, and Cu by maize grain. This is consistent with the present study that poultry litter enhanced nutrient uptake of A. vera in addition to increasing nutrient status in soil. Kumar and Chopra [74] also reported higher contents of Fe, Mn and Zn in French bean (Phaseolus vulgaris) leaves of A. vera due to the application of 10 kg N as ammonium sulphate.

### 3.1.9. Mn concentration and uptake

Integrated levels of IF and PM brought a significant variation on the Mn concentration and uptake by A. vera leaf gel (Table 2). The highest Mn concentration (223 μg g⁻¹) was obtained from the 100% PM treatment which was statistically identical with the Mn concentration of the leaves of A. vera plant fertilized with 75% PM along with 25% IF. The lowest Mn concentration (67 μg g⁻¹) was obtained from the plants receiving no fertilizer. Mn uptake varied from 11-82 mg plant⁻¹ across the treatments. The uptake of Mn was maximum in 75% PM plus 25% IF treated plant followed by 100% IF which was identical with 100% PM and 75% IF plus 25% PM. The lowest Mn uptake was observed in the control treatment. Swarup [75] and Chaudhary and Narwal [76] reported that the incorporation of manures brought about a remarkable improvement in the availability of native and applied micronutrient cations (Zn, Fe and Mn) in soil. Abdalla et al., [77] also reported significantly higher contents of Fe, Zn, Mn and Co except Cu in forage due to the application of poultry manure.

### 3.2. Protein and chlorophyll contents of A. vera

#### 3.2.1. Protein content

Protein which would be serving as enzymatic catalyst, mediate cell responses, control growth and cell differentiation [78] is considered as the third highest (10.50%) parameter of A. vera [79]. The result reveals that the protein content of A. vera leaf gel was significantly influenced by different combinations of IF and PM (Figure 1). Maximum protein content (15.85%) was observed in IF100PM0 treated plants and minimum content (10.94%) was found in control. On the other hand, the plants treated with IF100PM0 and IF25PM75 biosynthesized statistically identical percent of protein. IF25PM75 and IF50PM50 treated plants also showed identical protein content. These results are in accordance with the findings of a study [80]. They reported 7.56-15.4% crude proteins on compositional features of A. vera tissues. Saleha [81] observed an increase in the total carbohydrate, protein and ascorbic acid and a decrease in the crude fiber content of okra due to the application of 10 kg N as ammonium sulphate + 50 kg N as poultry manure.

#### 3.2.2. Chlorophyll concentrations

Combined application of IF and PM had significant effect on the chlorophyll concentrations but the trend and peaks were different than their individual applications. This may be due to the complimentary effect of IF and PM resulting in better nutrient availability. Different manuring treatment also significantly affected the chlorophyll concentration of A. vera in this study (Table 3). The highest chlorophyll a (0.29 mg g⁻¹ FW) and chlorophyll b (0.116 mg g⁻¹ FW) concentrations at harvest were observed in the treatment where 25% IF along with 75% PM was applied which was statistically superior to other treatments. The second highest chlorophyll a and chlorophyll b concentrations were observed in the plants treated with 100% PM followed by application of 50% PM with or without IF. The lowest chlorophyll a and chlorophyll b were observed in the control pot where no IF and PM was applied (Table 3). An increased trend of both chlorophyll a and chlorophyll b was observed with the increased levels of PM. It could be due to the beneficial effect of organic matter in soil properties and plant growth [82, 83, 84].

### 3.3. Pharmaceutical compounds of A. vera

#### 3.3.1. Aloin concentration

A. vera has different secondary metabolites and the most important of them is aloin. It is the active component that has anti-ulcer, inhibiting action against some bacteria and fungi-inflammation, healing ability of skin burns and cutaneous injuries properties. As shown in Table 3, integrated application of IF and PM significantly increased aloin concentration of A. vera leaf. Maximum aloin concentration (492.4 μg g⁻¹) was biosynthesized in the plants treated with IF50PM50 which was identically followed by the amounts of leaf aloin content of the plants having the treatments IF25PM75 (467.8 μg g⁻¹) and IF50PM50 (456.9 μg g⁻¹).

### Table 2. Integrated effects of IF and PM on Ca, Mg, Fe, Zn and Mn concentration and their uptake by A. vera.

| IF and PM levels | Calcium (Ca) | Magnesium (Mg) | Iron (Fe) | Zinc (Zn) | Manganese (Mn) |
|------------------|-------------|---------------|-----------|-----------|----------------|
|                  | Conc. (μg g⁻¹) | Conc. (μg g⁻¹) | Uptake (μg g⁻¹) | Conc. (μg g⁻¹) | Conc. (μg g⁻¹) | Conc. (μg g⁻¹) |
|                  | (g plant⁻¹)   | (g plant⁻¹)   | (plant⁻¹) | (plant⁻¹) | (plant⁻¹) | (plant⁻¹) |
| IF0PM0           | 0.13 ± 0.02d  | 0.21 ± 0.03c  | 0.22 ± 0.1b | 0.36 ± 0.02d | 119 ± 6.79e | 112 ± 1.42d | 67± 2c | 11.4 ± 1.2d |
| IF100PM0         | 0.19 ± 0.01d  | 0.78 ± 0.18b  | 0.24 ± 0.03b | 1.01 ± 0.15c | 139 ± 5.93d | 58 ± 7.96c | 75 ± 2.67 | 31 ± 3.1a | 84 ± 2.5c | 34.89 ± 2.9c |
| IF100PM0         | 0.14 ± 0.03cd | 0.47 ± 0.09b  | 0.29 ± 0.03b | 0.98 ± 0.17c | 154 ± 7.18d | 53 ± 6.58d | 77 ± 2.82 | 26 ± 1.4b | 113 ± 1.58c | 38.25 ± 3.7bc |
| IF100PM0         | 0.22 ± 0.04bc | 0.71 ± 0.06b  | 0.32 ± 0.02b | 1.03 ± 0.11c | 213 ± 4.83c | 68 ± 5.11c | 77 ± 5.19 | 25 ± 1.8bc | 132 ± 2.1b | 42.09 ± 4.0c |
| IF100PM0         | 0.25 ± 0.03a  | 1.03 ± 0.05a  | 0.36 ± 0.02a | 1.50 ± 0.14a | 250 ± 11.62b | 105 ± 2.95a | 79 ± 8.56 | 34 ± 0.7a | 194 ± 4.6a | 81.61 ± 5.2a |
| IF100PM0         | 0.17 ± 0.01b  | 0.48 ± 0.11b  | 0.41 ± 0.04a | 1.14 ± 0.24b | 331 ± 13.01a | 89 ± 8.58b | 80 ± 2.69 | 22 ± 3.2c | 223 ± 3.5a | 60.95 ± 6.6bc |
| CV%              | 12            | 16             | 8.83       | 15         | 4.27           | 9.17           | 6.93       | 8.74     | 2.14       | 9.52         |
| LSD0.05          | 0.04**        | 0.17**         | 0.05**     | 0.27**     | 17**           | 10.7**         | 10.7**     | NS       | 3.7**      | 5.2**        | 7.59**       |

IF = Inorganic fertilizers; PM = Poultry manure; NS = not significant; LSD = Least significant difference; CV = Coefficient of variance. Means within the same column followed by the different letter(s) were significantly different according to DMRT (*P < 0.01; *P < 0.05). Values are mean ± SD.

![Figure 1. Effects of IF and PM on the protein content of A. vera.](image-url)
Aloin concentration of leaf was increased by 42.44% over control. Previous report [12] confirmed that application of PM along with IF significantly increased aloin concentration of A. vera leaf. The result of the present study was in harmony with those obtained by [85] in A. vera plant. PM contains a higher amount of plant nutrients at the same time it regulates the physiochemical environment of soil ecosystem. The amount of aloin was enhanced in A. vera with the increasing rate of nitrogen [86] and antiplasmodial activity was increased with increase in the concentration of aloin and aloe-emodin [5].

3.3.2. Total phenolic and flavonoid concentrations

Free radical may cause many disease conditions such as cancer and coronary heart disease in human [87, 88]. Many plants extracts containing bioactive compounds including phenolics and flavonoids exhibit efficient antioxidant properties and prevent from free radical damage [88, 89]. Due to above mentioned reasons, total phenolic and flavonoid concentrations in A. vera leaf gel at different doses of fertilization had been determined. Those phytochemical compositions of A. vera is influenced by various environmental factors and nutrition [91, 92, 93].

Data for phenolic and flavonoid concentrations in different IF and PM treated plants are presented in Table 3. Integrated application of IF and PM at different combinations significantly influenced the total phenolic and flavonoid concentrations of A. vera leaf gel. The highest phenolic concentration (29.08 mg g\(^{-1}\) FW) was found in IF25PM75 treated plants which were identically followed by 50% IF and PM treated plants. Lowest phenolic concentration (24.54 mg g\(^{-1}\) FW) was obtained from the control treatment. The highest amount of flavonoids concentration (19.61 mg 100 g\(^{-1}\) FW) was obtained in IF25PM75 treatment and the lowest (5.68 mg 100 g\(^{-1}\) FW) in the control treatment.

These results are in agreement with those reported by [87] in Ocimum basilicum and [94] in selected herbs. Zheng and Wang [94] reported that application of vermicompost increased total phenolic and flavonoid concentrations in A. vera leaf gel at different doses of fertilization had been determined. Those phytochemical compositions of A. vera is influenced by various environmental factors and nutrition [91, 92, 93].

| IF and PM levels | Aloin (μg g\(^{-1}\) FW) | Chlorophyll (mg g\(^{-1}\) FW) | TFC (mg QE100 g\(^{-1}\) FW) | TPC (mg GAEg\(^{-1}\) FW) |
|----------------|------------------|------------------|------------------|------------------|
| IF\(_0\)PM\(_0\) | 345.7 ± 9.7 e | 11.79 ± 1.00 c | 0.068 ± 0.01c | 5.68 ± 0.32 e  |
| IF\(_{100}\)PM\(_0\) | 388.8 ± 14.9 d | 0.26 ± 0.11ab | 0.095 ± 0.01b | 9.58 ± 0.53 d  |
| IF\(_{75}\)PM\(_{25}\) | 415.6 ± 10.5 c | 0.23 ± 0.01bc | 0.091 ± 0.00 b | 12.28 ± 1.45 c |
| IF\(_{50}\)PM\(_{50}\) | 456.9 ± 9.5 b  | 0.25 ± 0.00 ab | 0.093 ± 0.00 b | 16.35 ± 0.61 b |
| IF\(_{25}\)PM\(_{75}\) | 467.8 ± 11.7 b | 0.29 ± 0.01a  | 0.116 ± 0.01a  | 19.61 ± 1.04 a |
| IF\(_0\)PM\(_{100}\) | 492.4 ± 14.0 a | 0.287 ± 0.00 a | 0.100 ± 0.00 b | 15.89 ± 0.24 b |
| CV | 2.79 | 11.47 | 9.47 | 6.17 |
| LSD\(_{0.05}\) | 21.2** | 0.05* | 0.02** | 1.45** |

IF = Inorganic fertilizers; PM = Poultry manure; NS = Not significant; TFC = Total flavonoid concentration, TPC = Total phenolic concentration; QE = Quercetin equivalent; GAE = Gallic acid equivalent; FW = fresh weight; LSD = Least significant difference; CV = Coefficient of variance. Means within the same column followed by the different letter(s) were significantly different according to DMRT (**P < 0.01; *P < 0.05), Values are mean ± SD.

Figure 2. Relationships between (A) N and P concentrations (B) P and K concentrations (C) P and S concentrations (D) S and K concentrations (E) Mg and Mn concentrations (F) Mg and Fe concentrations of A. vera as influenced by IF and PM (n = 18).
concentrations at 30% and 15% vermicompost, respectively. Another study [96] reported that application of PM along with IF increased the phenolic compounds in latex of A. vera leaves. Increasing nutrient elements in the soil treated with PM led to more secondary metabolites synthesis. The increase in phenolic concentration is related to the balance between carbohydrate sources and sinks. Thus, when there are more carbohydrates, there are also more phenolic compounds. However, excessive use of IF and PM increased a substrate's imbalance which has inhibitory effect on plant's activity. This could reduce the amount of phenolic compounds in high percentages of combination of IF and PM.

3.4. Correlation studies among different parameters of A. vera

Correlation studies give the amount of association between any pair of parameters. Interaction between mineral nutrients in crop plants occurs when the supply of one nutrient affects the absorption and utilization of other nutrients. There was a direct significant and positive relationship between N and S with P concentrations and P and S with K concentrations of A. vera at 5% level of probability (Figure 2). The positive interaction of nutrients on fresh weight of A. vera was an indication of the importance of the nutrient elements in plant nutrition. These findings are consistent with earlier findings of [97, 98, 99] and [100] in their studies of crop production. The results presented in Figure 3 show that there were significant and positive correlations between Mg, Fe and Mn concentrations with the pharmaceutical compounds of A. vera leaves. With the increase of Mg, Fe and Mn concentrations, concentration of pharmaceutical compounds (i.e. aloe, total phenolic and flavonoid concentrations) of A. vera leaf gradually increased.

4. Conclusions

The integrated effects of IF and PM on A. vera is our first work in the context of Bangladesh. From this investigation, it was noticed that different levels of IF and PM significantly affect the mineral nutrient concentrations and their uptake, chlorophyll, aloe, total phenolic and flavonoid concentrations of A. vera leaf. Concentrations and uptake of the concerned nutrients were gradually increased with the increased levels of PM except NPKS which were highest in sole application of IF. Highest chlorophyll, total phenolic and flavonoid concentrations were found in the plants receiving the treatment IF25PM75 except protein content which was obtained from IF100PM0. Aloe concentration of leaf was increased with the increased application of PM and by 42.44% over control. Relationships between nutrient and pharmaceutical compounds with mineral concentrations were found positively correlated. Among the mineral nutrients, N, P, K and S concentrations were positively correlated. Mg, Fe and Mn concentrations also positively influenced the aloe content, total phenol and flavonoid concentrations of A. vera leaf gel. Farmers may be advised to cultivate A. vera applying 75% PM at the rate of 5 t ha⁻¹ along with 25% IF (N, P, K and S at the rate of 150, 80, 120 and 30 kg ha⁻¹, respectively) for obtaining better quality A. vera leaf in terms of nutrients and pharmaceutical compounds under the agro-climatic conditions of the study area.
Declarations

Author contribution statement

Tanzin Chowdhury: Performed the experiments; Wrote the paper. Md. Akhter Hossain Chowdhury: Conceived and designed the experiments; Performed the experiments; Wrote the paper. Wang Qingyue: Contributed reagents, materials, analysis tools or data; Wrote the paper. Christian Ebere Enyoh: Analyzed and interpreted the data; Wrote the paper. Weiqian Wang, Md. Sirajul Islam Khan: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] A. Sofowora, E. Ogbonnobele, A. Oyase, The role and place of medicinal plants in the strategies for disease prevention, Afr. J. Tradit., Complementary Altern. Med. 10 (5) (2013) 210–229.
[2] World Health Organization, The Promotion and Development of Traditional Medicine: Report of a WHO Meeting [held in Geneva from 28 November to 2 December 1977], World Health Organization, 1978.
[3] M. Hasnuzzaman, K.U. Ahamad, K.M. Khalequzzaman, A.M.M. Shamsuzzaman, K. Nahar, Plant characteristics, growth and leaf yield of Aloe vera as affected by organic manure in pot culture, Aust. J. Crop. Sci. 2 (3) (2008) 158–163.
[4] S. Kumar, M. Yadav, A. Yadav, J.P. Yadav, Impact of spatial and climatic conditions on physicochemical diversity and in vitro antioxidant activity of Indian Aloe vera (L.) Burm. f, South Afr. J. Bot. 111 (2017) 50–59.
[5] M.L. Jackson, Soil Chemical Analysis, prentice, Hall of India Private Limited, New Delhi, 1973, p. 498.
[6] M.D. Boudreau, F.A. Beland, An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera, J. Environ. Sci. Health Part C 24 (1) (2006) 103–154.
[7] M.T.H. Darini, D. Indradewa, D. Shiddieq, A. Purwantoro, Response growth and Aloe vera contains aloe polyherbs plantlets from different sources, J. Sci. 4 (2013) 5–12.
[8] B.C. Ghosh, B.N. Mittra, R. Saha, S. Palit, February. Performance of Aloe vera as influenced by organic and inorganic sources of fertilizer supplied through fertigation, in: III WOCMAP Congress on Medica l and Aromatic Plants-Volume 2: Conservation, Cultivation and Sustainable Use of Medicinal and 676, 2003, pp. 171–175.
[9] L.C. Xin, W. Changhui, F. Yongmei, L. Zhaoqiu, Effect of heat treatment and dehydration on bioactive polysaccharide acomannan and cell wall polymers from Aloe barbadensis Miller, J. Food Eng. 75 (2006) 245–250.
[10] A. Vega, E. Uribe, R. Lema, M. Miranda, Hot-air drying characteristics of Aloe vera (Aloe barbadensis Miller) and influence of temperature on kinetic parameters, LWT - Food Sci. Technol. 40 (10) (2007) 1698–1707.
[11] A. Vega-Gálvez, E. Notte-Cuello, R. Lema-Mondaca, L. Zura, M. Miranda, Mathematical modelling of mass transfer during dehydration process of Aloe vera (Aloe barbadensis Miller), Food Bioprocess. Proc. 87 (4) (2009) 254–265.
[12] K. Esbo, Q. He, Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review, Crit. Rev. Food Sci. Nutr. 44 (2) (2004) 91–96.
[13] T. Reynolds (Ed.), Aloe: the Genus Aloe, CRC press, Boca Raton. Florida, USA, 2004, pp. 39–74.
[14] J.H. Hamman, Composition and applications of Aloe vera leaf gel, Molecules 13 (8) (2008) 1599–1616.
[15] L.H. Hernández-Cruz, R. Rodríguez-García, R.D. Jasso, J.L. Angulo-Sánchez, Aloe vera response to plastic mulch and nitrogen, in: J. Janick, A. Whistey (Eds.), Trends in New Crops and New Uses, ASHS Press, Alexandria, VA, 2002, pp. 570–574.
[16] C.T. Ramachandra, P.S. Rao, Processing of Aloe vera leaf gel: a review, Am. J. Agric. Biol. Sci. 5 (2) (2006) 502–510.
[17] E.B. S, Effect of poultry dung and cattle manure on chemical properties of clay and sandy clay loam soil, J. Anim. Vet. Adv. 4 (10) (2005) 839–841.
[18] M.M. Zaman, K. Nahar, T. Chowdhury, M.A.H. Chowdhury, Growth, leaf biomass yield of stevia and post-harvest soil fertility as influenced by different levels of poultry manure, J. Bangladesh Agric. Univ. 15 (2) (2017) 212–218.
[19] E.O. Adeleye, L.S. Ayeni, S.O. Ojeyeni, Effect of poultry manure on soil physico-chemical properties, leaf nutrient contents and yield of yam (Dioscorea rotundata) on alfisol in southwestern Nigeria, Am. J. Sci. 6 (10) (2010) 871–878.
[20] S.S. Ningsih, Effect of chicken manure and ZA fertilizer to mustard growth and yield performance of Aloe vera grown in different soil types of Bangladesh, J. Bangladesh Agric. Univ. 15 (2) (2017) 212–218.
[21] E. B S, Effect of poultry dung and cattle manure on soil chemical properties of clay and sandy clay loam soil, J. Anim. Vet. Adv. 4 (10) (2005) 839–841.
[22] M.M. Zaman, K. Nahar, T. Chowdhury, M.A.H. Chowdhury, Growth, leaf biomass yield of stevia and post-harvest soil fertility as influenced by different levels of poultry manure, J. Bangladesh Agric. Univ. 15 (2) (2017) 212–218.
[23] E.O. Adeleye, L.S. Ayeni, S.O. Ojeyeni, Effect of poultry manure on soil physico-chemical properties, leaf nutrient contents and yield of yam (Dioscorea rotundata) on alfisol in southwestern Nigeria, Am. J. Sci. 6 (10) (2010) 871–878.
[24] S.S. Ningsih, Effect of chicken manure and ZA fertilizer to mustard growth and yield performance of Aloe vera grown in different soil types of Bangladesh, J. Bangladesh Agric. Univ. 15 (2) (2017) 212–218.
[25] M. Blumenthal, W.R. Busse, A. Goldberg, J. Gruenwald, T. Hall, C.W. Riggins, The Complete German Commission E Monographs: Herbal Medicines, Communications, Austin, TX/Boston, MA, 1998.
[26] M. Hasanuzzaman, K.U. Ahamad, K.M. Khalequzzaman, A.M.M. Shamsuzzaman, K. Nahar, Plant characteristics, growth and leaf yield of Aloe vera as affected by organic manure in pot culture, Aust. J. Crop. Sci. 2 (3) (2008) 158–163.
[27] A.H. Van Schaik, P.C. Struik, T.G. Damian, Effects of irrigation and N on the vegetative growth of Aloe barbadensis Mill. in Aruba, Trop. Agric. 74 (2) (1997) 104–109.
[28] S.K. Chandavel, J. Manssakti, M.R. Choudhary, K.N. Gupta, Effect of nitrogen and spacing on growth and yield of Indian aloe (Aloe barbadensis L.), J. Med. Aromat. Plant Sci. 31 (3) (2009) 203–205.
[29] IUS Working Group W6R, World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106, FAO, Rome IT EU, 2015, p. 192.
[30] A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Method of Soil Analysis, Part-2 Chemical and Microbiological Properties, second ed, American Society of Agronomy Inc., Madison, Wisconsin, USA, 1982.
[31] M.J. Jackson, Soil Chemical Analysis prentice, Hall of India Private Limited, New Delhi, 1973, p. 498.
[32] S.P. Olsen, L.E. Sommers, Phosphorus, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Method of Soil Analysis, Part-2 Chemical and Microbiological Properties, American Society of Agronomy Inc., Madison, Wisconsin, USA, 1982.
[33] M.L. Jackson, Soil Chemical Analysis prentice, Hall of India Private Limited, New Delhi, 1973, p. 498.
[34] S.P. Olsen, L.E. Sommers, Phosphorus, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Method of Soil Analysis, Part-2 Chemical and Microbiological Properties, American Society of Agronomy Inc., Madison, Wisconsin, USA, 1982.
[35] S.P. Olsen, L.E. Sommers, Phosphorus, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Method of Soil Analysis, Part-2 Chemical and Microbiological Properties, second ed, American Society of Agronomy Inc., Madison, Wisconsin, USA, 1982.
[36] M.J. Jackson, Soil Chemical Analysis prentice, Hall of India Private Limited, New Delhi, 1973, p. 498.
[37] S.P. Olsen, L.E. Sommers, Phosphorus, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Method of Soil Analysis, Part-2 Chemical and Microbiological Properties, American Society of Agronomy Inc., Madison, Wisconsin, USA, 1982.
K. Ghosh, M.A.H. Chowdhury, M.H. Rahman, S. Bhattacherjee, Effect of integrated K. Manoj, L.K. Baishaya, D.C. Ghosh, V.K. Gupta, S.K. Dubey, D.D. Anup, P. Patel, M. Islam, G.C. Munda, Effect of organic and inorganic fertilizer on growth, T. Chowdhury et al. Heliyon 7 (2021) e07464 M.P. Sharma, S.Y. Bali, D.K. Gupta, Crop yield and properties of inceptisol as R.K. Mapp, A comparative investigation of methods used to estimate aloin and W. Gong, X. Yan, J. Wang, T. Hu, Y. Gong, Long-term applications of chemical and P.V. Jeegadeeswari, P.S.S. Kumar, K. Kumaraswamy, Potassium balance and uptake T. Sreelatha, A.S. Raju, A.P. Raju, Effect of different doses of farm yard manure and F.O. Olasantan, Response of tomato and okra to nitrogen fertilizer in sole cropping M.M. Zaman, Nutrient Requirement Leaf Yield and Stevioside Content of Stevia B. Prasad, A.P. Singh, M.K. Sinha, Effect of poultry manure as a source of zinc, iron and M.N. Faiyad, M.M. Shehata, S.W. Barsoon, Response of faba bean grown on sandy soil to organic and inorganic nitrogen fertilization, Egypt. J. Soil Sci. 31 (1991) 343–355. B. Prasad, A.P. Singh, M.K. Sinha, Effect of poultry manure as a source of zinc, iron and M. Faiyad, M.M. Shehata, S.W. Barsoon, Response of faba bean grown on sandy soil to organic and inorganic nitrogen fertilization, Egypt. J. Soil Sci. 31 (1991) 343–355. A. Swarm, Effect of micronutrients and farmyard manure on the yield and micronutrient content of rice and wheat grown on a sodic soil, J. Indian Soc. Soil Sci. 52 (2) (1992) 397–399. M. Chaudhury, R.P. Narwal, Effect of long-term application of farmyard manure on soil micronutrient status, Arch. Agron Soil Sci. 51 (3) (2005) 351–359. M.A. Abdalla, N.O. Salih, A.A. Hassabo, A.G. Mahala, Effect of application of organic amendments on quality of forage sorghum (Sorghum bicolor L.) in the semi-arid tropics, Arch. Agron Soil Sci. 53 (5) (2007) 529–538. E.N. Whitney, S.R. Rolifes, Nutrition Explorer: to Accompany Understanding Nutrition, tenth ed., Thomson Wadsworth, Belmont, CA, 2005, pp. 152–157. G. Haque, M.B. Islam, M.A. Jali, M.Z. Shahfiq, Proximate analysis of aloe vera leaves. J. Appl. Chem. 7 (6) (2014) 36–40. A. Femenia, E.S. Sanchez, S. Simal, C. Roselló, Compositional features of polysaccharides from Aloe vera (Aloe barbadensis Miller) plant tissues, Carbohydr. Polym. 39 (2) (1999) 109–117. A. Saleha, Studies on the effects of organic vs. inorganic form of nitrogen on the quality of okra, J. Maharashtra Agric. Univ. 17 (1992) 133–134. A.R. Dexter, Advances in characterization of soil structure, Soil Tillage Res. 11 (3-4) (1989) 199–226. J.M. Tisdall, J.M. Oades, Organic matter and water-stable aggregates in soils, J. Soil Sci. 33 (2) (1982) 141–163. R. Uysano, U. Cetin, M. Zengin, K. Gur, Effect of different organic wastes on nitrogen mineralization and organic carbon contents of soil: international conference at Canakkale, Sustainable Land Use Manage. Int. Turkey, 2002, pp. 223–228. K.A. Shadid, Effect of Planting Distances on vegetative growth, chemical composition and active constituents of Aloe vera l, in: Fourth Arab Conf. for Horticultural Crops, El-Minia, Egypt, 1996. W. Ji-Dong, L. Zaho-pu, Z. Qing-song, L. Ling, P. Feng-zhi, Effects of different nitrogen levels on seedling growth, nitrat and its secondary metabolites in Aloe vera seedling, Plant Nutr. Fert. Sci. 12 (2006) 864–868. J. Javanmardi, C. Stuhlhoff, E. Locke, J.M. Vivanco, Antioxidant activity and total phenolic content of Iranian Ocmium accessions, Food Chem. 83 (4) (2003) 547–550. J. Løliger, The use of antioxidants in food, in: Hn O.I. Aruoma, B. Halliwell (Eds.), Free Radicals and Food Additives, Taylor and Francis, London, 1991, pp. 129–150. R.A. Larson, The antioxidants of higher plants. Phytochemistry 27 (4) (1988) 969-978. S. Kumar, M. Yadav, A. Yadav, J.P. Yadav, Comparative analysis of antimicrobial activity of methanolic extracts of Aloe vera and quantification of aloe-emodin collected from different climatic zones of India, Arch. Clin. Microbiol. 6 (2) (2015), S. Kumar, L. Budhwar, A. Yadav, M. Yadav, J. Parkash Yadav, Photocatalytic screening and antibacterial activity of Aloe vera collected from different climatic regions of India, Nat. Prod. J. 6 (1) (2016) 73–82. S. Kumar, A. Yadav, M. Yadav, J.P. Yadav, Effect of climate change on photocatalytic diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f. BMC Res. Notes 10 (11) (2017) 1–12. W. Zheng, S.Y. Wang, Antioxidant activity and phenolic compounds in selected herbs, J. Agric. Food Chem. 49 (11) (2001) 5165-5170. V.S.P. Sarasdi, S. Khaman, B.G. Shivanna, T.V. Kumar, T.N. Shivanna, Effect of NPK fertilizers on chemical constituents of Aloe vera leaves, J. Nat. Remedies 7 (2) (2007) 258–262. D.D. Mankar, R.N. Sato, Influence of nitrogen and phosphorus on growth, yield and yield attributes of yam, Punbharas Krishi VidyaParsheshth Res. J. 19 (1995) 79–80. A. Ray, S.M. Awastha, An analysis of the influence of growth periods on physical appearance, and ascmanum and elemental distribution of Aloe vera L. gel, Ind. Crop. Prod. 48 (2013) 36-47. B.B. Babaji, R.I. Ali, R.A. Yahaya, M.A. Mahadi, A.I. Sharifini, and phosphorus nutrition of sesame, in: Proceedings of the 31st Annual Conference of the Soil Science Society of Nigeria 12–17 November, 2006, pp. 329–336. R.A. Abdalsalam, O.A. Ogunduro, Response of Abelmoschusesculentus to nitrogen and phosphorus fertilization, in: Proceedings of the 31st Annual Conference of the Soil Science Society of Nigeria, 12 – 17 November, 2006, Zaria, Nigeria, 2006, pp. 312–315.