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Research article

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

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ABSTRACT

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.*, $IF_{00}PM_{00}$ (IF = 0%, PM = 0%), $IF_{100}PM_{00}$(IF = 100%, PM = 0%), $IF_{75}PM_{25}$ (IF = 75%, PM = 25%), $IF_{50}PM_{50}$ (IF = 50%, PM = 50%), $IF_{25}PM_{75}$ (IF = 25%, PM = 75%) and $IF_{00}PM_{100}$ (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 $IF_{25}PM_{75}$ except protein content which was obtained from $IF_{100}PM_{00}$. 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 glucomannan [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, antiulcer, 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, increasing the soil's water and nutrient-holding capacities [21]. The use of poultry manure as a soil amendment to sustain adequate crop yields has been found effective for many crops like stevia, maize and yam etc. [22, 23]. Ningsih [24] in his research found that the provision of chicken manure affects the growth and production of mustard plants. The incorporation of organic material into soil is considered a good management practice because it stimulates soil 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. 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*., $IF_{00}PM_{00}$, $IF_{100}PM_{00}$, $IF_{75}PM_{25}$, $IF_{50}PM_{50}$, $IF_{25}PM_{75}$ and $IF_{00}PM_{100}$. Inorganic fertilizer (IF) consisting of N, P, K and S at the rate of 150, 80, 120, 40 kg ha^{-1}, respectively from urea, TSP, MoP and gypsum and poultry manure (PM) at the rate of 5 ton ha-1 were applied. PM contained 16.86, 2.20, 1.72, 0.42, 0.27, 0.21 and 0.016% organic C, total N, P, K, S, Ca and Mg, respectively. Each earthen pot was filled with 10 kg of processed soil. Soils used in this study collected from *Fulbaria* (Mymensingh) belonged to Non-Calcareous Dark Grey Floodplain soil under the Agro-Ecological Zone of Old Brahmaputra Floodplain and classified as Vertic Endostagnic Cambisols according to World Reference Base (AEZ-9) [34] which were clayey in texture, acidic in nature, moderate in organic carbon, medium in available N, moderate in P and moderately high in available K. Eighteen months old *A. vera* seedlings collected from the local farm of *Shomvogonj*, Mymensingh were used for the experiment. Time to time necessary intercultural operations had been done. After 180 days, the plants were harvested, cleaned, weighed and analyzed to determine the concentration of mineral nutrients and pharmaceutical compounds. Mineral nutrient concentrations of *A. vera* leaf gel were determined following standard methods [35].

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 (HNO_3:$HClO_4$ = 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 leaf gel samples were digested with conc. H_2SO_4 in presence of K_2SO_4 catalyst mixture (K_2SO_4:$CuSO_4.5H_2O$:Se = 10:1:0.1). Nitrogen in the digest was collected by distillation with 40% NaOH. The distilled ammonia was trapped in 4% H_3BO_3 solution and 4 drops of mixed indicator (Methyl red and Bromocresol green) solution. Finally the distillate was titrated with standard H_2SO_4 (0.005M) until the color changed from green to pink. The amount of N was calculated using the following formula:

$$\text{Total N (\%)} = (T-B)*M*1.4/S$$

Where, T = Sample titration, mL.standard H_2SO_4; B = Blank titration, mL.standard H_2SO_4; M = Molarity of H_2SO_4 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 molybdate 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 PFP7) [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 ($BaCl_2.2H_2O$) 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 Na_2-EDTA as a complexing agent where calcon and eriochrome black T were used as indicators at pH 12 (Using 10% NaOH solution) and 10 (Using NH_3-NH_4 buffer solution), respectively [39]. 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 Na_2-EDTA (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 was determined with 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% Na_2CO_3 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 determination was repeated three times for precision and values were expressed in mean + standard deviation in terms of total phenolic concentration (Gallic acid equivalent, GAE) per g of fresh weight.

2.2.11. Determination of total flavonoid concentration

Total flavonoid concentration of *A. vera* leaf gel was determined by aluminium chloride method [46] using quercetin as a standard. 1mL of test sample and 4 mL of water was added to a 10 mL volumetric flask. After 5 min, 0.3 mL of 5 % $NaNO_2$, 0.3 mL of 10% $AlCl_3$ was added. After 6 min incubation at room temperature, 1mL of 1 M NaOH was added to the reaction mixture. Immediately the final volume was made up to 10 mL with distilled water. Absorbance of sample was measured against the blank at 510 nm using a spectrophotometer (Model: TG-60 U, UK). All the determination was repeated three times for precision and values were expressed in mean ± standard deviation in terms total flavonoid concentration (TFC) (Quercetin equivalent, QE) per 100g of fresh weight.

2.2.12. Determination of aloin concentration

Aloin concentration of *A. vera* leaf gel was determined by using McCarthy method [47]. In the filtered juice, 1% $Ca(OH)_2$ was added and centrifuged. After the centrifugation, residues containing aloin settle down. Then supernatant was decanted and residues were weighed. Aloin content of leaves were determined and expressed as mg kg^{-1} fresh weight.

2.3. Statistical analysis

All the chemical analyses were done in the laboratories of the departments 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 $IF_{25}PM_{75}$ 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.

Table 1. Integrated effects of IF and PM on N, P, K and S concentrations and their uptake by *A. vera*.

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})	Conc. (%)	Uptake (g plant^{-1})
IF_0PM_0	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 ± 0.29e
$IF_{100}PM_0$	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
$IF_{75}PM_{25}$	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
$IF_{50}PM_{50}$	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
$IF_{25}PM_{75}$	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
IF_0PM_{100}	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
$LSD_{0.05}$	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 NO_3-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].

3.1.2. P concentration and uptake

Different combinations of IF and PM significantly influenced the P concentration and its uptake by *A. vera* leaf gel (Table1). The highest concentration (0.34%) was obtained from $IF_{100}PM_{00}$ treated plants which was statistically identical with the P concentration of the leaves of *A. vera* plant fertilized with $IF_{25}PM_{75}$ but significantly different from other treatments. The lowest P concentration (0.13%) was obtained from the plants receiving no fertilizer. P uptake varied from 0.21-1.43 g plant^{-1}. The uptake of P was highest when the plant was treated with $IF_{100}PM_{00}$ which was identical with $IF_{25}PM_{75}$. 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].

3.1.3. 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 $IF_{100}PM_{00}$ which was statistically identical with the K concentration of the leaves of *A. vera* plant fertilized with $IF_{75}PM_{25}$ but significantly different from other treatments. The minimum K concentration was recorded in case of no fertilizer treatment. The maximum K (7.81 g plant^{-1}) uptake by *A. vera* leaf was recorded in only IF treated plants and the minimum (1.7 g plant^{-1}) as recorded in case of control ($IF_{00}PM_{00}$). The second highest K concentration (6.18 g plant^{-1}) was obtained from $IF_{75}PM_{25}$ treated plants which was identical with others such as $IF_{50}PM_{50}$ and $IF_{25}PM_{75}$. Reference [60] and [57] showed that combined use of organic and mineral fertilizers significantly increased the plant K uptake. Sheoran et al., [61] also observed that combined application of N based mineral fertilizers and vermicompost had significant effect on K uptake. Jeegadeeswari et al., [62] reported that K uptake in rice plant was higher in urban compost treated plots over green manure and controlled treatment.

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 (0.24%) was obtained from the treatment $IF_{100}PM_0$ which was statistically identical with the S concentrations of the leaves of *A. vera* plant fertilize with all other treatments except sole application of PM. The lowest S concentration was obtained from the plants receiving no S 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 [65] 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 in comparison with FYM. Higher nutrients uptake with the application of organic fertilizer might be due to higher dry matter production along with higher nutrient concentration [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

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

IF and PM levels	Calcium (Ca)		Magnesium (Mg)		Iron (Fe)		Zinc (Zn)		Manganese (Mn)	
	Conc. (%)	Uptake (g plant $^{-1}$)	Conc. (%)	Uptake (g plant $^{-1}$)	Conc. (μg g^{-1})	Uptake (mg plant $^{-1}$)	Conc. (μg g^{-1})	Uptake (mg plant $^{-1}$)	Conc. (μg g^{-1})	Uptake (mg plant $^{-1}$)
IF_0PM_0	0.13 ± 0.02d	0.21 ± 0.03c	0.22 ± 0.0.1b	0.36 ± 0.02d	119 ± 6.79e	20 ± 1.22e	73 ± 3.24	12 ± 1.6d	67±2d	11.14 ± 1.2d
$IF_{100}PM_0$	0.19 ± 0.01d	0.78 ± 0.18b	0.24 ± 0.03b	1.01 ± 0.15c	139 ± 5.93d	58 ± 7.96cd	75 ± 2.67	31 ± 3.1a	84 ± 2.5c	34.89 ± 2.9b
$IF_{75}PM_{25}$	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 ± 1.5bc	38.25 ± 3.7bc
$IF_{50}PM_{50}$	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
$IF_{25}PM_{75}$	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
IF_0PM_{100}	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
$LSD_{0.05}$	0.04**	0.17**	0.05**	0.27**	17**	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.

concentration (80 μg g^{-1}) was obtained from IF_0PM_{100} 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, Zn, Fe 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* L.) amended with sewage sludge.

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 by100% 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 $IF_{100}PM_0$ treated plants and minimum content (10.94%) was found in control. On the other hand, the plants treated with $IF_{100}PM_0$ and $IF_{25}PM_{75}$ biosynthesized statistically identical percent of protein. $IF_{25}PM_{75}$ and $IF_{50}PM_{50}$ 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.

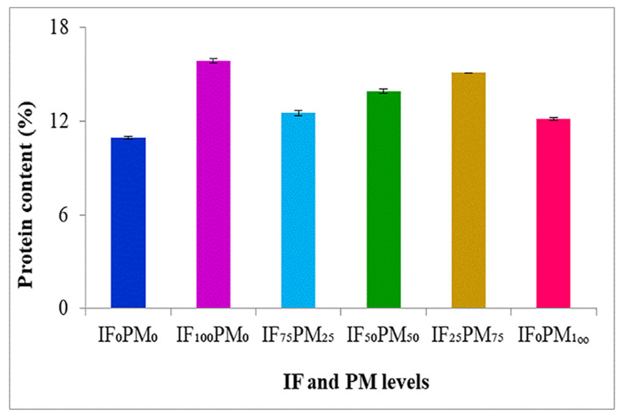

Figure 1. Effects of IF and PM on the protein content of *A. vera*.

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 IF_0PM_{100} which was identically followed by the amounts of leaf aloin content of the plants having the treatments $IF_{25}PM_{75}$ (467.8 μg g^{-1}) and $IF_{50}PM_{50}$ (456.9 μg g^{-1}).

Table 3. Integrated effects of IF and PM on aloin, chlorophyll, total phenolic and total flavonoid concentrations of *A. vera*.

IF and PM levels	Aloin ($\mu g\ g^{-1}$ FW)	Chlorophyll ($mg\ g^{-1}$ FW) "A"	Chlorophyll ($mg\ g^{-1}$ FW) "B"	TFC (mg QE100 g^{-1} FW)	TPC (mg GAEg^{-1} FW)
IF_0PM_0	345.7 ± 9.7 e	0.19 ± 0.01c	0.068 ± 0.01c	5.68 ± 0.32 e	15.63 ± 0.53 d
$IF_{100}PM_0$	388.8 ± 14.9 d	0.26 ± 0.11ab	0.095 ± 0.01b	9.58 ± 0.53 d	20.98 ± 0.98 c
$IF_{75}PM_{25}$	415.6 ± 10.5 c	0.23 ± 0.01bc	0.091 ± 0.00 b	12.28 ± 1.45 c	22.57 ± 0.69 bc
$IF_{50}PM_{50}$	456.9 ± 9.5 b	0.25 ± 0.00 ab	0.093 ± 0.00 b	16.35 ± 0.61 b	27.9 ± 1.11 a
$IF_{25}PM_{75}$	467.8 ± 11.7 b	0.29 ± 0.01a	0.116 ± 0.01a	19.61 ± 1.04 a	29.08 ± 1.20 a
IF_0PM_{100}	492.4 ± 14.0 a	0.287 ± 0.00 a	0.100 ± 0.00 b	15.89 ± 0.24 b	24.54 ± 2.21 b
CV	2.79	11.47	9.47	6.17	5.30
$LSD_{0.05}$	21.2**	0.05*	0.02**	1.45**	2.21**

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.

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}) was found in $IF_{25}PM_{75}$ treated plants which were identically followed by 50% IF and PM treated plants. Lowest phenolic concentration (24.54 mg g^{-1}) was obtained from the control treatment. The highest amount of flavonoids concentration (19.61 mg 100 g^{-1} was obtained in $IF_{25}PM_{75}$ treatment and the lowest (5.68 mg 100g^{-1}) 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

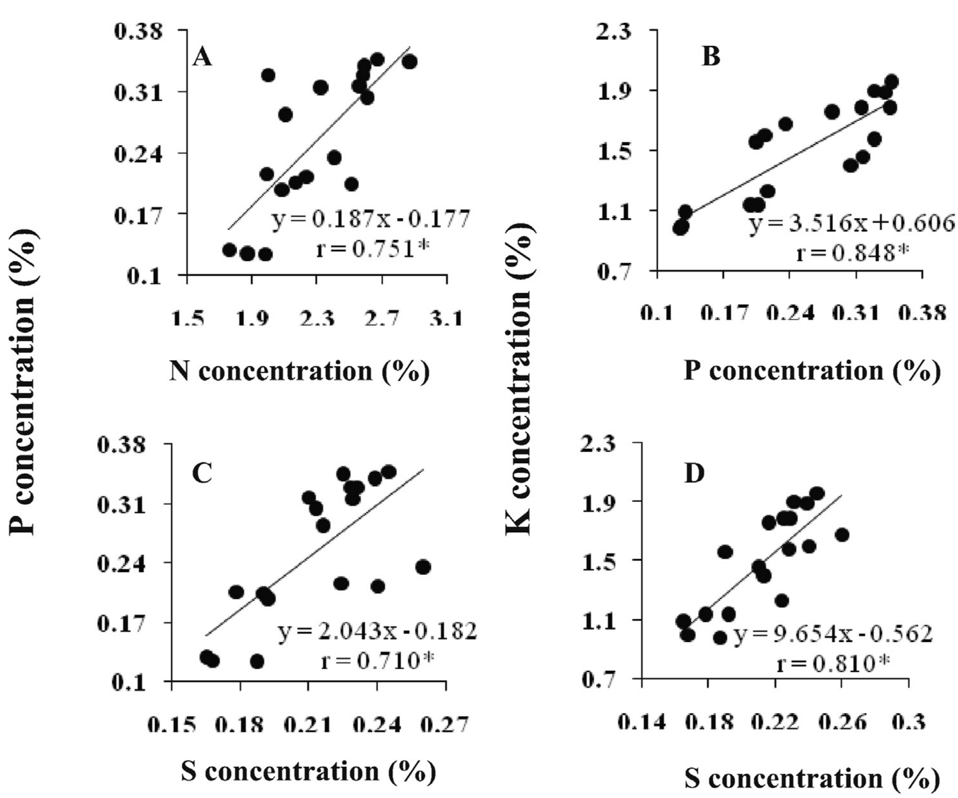

Figure 2. Relationships between (A) N and P concentrations (B) P and K concentrations (C) P and S concentrations (D) S and K concentrations (E) Mg and Mn concentrations (F) Mg and Fe concentrations of *A. vera* as influenced by IF and PM (n = 18).

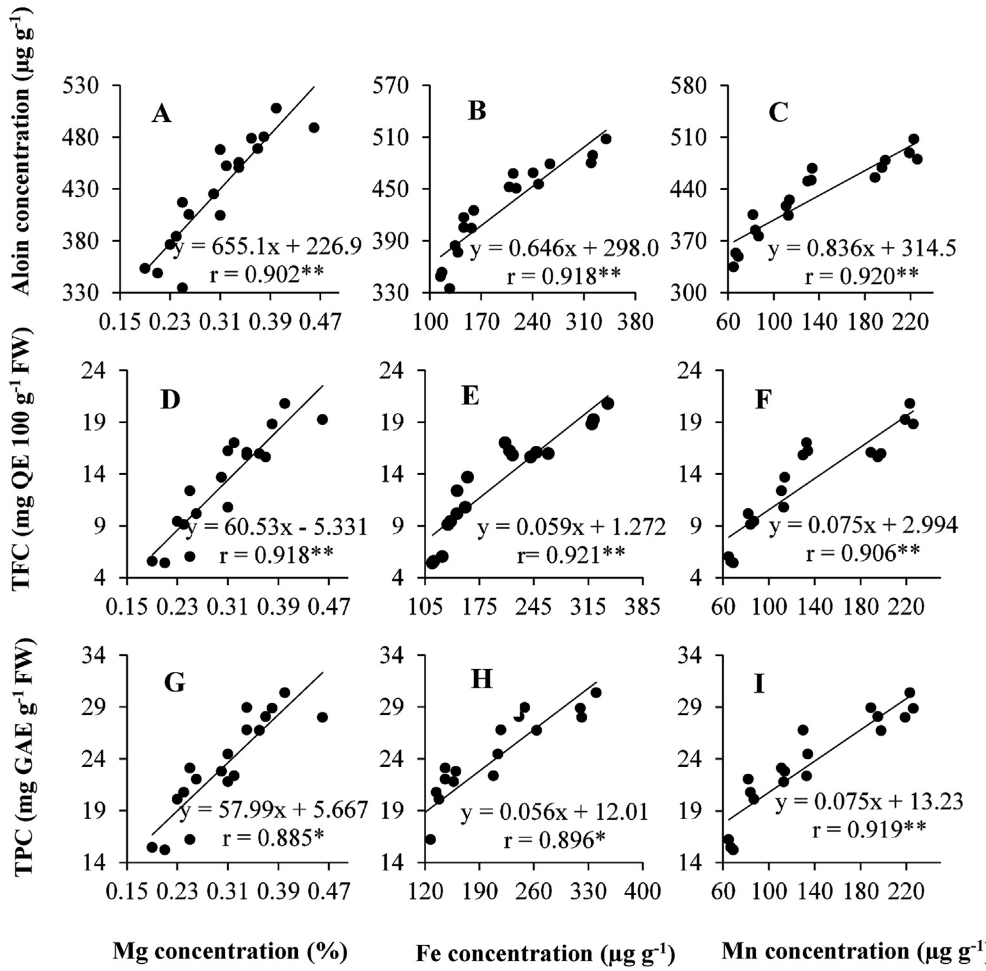

Figure 3. Relationships between (A) Mg and Aloin concentrations (B) Fe and Aloin concentrations (C) Mn and Aloin concentrations (D) Mg concentration and TFC (E) Fe concentration and TFC (F) Mn concentration and TFC (G) Mg concentration and TPC (H) Fe concentration and TPC (I) Mn concentration and TPC of *A. ver a*as influenced by IF and PM.TPC = Total phenolic concentration, TFC = Total flavonoid concentration (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. aloin, 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, aloin, 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 $IF_{25}PM_{75}$except protein content which was obtained from $IF_{100}PM_0$. Aloin 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 aloin 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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