Effect of Enzymatic Chicken manure-based fertilizer and Organic Humic acid - Fulvic acid foliar application impact on fodder and antioxidant activities of Alfalfa (*Medicago Sativa*) grown on the sandy soils of Sultanate of Oman

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Abstract
A wide variety of crops, forages and fruits are cultivated in the Sultanate of Oman, a subtropical arid country in the Middle East. After dates, lime and mango, alfalfa occupies the largest part of cultivated land. In this study, the effect of VIRENXIA formulation consisted of five levels of chicken manure (CM) 0.5 t/ha (C1), 1.0 t/ha (C2), 1.5 t/ha (C3), 2.0 t/ha (C4), 2.5 t/ha (C5) along with two enzymes, enzyme 1 and 2 and foliar application of organic humic acid - fulvic acid (OHA-FA) was investigated on Alfalfa M. Sativa crop and compared against control without CM (C0) and humic acid foliar application at different cuttings under the cut-and-carry system. Chicken manure at the rate of 1.5 t/ha significantly improved fodder growth parameters, biomass yield, antioxidant activity by increasing the crude protein (CP), total phenolic content (TPC), total flavonoid content (TFC), nitrogen content and also by decreasing neutral detergent fiber (NDF) and acid detergent fiber (ADF). Similar results were observed in other plant growth parameters.

Hence, our study revealed that VIRENXIA's developed chicken manure- organic humic acid - fulvic acid formulation (CH+OHA) of doses of 1.5 t/ha can be recommended for the cultivation of Alfalfa M. Sativa crop in the southern region of Oman. It can be concluded that enzymatic manure and organic humic acid - fulvic acid are effective tools to boost production in arid saline soils when an appropriate level is used.

Keywords: Chicken manure, Humic acid, Fulvic acid, Alfalfa, Antioxidants, NDF, ADF.

Introduction

Chicken manure is extensively used as an efficient organic fertilizer due to its excellent source of nutrition (NPK) and richness in organic matter, its ability to improve soil structure, vegetables and pastures growth[5]. In addition to this, chicken manure is also known for its efficacy to reduce the population of nematodes and root galling in vegetables[10]. Similarly, humic and fulvic acid is well recognized to improve the growth of vegetable and non- vegetable crops due to beneficial effects on plant growth, if applied in the right concentration. It has several benefits such as improved nutrient uptake, increased root vitality, increased organic matter addition etc. One of the important mechanisms of humic and fulvic acid to improve nutrient uptake is chelation. It basically forms chelates with micronutrients and releases to plants slowly when required.

According to the report of Kadam et al,[23] humic acid avoids the leaching and precipitation of micronutrients through the mechanism of chelation. The chicken litter contains varying amounts of water, mineral nutrients and organic matter[12]. While the use of organic wastes as manure has been in practice for centuries worldwide[37] and in the recent times[30], there is still a need to evaluate the efficacy and impact of chicken manure, humic and fulvic acid on soil physicochemical properties and crop yield and in particular evaluating the critical application levels.

Several research frequently reported the positive effect of chicken manure on many crops such as tomatoes[7], spinach[11], lettuce[15], sunflower[1], brassicas[28] and peas[40].

Genetic modification of plants could be one of the futuristic approaches to yield a better quality cell wall with less lignin content which serves as better feedstock for forage production. However, earlier studies reported that cell wall genetic modifications lead to defects in plant growth, physiology and biochemistry thereby reducing biomass yield and survivability[38]. Hence it is very crucial to find out a long-lasting, sustainable solution to maintain the quantity and quality of biomass under stressful conditions. To satisfy the rising demand for Alfalfa forage, to reduce the application of NPK fertilizer and to facilitate the degraded land use, the application of chicken manure, humic and fulvic acid can be adopted as an effective method.

85473.10 ha of The Sultanate of Oman's agricultural lands are under cultivation. The majority of agricultural land is occupied by fruits (36.11%) followed by fodder crops (39.40%)[2]. Amongst all fodder crops, the Alfalfa crop is the common one and grown by almost every farmer of the Sultanate.

The country receives an average rainfall of about 0-100 mm between November to February while some regions such as the Dhofar region receive monsoon based rainfall (200-250 mm) during Kharif season (July-September)[16,27]. Recently due to unforeseen climatic conditions and abiotic stresses such as drought, flood, fewer rainy days, salinity and extreme temperature conditions, the farmers of the country encountered various problems in Alfalfa cultivation such as water scarcity, fragile ecosystems, low land productivity, limited good quality irrigation water and non-sustainable farming systems. Moreover, the extreme temperature leads to quick evaporation rates of rainfall which makes the majority of cultivable land depend on saline irrigation.

Moreover, this saline and harsh climatic condition hinder the growth and productivity of Alfalfa crops. Several studies reported that such adverse (Saline and drought) conditions create a hindrance for plant growth and productivity through morphological, physiological and biochemical stress such as water conservation (by closing stomata, sunken stomata, boundary layer effect), electrolyte leakage, limitation of nutrient uptake, synthesis of osmolytes and ethylene and increase in lignin content. Antioxidant production decreases the damaging effects of reactive oxygen species (ROS) and by activating a wide range of proteins and stress-responsive genes[6,9,38].

Abiotic stresses such as drought and salt induce oxidative stress in plants due to enhanced synthesis of reactive oxygen species (ROS). These ROS are cytotoxic in nature and cause peroxidation of membrane lipids, denaturation of proteins, mutation of DNA and several types of oxidative damages. Plant cells are protected against the detrimental effects of ROS by a complex antioxidant system consisting of enzymic or nonenzymic antioxidants[4]. Plants with the high level of antioxidants, either constitutive or induced, have been reported to provide sufficient resistance against oxidative damage[14,31].

Earlier under a similar situation, to cope with the increasing demand and attain self-sustainability, the Oman agrarian system adopted several new initiatives. Chemical fertilizers played a significant role in improving agricultural productivity, but their excess usage severely affected the soil health by disturbing microbial population and total organic carbon[38]. Another major impact of chemical fertilizer is decrease in agriculture productivity and an increase in the degree of land degradation.

In addition to usage of chemical fertilizers, climate change problems such as altered rain pattern, drought, salinity and extreme temperatures impose a threat to plant productivity[35,38].

On the other hand, apart from chemical fertilizers, the fodder crisis is a major problem for several developed as well as developing countries such as Oman. Livestock is one of the major sources of livelihood and it has a direct influence on agricultural production as it is a source of crop nutrition and human nutrition and a valuable asset. There is an enormous gap between the supply and demand of the fodder situation in Oman[26]. Feed shortages constitute a threat to the efficient production of small and large ruminants in the Sultanate.

The economic productivity of livestock is dependent on the quality and quantity of nutritious forage, but in Oman, livestock is still dependent on cereal crop residues (1.25%), by-products flour mill (wheat bran) (12.5%), agro-industrial by-products (date processing) (1%), inferior quality dates fruit (2.75%) and sun-dried sardines fish (2.5%) for their diet, as they are not getting good quality green fodder[26].

Hence, more emphasis should be given to research pertaining to the development of fodder varieties with properties such as high yielding with better nutrient value, stress resistance and an ability to grow on marginal and degraded land.

In this regard, Alfalfa *Medicago Sativa* (Family-Poaceae) a leguminous perennial autotetraploid crop often called "Queen of the Forages" could be a potential source of forage production. Alfalfa is one of the oldest leguminous crops to be used as forage worldwide[43]. Due to its multicult nature, ease of propagation, fast growth, good yield, low fertilizer requirement and wide adaptability under different agroclimatic conditions of Oman, good palatability, high digestibility, high protein content, excellent calcium, magnesium, phosphorus, vitamin D content, low in the cell wall and neutral detergent, this crop could offer a sustainable and economic alternative option for forage production[35].

Besides, Alfalfa is well known for its ability to improve soil structure, reduce soil erosion and act as a source of nitrogen for neighbouring plants[43]. However, more studies are required to assess the quality and quantity of Alfalfa *M. Sativa* under wasteland, as not very much information is available on the effect of stress conditions on Alfalfa *M. Sativa*.

Wong et al[42] found that excess chicken manure causes acidity in the soil which severely affects root growth and seed germination. So, it is very important to apply chicken manure correctly and if applied in correct quantities, chicken manure can act as a good soil fertilizer/ conditioner (e.g. provides N, P and K) and subsequently can also increase the soil and leaf N, P, K Ca and Mg concentrations.

Numerous reports have been published to identify the effect of chicken manure, organic humic and fulvic acid on various crops while very less research has been conducted on Alfalfa crops at multiple cuts and doses of application in subtropical arid climates such as that of Oman[1,11]. In this research, the study aimed to study the enhanced fodder growth, antioxidant activity and micronutrients characteristics of Alfalfa perennial crops as affected by chicken manure and organic humic acid - fulvic acid foliar application at different levels and cuts for the southern region of Oman. This experiment informs the need for an assessment to ascertain, if the applied manure amendment would suffice the nutritional requirement of Alfalfa *M. Sativa*.

The findings of this research experiment would offer us an opportunity to exploit the potential of chicken manure and organic humic acid - fulvic acid as fertilizer towards increasing the quality biomass yield of Alfalfa *M. Sativa* on semiarid and degraded lands of Oman and further their utilization for forage production.

Material and Methods

Under field conditions, a pot experiment was conducted to study the effect of different levels of VIRENXIA developed enzymatic chicken manure followed by foliar application of organic humic acid - fulvic acid application on Alfalfa crop growth promotion at multiple cuttings. To study the effect of treatments on various parameters, at all cuttings, the same plants of the same pot were harvested.

Experimental site: A pot study was carried out at the 36 ha VIRENXIA farm located at Shaser in the Dhofar region of the Sultanate of Oman (18.161194 N, 53.761519 E) during the period of November 2019 to March 2020. The experimental region is characterized by a warm humid tropical climate during Kharif, high temperature during summers and an annual rainfall of about 100 mm. The monsoon season (locally known as Khareef) starts from late June to early September. The average temperatures during the experiment period were between 25 - 35ºC. The soil of this region is bristly coarse (sandy or coarse loamy) with low water holding capacity. The soil pH is sober to strong alkaline and the content of organic matter is very less. These make this soil non-saline to slightly saline with low quality.

Procurement of chicken manure and Biological samples: Chicken manure (CM) used in the experiment was collected from a commercial poultry farm A'Saffa located in Oman to be used as fertilizer. Ziplock plastic bags were used for collection to avoid moisture and mineral loss. The CM was allowed three weeks of decomposition prior to application.

Humic and fulvic acid applied in this study were extracted from the highest-grade Russian peat and sapropel through a patented 100% organic process called "Hydrodynamic Pulse Technology" which uses high intensity pulse to change organic material without chemical additives and break humic substances to nano and micron level size.

Seeds of Alfalfa *Medicago Sativa* cultivar CUF101 and WL903 certified non-GMO were procured from seed stock maintained at Seed Distribution Centre Oman. The seeds were surface sterilized with 1% sodium hypochlorite for 1 min followed by three-time washing with tap water, blot-dried and used through the experiment[38].

Analysis of Chicken manure and soil: Before the start of the experiment, the soil and chicken manure used in the experiment were analyzed to determine their nutrient compositions after being air-dried and sieved using a 2- mm sieve. The analysis has been done for the following properties: Electrical conductivity (EC) was estimated by EC meter (Scientific Make) using soil to water suspension of 1:5. pH was measured by pH meter (Scientific Make) by using soil: water suspension of 1:10. Total nitrogen and organic carbon content were determined by CHN analyzer (CHNOS Elementar, Vario EL III model), P by Olsen's method[29], potassium (K) was done through flame photometer using potassium chloride as a standard[20], dry matter as per ISO 712:2009 and organic matter by the following method of AOAC[3].

Experiment design: About 500mg (~25 seeds) Alfalfa seeds were sown densely in pots (32 cm X 20cm) containing pre-sterilized soil (Table 1) and maintained in a poly house with natural light, at a temperature varying from 40ºC (day) to 27ºC (night) and relative humidity of 65–80%. To maintain optimal moisture level, pots were watered regularly[38]. Seed germination started after the third day of sowing and 10 days after sowing, the seedlings were thinned to ten per pot.

An experiment was carried out by using enzymatic chicken manure-based fertilizer formulated by VIRENXIA. The formulation was a mixture of chicken manure, lime, gypsum and bentonite in a ratio of 100:1:1:0.1 followed by the addition of enzyme 1(70ml/ton) and then further irrigated by enzyme 2.

Table 1
Physicochemical chemical characteristics of soil and chicken manure used for the experiments

Physicochemical characteristics	Values obtained	
	Soil	Chicken Manure (CM)
pH	8.68± 0.05	8.48±0.05
Electrical Conductivity (µS/cm)	4032±0.03	6312±0.02
Total Nitrogen (%)	0.41±0.05	3.94±0.04
Exchangeable Potassium (%)	1.9±0.03	2.2±0.01
Available Phosphorus (%)	0.08±0.05	3.63±0.02
Organic Carbon (%)	1.72±0.03	47.8±0.07
Dry Matter (%)	92.86±0.01	90.83±0.02

Our experiment consisted of five different level chicken manure treatments named C1, C2, C3, C4 and C5 along with control C0 (Untreated) mixed with exclusive enzyme 1 of VIRENXIA. Further, the irrigation has been done by enzyme 2 solution (@4.5L/ha), prepared by adding 4.4ml of enzyme 2 along with 1.5mg of molybdenum to 8L of water by constantly stirring to make a homogenous mixture. The pots were maintained in a completely randomized block design with ten replications of five treatments and control (Untreated), with a total of 60 pots. Organic humic acid - fulvic acid foliar application was done @10ml/L (VIRENXIA product Asaava contains 5% humic acid, 3% fulvic acid, >17 amino acids, growth hormones). First foliar application was carried out after 30 days followed by second application after 45 days.

- C0 - Untreated control (Without chicken manure and enzymes)
- C1 - 0.5 tons chicken manure/ha + Enzyme 1 + Enzyme 2 followed by Organic Humic acid - Fulvic Acid foliar application at 30th and 45th day
- C2 - 1.0 tons chicken manure/ha + Enzyme 1 + Enzyme 2 followed by Organic Humic acid - Fulvic Acid foliar application at 30th and 45th day
- C3 - 1.5 tons chicken manure/ha + Enzyme 1 + Enzyme 2 followed by Organic Humic acid - Fulvic Acid foliar application at 30th and 45th day
- C4 - 2.0 tons chicken manure/ha + Enzyme 1 + Enzyme 2 followed by Organic Humic acid - Fulvic Acid foliar application at 30th and 45th day
- C5 - 2.5 tons chicken manure/ha + Enzyme 1 + Enzyme 2 followed by Organic Humic acid - Fulvic Acid foliar application at 30th and 45th day.

The chicken manure (composition percentage on dry weight basis given in table 1) was broadcasted and irrigated two weeks before the sowing date to avoid the effect of fermentation heat on seed germination.

All the pot experiments were conducted from November 2019 to March 2020. A total of five cuttings were done from the same pot. First harvesting has been done after 70 days of sowing followed by next cutting in 30 days interval. All the harvested seedlings from each cutting were analyzed for plant growth promotion studies and forage potential.

The first cutting was done after 45 days of thinning (in November 2019) and subsequent cuttings were also done at the initiation of 10% flowering (after ∼24-30 days for each cutting) i.e. December 2019, January 2020, February 2020 and March 2020 respectively. However, no cuttings were obtained from April to October owing to extreme high temperature and in total, five cuttings were obtained over the period of one year to maintain universal experimental balance. For the assessment of fodder production and quality in the cut-and-carry system at each cutting, five plants of each treatment were cut for further analysis.

After taking data on plant height, number of tillers per pot, number of leaves per plant per pot and fresh weight, the samples were dried at 60°C to constant weight to determine total dry weight. The dried samples were ground for the estimation of fodder quality.

Plant growth promotion studies: Ten plants were randomly selected from the first cutting slot for the determination of plant growth promotion parameters such as plant height, the number of leaves, stem diameter, shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW) and leaf area index.

Five plants from each pot were selected to measure plant height by measuring from the ground level to the tallest leaf apex using a ruler. The number of leaves and tillers were measured by counting. The stem diameter was measured from the same five plants that were randomly selected for the plant height using a digital vernier caliper. For shoot and root fresh weight, Alfalfa crop plant was uprooted carefully and soil adhered to root was removed by washing under running tap water and blot-dried followed by weight was taken.

While for SDW and RDW, the plant materials were dried at 50 °C for 2–3 days (until the plant materials attain constant weight) and DW was calculated. leaf area index (LAI) was determined according to the Watson and Watson method[41].

Chemical analysis of Alfalfa biomass for forage potential: Neutral detergent fiber (NDF) content in the Alfalfa *M. Sativa* was estimated by refluxing of the sample with a solution made up of sodium lauryl sulfate, disodium dihydrogen EDTA, sodium borate (decahydrate), disodium hydrogen phosphate and ethoxyethanol while in ADF, samples were refluxed in cetyl trimethyl ammonium bromide reagent made in 1 N H_2SO_4. The determination of lignin was done by the method of Goering and Van Soest[19]. Crude protein (CP) content in plant biomass was determined by multiplying 6.25 by the plant N content. Nitrogen was estimated by the micro-Kjeldahl method[33,38].

Antioxidant analysis of Alfalfa biomass: Alfalfa biomass was analyzed for the total phenol and flavonoids activities. Total phenol was quantified by the Folin- Ciocalteu method[34]. For flavonoids, the modified procedure of Leontowicz et al[25] was used.

Macronutrient analysis of Alfalfa biomass: Alfalfa biomass was analyzed for the organic N, P, K, Ca and Mg content. Total nitrogen content was estimated through the Kjeldahl method involving digestion and distillation steps[22]. Available P was determined as described by Olsen et al.[29]

The phosphorus was measured by ammonium molybdate colorimetric methods. Available K was determined as described by Carson[8] and was measured using flame emission spectrophotometry.

Experimental design and Data analysis: All Alfalfa crop parameters were analyzed in triplicate and repeated thrice and the average value is represented. Under environmental conditions, each treatment (Control and Treated) contained ten sets of pots and were arranged in randomized order. Environmental experiments were repeated thrice and the value obtained was averaged and tabulated.

All data obtained from laboratory and environmental experiments were statistically analyzed through Analysis of Variance (ANOVA) using *SPSS* Windows (version *16.0*). Probabilities of significant difference from ANOVA were used to test the significance among treatments ($P \leq 0.05$)[4].

Results and Discussion

Alfalfa crop growth promotion parameters: The results of this experiment indicated positive response of the Alfalfa crop to increasing levels of poultry manure at different cutting. The medium level of application (1.5t/ha) was the best in growth enhancement and forage yield. The soil texture at the study site was classified as a sandy loam (Table 1). The pH of soil and CM was slightly alkaline. Mineral content (N, P, K) was comparatively high in CM.

Five levels of CM (C1, C2, C3, C4 and C5) were used to evaluate their potential to enhance the growth of Alfalfa under normal conditions at polyhouse. Data pertaining to the effect of CM on growth parameters at different cuttings are summarized in fig. 1 and 2. Analysis of data showed that CM treatment showed significant ($P \leq 0.05$) impact concerning plant height, shoot and root dry weight (SDW, RDW), shoot and root fresh weight (SFW, RFW), no. of leaves, leaf area index and stem diameter in comparison to control (Untreated). All the tested CM treatment levels recorded a varied level of plant growth promotion in comparison to control. However medium concentration of CM level i.e. 1.5t/ha showed significant results for most of the parameters.

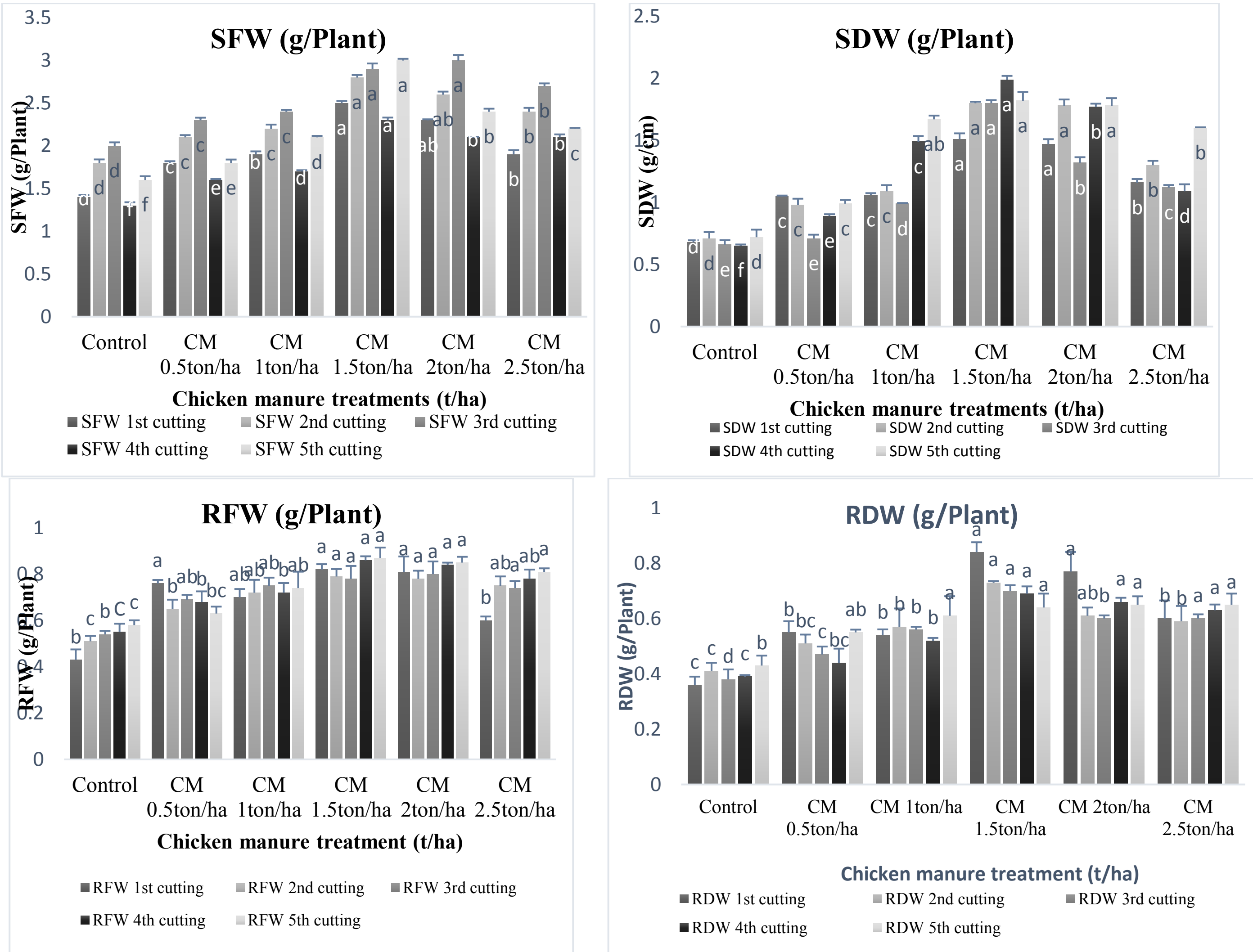

Fig. 1: Effect of Chicken manure and Organic Humic acid - Fulvic Acid foliar application treatment on growth parameters of Alfalfa *M. Sativa*
Data presented in the table represented as superscripts in each column are not significantly different ($P \leq 0.05$). A plant is grown with and without CH+HA treatment at different cuttings. SFW: Shoot fresh weight; SDW: Shoot dry weight; RFW: Root fresh weight; RDW: Root dry weight

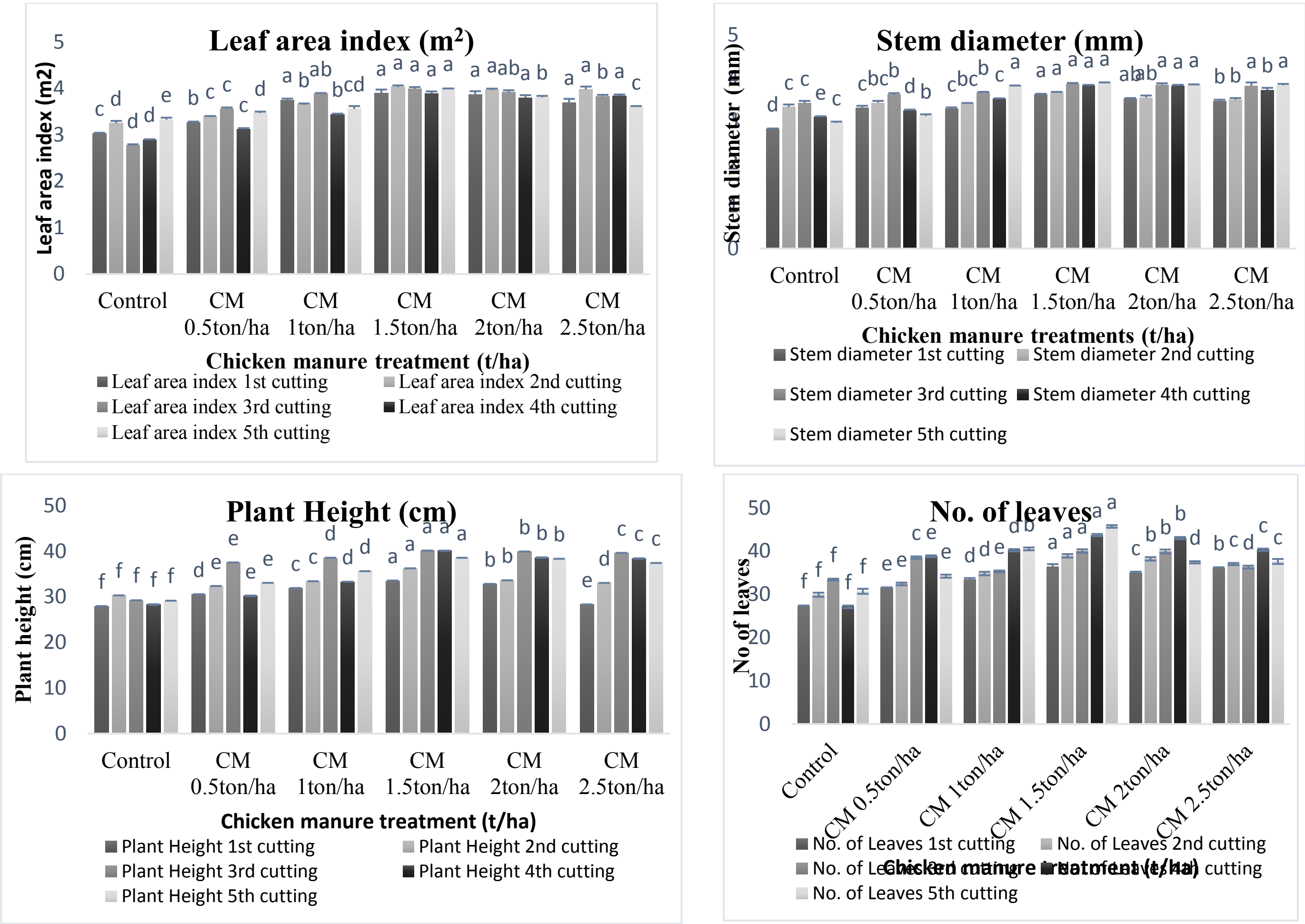

Fig. 2: Effect of Chicken manure and Organic Humic acid - Fulvic Acid foliar application treatment on growth parameters of Alfalfa *M. Sativa* Data presented in the table represented as superscripts in each column are not significantly different (P≤0.05). A plant is grown with and without CH+HA treatment at different cuttings. SFW: Shoot fresh weight; SDW: Shoot dry weight; RFW: Root fresh weight; RDW: Root dry weight

Among all the CM level (C1, C2, C3, C4 and C5), tested C3 was able to increase the plant length significantly (P≤0.05) (33.5 cm (1st cut), 36.2cm (2nd cut), 40.1 (3rd cut), 40.1cm (4th cut),38.5 (5th cut)) in all cuttings followed by C4 treatment. Similarly, for SDW, best results were recorded by treatment C3 in every cuts 1.51g/plant (1st cut), 1.8g/plant (2nd cut), 1.8g/plant (3rd cut), 1.99g/plant (4th cut) and 1.82 g/plant (5th cut) whereas in case of RDW treatments, C3 to C5 showed significantly high (P≤0.05) dry weight and maximum output was found with C3 in initial three cutting while for rest two cuttings all three treatment C3 to C5 were equally effective.

Similarly, SFW was found to be increased significantly (P≤0.05) in plant biomass raised from C3 treatment closely followed by treatment C4>C5 (Fig. 1) in comparison to control C0 (Untreated) in all five cuts 1.4g/plant (1st cut), 1.8g/plant (2nd cut), 2g/plant (3rd cut), 1.3g/plant (4th cut) and 1.6g/plant (5th cut).

All the five CM treatments improved RFW in every cut over control (untreated) but significant (P≤0.05) differences were detected in C3 and C4 treatment 0.82 and 0.81 g/plant (1st cut), 0.79 & 0.78 g/plant (2nd cut), 0.78 and 0.8 g/plant (3rd cut), 0.86 and 0.84 g/plant (4th cut) and 0.87 and 0.85 g/plant (5th cut) followed by C5>C2>C1>C0. Amongst all five CM levels, C3 significantly increased the RDW in all five cuttings 0.55g/plant (1st cut), 0.61g/plant (2nd cut), 0.64g/plant (3rd cut), 0.65g/plant (4th cut) and 0.65g/plant (5th cut) in comparison to control.

However, a varied level of response was observed in the case of no. of leaves but all the CM treatments were able to increase the no. of leaves significantly (P≤0.05) in Alfalfa crops. Significant results were observed with C3 treatment and maximum no. were observed in 4th (43.7) and 5th (45.7) cutting followed by C4 treatment and similarly the maximum no. of leaves were found in 4th (43) and 5th (37.4).

C3 treatment was most effective in the case of leaf area index and stem diameter as well with 3.9m^2 in 1st cut, 4.03m^2 in 2nd cut, 3.99m^2 in 3rd cut, 3.89m^2 in 4th cut and 4m^2 in 5th cut and 3.6mm (1st cut), 3.65mm (2nd cut), 3.85mm (3rd cut), 3.82mm (4th cut) and 3.87mm (5th cut) respectively in comparison to C0 (untreated).

VIRENXIA derived enzymatic chicken manure based fertilizer and hydrodynamically extracted organic humic acid - fulvic acid foliar application significantly increased plant height, the number of leaves per plant, shoot fresh and dry weight, root fresh and dry weight, stem diameter and leaf area index. The beneficial effect of chicken manure and humic acid could be due to its positive effects on soil physical conditions, increased nutrient uptake, water and root penetration, photosynthesis and other physiological factors that increase protein synthesis[18].

Generally, fertilizers boost crop yields, but intensive agricultural systems have increasingly negative effects on the environment. Chicken manure, either alone or in combination with microbial inoculation, may prove to be a better alternative to supply nitrogen to the cropping systems of the future [21].

Chicken manure and humic acid treatments significantly increased plant height in all cuts. This could be due to the fact that chicken manure consists of different nutrients that improve soil fertility and increase the microbial population[17]. Apart from this, another reason for significant improvement could be due to the addition of enzymes derived by VIRENXIA. The enzyme addition in formulation results in soil regeneration and conditioning, soil carbon and microbial preservation and sustenance. Both enzymes promote the development of the microorganisms in the rhizosphere of the plants, contribute due to its catalytic properties to the accelerated processing of the root hairs which die off leaving behind them the channels for the penetration of air and moisture.

Another significant reason could be due to foliar application of organic humic acid - fulvic acid, the auxin activity it induces in the catalytic activity of cell membrane and increased uptake of nutrients. According to the report of Turkmen et al[39], it was reported that HA increased the root and shoot dry matter when applied to the growth medium. Similarly, in their experiment, Pertuit et al[32] indicated that the humic acid increased shoot dry matter content in tomato when added into the sand medium. Our results support the previous reports.

Fodder Characteristics: Alfalfa *M. Sativa* plants were analyzed for their forage potential. Under stressful conditions, control plants (untreated) were found to reduce forage quality very quickly which indicates their fiber content. When VIRENXIA derived fertilizer treated plants were analyzed for their NDF, ADF, crude protein and lignin content, all the treated plants improved the fodder quality by decreasing the fiber, lignin content and by enhancing the crude protein content. Treatment C3 showed significant ($P \leq 0.05$) result with 34.1% NDF, 27.1% ADF, 29.86% crude protein and 4.8 % lignin in first cut followed by C4 treatment in comparison to control C0 (36.7% NDF, 30.2 % ADF, 26.11% crude protein and 7.11 % lignin). The data pertaining to NDF, ADF, crude fiber and lignin for all treatments along with control (Untreated) are summarized in table 2. Similarly, chicken manure and organic humic acid - fulvic acid treatments significantly improved forage fresh yield in all cuts. A maximum increase in forage fresh yield was obtained with 1.5 tons of chicken manure/ha treatment.

According to Giarpini et al[13], chicken manure alone supplies adequate levels of nutrients to the crop and increased the concentration of N, P and K in the soil. However, high levels of manure can result in adverse ecological effects as pointed out by Elsheikh et al.[13] The chicken manure level and forage fresh yield indicate that the optimum dose of chicken manure is not necessarily the highest dose (2.5 ton manure/ha). These results are in line with those of Forawi and Elsheikh[17] who reported that yields showed a positive correlation with rates of chicken manure. Moreover, the application of organic humic acid - fulvic acid on 30th and 45th day helps in improving early plant growth and also promotes dry matter accumulation.

All CH+OHA-FA treatments significantly increased protein content and decreased crude fiber content compared to untreated plants. These results are in agreement with the findings of Elsheikh et al[13] who found that chicken manure along with microbial inoculation increases the protein content of alfalfa. The decrease in crude fiber content reflects the role of chicken manure in improving the quality of alfalfa forage. Chicken manure, humic and fulvic acid usually increase nitrogen and phosphorus in plants, improve soil physical properties, increase aggregation of soil particles and improve water holding capacity which may consequently enhance the nutrient uptake[13].

In our studies performed, we found a reduction in lignin content in all CM+OHA-FA treated plants at every cutting. Upon exposure to the stress, the secondary cell wall of the plants is strengthened by the incorporation of lignin and hemicellulose to avoid cell wall damage[24,38]. These observations were supported by increased activity of phenylalanine ammonia-lyase, a key enzyme in the lignin synthesis pathway[38]. If such biomass is used for forage application, their digestibility becomes more difficult[44]. These observations are in agreement with earlier reports[44]. Upon treatment with CM+HA, defense mechanism of crops becomes strong which suppresses the lignin synthesis pathway.

Antioxidant activities: Our results showed that the total phenolic content (TPC) was increased in plants treated with CM+HA over control in all five cuts. Maximum total phenolic content (TPC) was observed with treatment C3 3.96 mg/g, 3.7 mg/g, 3.8mg/g, 2.9mg/g and 2.9mg for 1st, 2nd, 3rd, 4th and 5th cut respectively (Table 3).

Similar results were found in case of total flavonoid contents (TFC) in which treatment C3 recorded maximum activity in all cuts except 4th one (7.8 mg/g, 7.9 mg/g, 6.2 mg/g, 5.1 mg/g and 5.4 mg/g for 1st, 2nd, 3rd, 4th and 5th cut respectively). Our crops were growing in sandy and salty soil of Oman which created abiotic stresses for them and several findings showed that the TPC and TFC were increased due to stress conditions[38].

Studies reported that organic fertilizers induce the secondary metabolite pathway in plants such as the acetate shikimate pathway which leads to high phenolic and flavonoid synthesis[38].

Table 2
Effect of Chicken manure and organic humic acid - fulvic acid foliar application treatment on NDF (%), ADF (%), lignin (%) and crude protein (%) at different cuttings

Treatments	NDF (%)					ADF (%)				
	1st cutting	2nd cutting	3rd cutting	4th cutting	5th cutting	1st cutting	2nd cutting	3rd cutting	4th cutting	5th cutting
Control	36.7± 0.023a	38.5± 0.02a	41.2± 0.05a	43.5± 0.025a	44.6± 0.026a	30.2± 0.03a	32.3± 0.025b	34.8± 0.05a	35.5± 0.03a	36.5± 0.023a
CM 0.5ton/ha	35.1± 0.04c	36.7± 0.017ab	38.6± 0.02d	40.44± 0.045c	40.2± 0.02d	29.2± 0.025c	31.7± 0.01d	33.1± 0.05d	34.6± 0.02b	36± 0.01b
CM 1.0ton/ha	34.2± 0.015d	35.2± 0.023c	37.7± 0.06e	37.3± 0.015e	38.4± 0.013e	27.2± 0.028e	30.8± 0.026e	32.9± 0.05e	34.5± 0.023b	35.1± 0.02d
CM 1.5ton/ha	34.1± 0.06e	34.5± 0.06e	37.5± 0.06f	37.6± 0.045d	39.5± 0.025f	27.1± 0.07e	30.5± 0.01f	32.7± 0.044f	32.1± 0.032c	34.9± 0.015e
CM 2.0ton/ha	34.2± 0.025d	34.7± 0.011d	40.2± 0.04c	40.6± 0.041b	43.2± 0.023c	28.8± 0.023d	32± 0.023c	34.3± 0.036b	34.6± 0.015b	35.1± 0.056d
CM 2.5ton/ha	36.3± 0.06b	36.3± 0.025b	40.9± 0.036b	40.2± 0.036c	43.5± 0.05b	29.8± 0.01b	33.5± 0.01a	33.8± 0.07c	34.5± 0.05b	35.7± 0.037c
	CP (%)					Lignin (%)				
Control	26.11± 0.02f	23.1± 0.036f	19.8± 0.041e	16.5± 0.07e	13.2± 0.015e	7.11± 0.035a	8.54± 0.07a	10.21± 0.01a	10.49± 0.023a	11.15± 0.05a
CM 0.5ton/ha	28.63± 0.01c	25.9± 0.035c	23.1± 0.036c	19.4± 0.015c	17.3± 0.036c	6.2± 0.045c	7.23± 0.015b	8.9± 0.023b	9.65± 0.036b	10.2± 0.037d
CM 1.0ton/ha	29.36± 0.035b	26.2± 0.01b	24.7± 0.02b	19.8± 0.041b	19.8± 0.01b	5.45± 0.026d	5.7± 0.017e	7.67± 0.01d	8.1± 0.05d	9.6± 0.023e
CM 1.5ton/ha	29.86± 0.04a	26.6± 0.02a	24.8± 0.015a	20.1± 0.047a	19.2± 0.035a	4.8± 0.01e	5.9± 0.02d	7.6± 0.01e	8.4± 0.06e	9.4± 0.035f
CM 2.0ton/ha	27.1± 0.03d	24.3± 0.02d	22.6± 0.03d	16.7± 0.07d	14.2± 0.05d	5.44± 0.01d	6.3± 0.05c	8.6± 0.023c	9.23± 0.01c	10.4± 0.041c
CM 2.5ton/ha	26.8±0.0 45e	23.7± 0.045e	22.6± 0.026d	16.3± 0.02f	12.3± 0.023f	6.88± 0.06b	7.3± 0.05b	8.9± 0.01b	9.1± 0.05c	10.6± 0.05b

Data presented in the table are mean (n=3) ± standard error. According to DMRT same letters represented as superscripts in each column are not significantly different (P≤0.05). NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein

Table 3
Effect of Chicken manure and organic humic acid - fulvic acid foliar application treatment on NDF (%), ADF (%), lignin (%) and crude protein (%) at different cuttings

Treatments	TPC (mg/g)					TFC (mg/g)				
	1st cutting	2nd cutting	3rd cutting	4th cutting	5th cutting	1st cutting	2nd cutting	3rd cutting	4th cutting	5th cutting
Control	2.11± 0.02f	2.2± 0.036f	2.3± 0.041e	1.9± 0.07e	1.6± 0.015e	4.11± 0.035a	4.54± 0.07a	4.21± 0.01a	4.49± 0.023a	4.15± 0.05a
CM 0.5ton/ha	3.63± 0.01c	3.2± 0.035c	2.9± 0.036c	2.4± 0.015c	1.9± 0.036c	5.2± 0.045c	5.23± 0.015b	4.9± 0.023b	4.65± 0.036b	4.2± 0.037d
CM 1.0ton/ha	3.36± 0.035b	3.5± 0.01b	3.3± 0.02b	2.5± 0.041b	2.2± 0.01b	6.45± 0.026d	6.7± 0.017e	5.67± 0.01d	5.4± 0.05d	4.6± 0.023e
CM 1.5ton/ha	3.96± 0.04a	3.7± 0.02a	3.8± 0.015a	2.9± 0.047a	2.9± 0.035a	7.8± 0.01e	7.9± 0.02d	6.2± 0.01e	5.1± 0.06e	5.4± 0.035f
CM 2.0ton/ha	3.3± 0.03d	3.0± 0.02d	2.8± 0.03d	2.7± 0.07d	2.4± 0.05d	5.44± 0.01d	7.3± 0.05c	5.6± 0.023c	5.23± 0.01c	4.7± 0.041c
CM 2.5ton/ha	3.5± 0.045e	2.7± 0.045e	2.6± 0.026d	2.3± 0.02f	2.3± 0.023f	6.88± 0.06b	6.3± 0.05b	5.9± 0.01b	5.1± 0.05c	4.6± 0.05b

Data presented in the table are mean (n=3) ± standard error. According to DMRT same letters represented as superscripts in each column are not significantly different (P≤0.05). TPC: Total phenolic content; TFC: Total flavonoid content

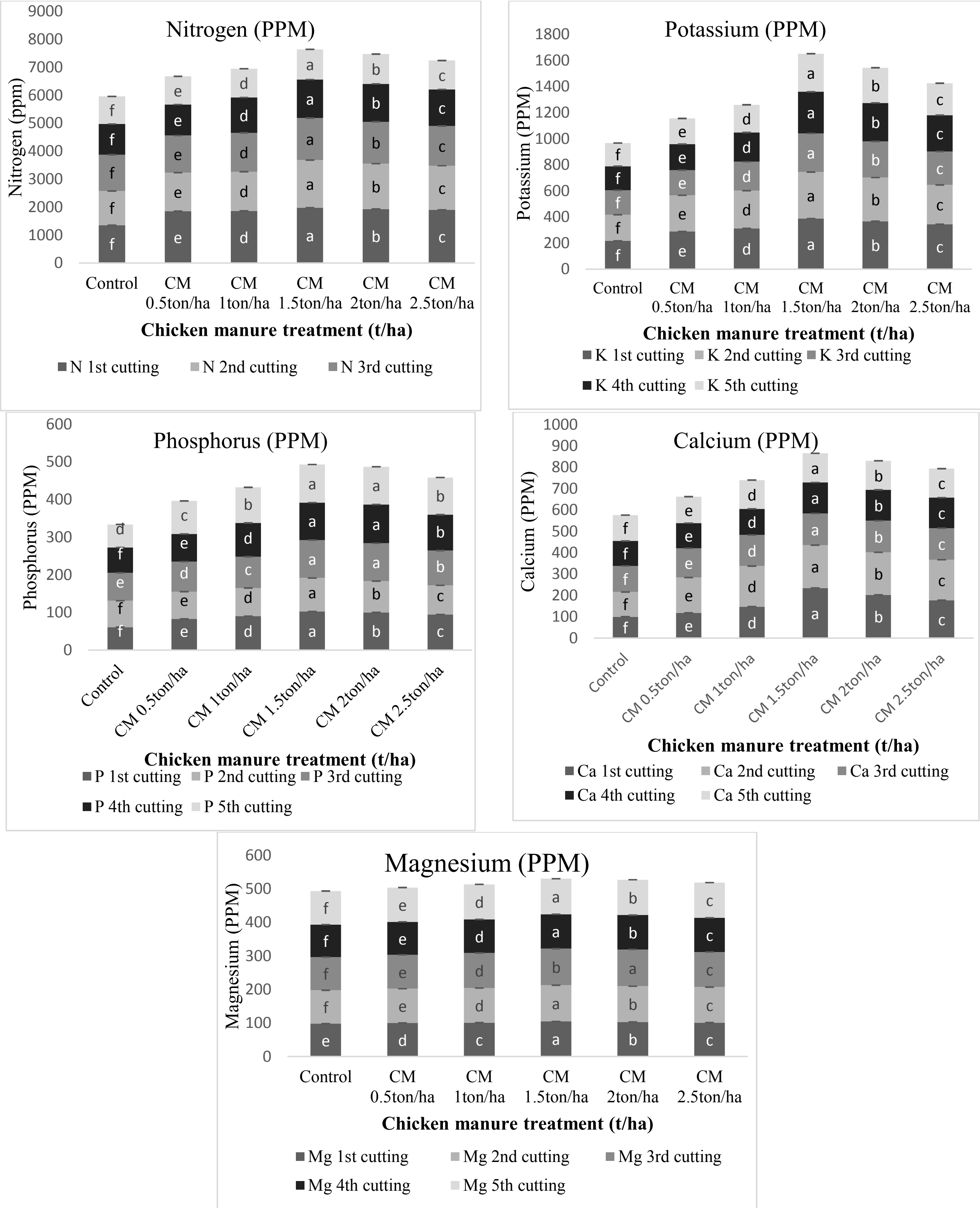

Fig. 3: Effect of Chicken manure and organic humic acid - fulvic acid foliar application treatment on the macronutrient content of Alfalfa *M. Sativa*

Data presented in the table represented as superscripts in each column are not significantly different (P≤0.05). The plant is grown with and without chicken manure treatment.

N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium

Overall, chicken manure combined with the foliar application of organic humic acid - fulvic acid promoted the TPC and TFC. This research will prove that crops grown with organic fertilizers and organic humic acid - fulvic acid contain higher concentration of secondary metabolites and might be more healthier option for livestock.

Earlier research indicated that rhizobacteria, PGPRs, mycorrhizal fungi play an important role in increasing these compounds. The reason behind this is that organically growing crop systems supply enormous amount of organic materials and improve nutrients mobility, which helps in enriching the soil with microbial diversity (such as bacteria, fungi and nematodes) that are beneficial to plant growth promotion. Several studies reported soil microflora and plant roots interactions.

Macronutrient content: Similarly, the significant effect of CM+ HA treatment was found for mineral content in comparison to control (Untreated) (Fig. 3). Maximum N (1978 ppm), P (102.6 PPM), K (388.1 PPM), Ca (234.4 PPM) and Mg (104.6 PPM) content were observed with C3 treatment in 1st cut followed by C4 treatment N (1932ppm), P (100.2 PPM), K (367.8 PPM), Ca (202.3 PPM) and Mg (102.8 PPM) while in control plants N (1350 ppm), P (60.9 PPM), K (217.2 PPM), Ca (100.2 PPM) and Mg (97.7 PPM) content were comparatively less. A similar pattern was found for rest of cuts as well. The results presented are also supported by several findings that chicken manure changes the morphology of roots so that they can uptake more water and nutrients from the soil[11].

The results of this investigation indicate that the CM+HA treatments are promising organic bio-fertilizers because they are low-cost, easily available and easy to handle. The efficiency of treatments could be improved by the addition of bio-inoculants and soil amendments. Moreover, chicken manure and organic humic acid - fulvic acid could provide a comparatively large amount of nutrients for long periods. Besides, it improves the alfalfa protein content and the physical and chemical properties of the soil.

Conclusion

The results concluded that chicken manure followed by organic humic acid - fulvic acid foliar application modifies the stress response of host-plant which leads to the improvement of biomass characters. Chicken manure treated plants showed a tendency to maintain the growth parameters and forage composition in low fertile soil. The current experiment offers an opportunity to understand chicken manure and organic humic acid - fulvic acid host interaction under stressful conditions and helps in improving the cultivation of Alfalfa crops on marginal and degraded land.

The laboratory and poly house experiments data clearly indicated that the best identified treatment C3 (1.5 ton/ha) could be used for their application in normal as well as marginal fields as a biofertilizer. The use of these chicken manure and organic humic acid - fulvic acid in sustainable agriculture is directly related to understanding their mode of action in growth promotion and suppression of abiotic and biotic stress in plants.

In the present investigation, inoculation of chicken manure followed by organic humic acid - fulvic acid foliar application with other beneficial traits such as high nutrient content, organic matter and capability to improve soil texture and relieving plant stresses and damages attributed to exposure of plants to infertile sandy soil was done. Further quality and quantity of Alfalfa were improved by CH+HA treatment and produced biomass was evaluated for fodder, antioxidant and macronutrient content. Plant growth promotion might have been due to less ethylene production at rhizosphere, solute accumulation, plant cell membrane stabilization, antioxidant production and maintenance of plant water status conferred by these treatments.

From this study, it may be concluded that without any genetic manipulation we can enhance the good quality and quantity of Alfalfa under infertile soil conditions. There is no doubt that a lot of work remains to be done before chicken manure and organic humic acid - fulvic acid becomes a mainstay of agricultural and horticultural practice. Despite significant advancements made in understanding the mechanisms, its performance under field conditions is inconsistent and chicken manure, organic humic acid - fulvic acid is yet to fulfill its promise as a potential alternative to agrochemicals. Hence more research should be carried out to develop strategies to maintain consistency of chicken manure performance under field conditions.

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