Response of the proximate composition, minerals, antioxidant leaf pigments, phenolics, and flavonoids content of Slender Amaranth to different levels of organic manures in different agro-ecological regions

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ABSTRACT

In this study, the experiments were conducted in three agro-ecological zones represented by Swat, Peshawar, and the Horticulture Farm at the University of Haripur, Pakistan, to determine the significant effects of farmyard manure and humic acid on growth, proximate composition, antioxidant leaf pigments, minerals, phytochemical composition and Ascorbic acid of *Amaranthus viridis* with different levels of FYM (5, 10, and 15 tons/ha) and Humic acid (15, 20, and 30 kg/ha), during the period from March-June 2021. The plots were arranged in randomized complete block design with a split-plot arrangement. Three replications were applied in each district. Results from the single FYM (15 tons/ha) trial indicate significantly affected stem height, root length, leaf area, yield, proximate composition, minerals, antioxidant leaf pigments, vitamin C, TPC, and TFC in all agro-ecological zones. In the case of Humic acid, maximum growth, root length, leaf area, moisture percentage, and dry matter yield proximate composition, minerals, antioxidant leaf pigments, vitamin C, TPC, and TFC were recorded in all regions when compared with FYM 5 ton/ha. In terms of ecological alternation, Overall, *Amaranthus viridis* performs best in Haripur, then Peshawar and Swat.

Key word: Agro-ecological zones, *Amaranthus Viridis,* antioxidant leaf pigments, FYM, humic acid, proximate composition.

INTRODUCTION

Amaranthus viridis is one of the plants in the *Amaranthaceae* family, that produces grains as well as leafy edible vegetables. The genus name, *Amaranthus*, is derived from the Greek word "amarantos, which means "unfading" or "non-wilting." They are drought resistant and adapt readily to adverse environmental conditions (Assad *et al*., 2017). *Amaranthus viridis* exhibits C4 photosynthesis, allowing it to use CO_2 more efficiently throughout a larger temperature range (25°C to 40°C), at higher light intensities, and under more moisture-stressed conditions (Lara *et al*., 2008; Gill *et al*., 2011). *Amaranthus viridis* has excellent geographic adaptability to a wide range of environmental conditions (Joshi *et al*., 2018).

It can be found in abundance in different ecological regions of Asia, Africa, America, Australia, and Europe. Leaves and succulent stems are excellent sources of protein with the essential amino acids lysine and methionine (Ruth *et al*., 2021). About 12.5 to 17% of the protein in *Amaranthus viridis* seeds is made up of amino acids, including lysine (0.73% to 0.84% of the total protein) and methionine and cysteine (0.73% to 0.84% of the total protein) (Assad *et al*., 2017; Sahoo, 2018). In *A. viridis* leaves, dry matter ensures the highest protein content of 17.5–38.3% (Dada et al., 2017). Particularly in Pakistan, where it is consumed as a wild leafy plant, its morphological, nutritional and pharmacognostic characteristics are not as well documented as in other countries (Abbas *et al*., 2020).

Additionally, Amaranthus leaves and stems include carotenoids, ascorbic acid, dietary fibre, and minerals such as calcium, magnesium, potassium, phosphorus, iron, zinc, copper and manganese (Sarker *et al*., 2014; Chakrabarty *et al*., 2018). It is an excellent source of proximate carotenoids, dietary fibre, and beneficial compounds that have a significant role as natural antioxidants, ROS scavenger minerals such as calcium, iron, zinc, and magnesium, and Phytopigments (Sarker *et al*., 2014; Chakrabarty *et al*., 2018). *Amaranthus viridis* leaves (both raw and cooked) contain high levels of many vitamins, including vitamin A (retinol), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B9 (folate), and vitamin C (Jiménez-Aguilar, and Grusak, 2017; Sarker and Oba, 2019; Sarker *et al*., 2020; Jahan *et al*., 2021). While the leaves of *Amaranthus viridis* have a similar flavour to spinach, they are more nutritious because they contain three times the vitamin C, calcium, and niacin found in spinach (Verma *et al*., 2017).

Amaranthus viridis is rich in antioxidants, that decrease the effect of free radicals and are crucial in the prevention of cancer and degenerative diseases (Arora and Ramawat, 2018). Essential oils, which include high concentrations of triterpenoids, saponins, flavonoids, steroids, phenols, and tannins, have a presence of antioxidants that prevent from various diseases and have antiallergic, anticancer and antihypertensive properties (Lodh and Swamy, 2019).

In many parts of the world, particularly in developing countries, the fertility of the soil is decreasing gradually (Lal, 2006). This will lead to increased crop productivity while also maintaining crop quality (Kumari and Singh, 2020; Usharani *et al*., 2019). Organic manures are essential for increasing soil fertility and productivity because they contain growth-promoting components such as enzymes and hormones which improve the growth and yield of plants (Premsekhar and Rajashree, 2009; Blanco-Canqui *et al*., 2013; Kannan *et al*., 2013). Slender amaranth is a new crop in Pakistan and optimizing its production requires adapting to genotypes grown under optimum crop management practices, including soil fertility, management. This study was therefore undertaken to determine appropriate levels of FYM and Humic acid for enhanced growth, proximate composition, *antioxidant leaf pigments* minerals, phytochemical composition and Ascorbic acid of slender amaranth production along the value chain for food security in different agro-ecological zones in Khyber Pakhtunkhwa.

MATERIAL METHOD

Study sites

The experiments were conducted in three agro-ecological zones represented by Swat, Peshawar, and the Horticulture Farm at the University of Haripur, Pakistan, to determine the significant effects of farmyard manure and Humic acid on growth, proximate composition, *antioxidant leaf pigments* minerals, phytochemical composition and Ascorbic acid of *Amaranthus viridis* with different levels of FYM (5, 10 and15 tons/ha) and Humic acid (15, 20 and 30 kg/ha), The experimental sites were located at different districts i.e Matta (1,120m above sea level and Latitude: 34.93070 N, Longitude: 72.41690 E) in District Swat and Horticulture farm at The University of Haripur, Haripur District (520 m above sea level and *Latitude*: 33.99460 N, *Longitude:* 72.91060 E) and District Peshawar (331m above sea level and *Latitude*: 34.0151° N, *Longitude:* 71.5249° E).

Experimental design and treatments:

A randomized complete block design having a split-plot arrangement with three replications was used in each location. The experiment has two factors. Factor A consisted of three agro-ecological regions (Matta, Peshawar, and Swat) and was randomly assigned to main plots, whereas Factor B contained organic manures at different rates, namely control, FYM (5. 10, 15 t/ha), and humic acid (10,20, 30 t/ha), which were randomly assigned to 1 x 1 m2 sub plots with 25 rows 25 cm apart. Proper irrigation was provided to maintain the normal growth of the crop. Leaf samples were collected 50 days after the sowing of the seed. Appropriate growing practices were maintained. The details of the meteorological data related to the temperature, relative humidity, and rainfall during the period of the experiment were collected from the Khyber Pakhtunkhwa Meteorological Department.

PLANT MATERIAL SOWING AND DATA COLLECTION

Phase 1

Seeds were collected from different locations (elevation of Peochar: 1828.8m, elevation of Matta: 1,120 m, elevation of Batkhela: 648 m) at Khyber Pakhtunkhwa province in august-sept 2019. The seeds were authenticated by Dr. Shah Masaud Khan, Associate Professor and Chairman, Department of Horticulture, The University of Haripur, Pakistan and stored at room temperature (25 ^{0}C) until used in the experiments. During the Phase I growing season, which runs from March to June 2020. Seed were tested separately in each location in the mentioned districts (Swat, Peshawar and Haripur). Batkhela seed performed the best growth and yield in all districts compared to the seed collected regions of Peochar and Matta.

Phase 2

The collected seed from the Batkhela region (648 m above sea level and *latitude*: 35.2227^{0} N, *longitude:* 72.4258 E) in Khyber Pakhtunkhwa Province. There was sown again in the same districts (Matta, Peshawar, Swat). Experimental fields and sowing were done from March-April, 2021. Data were collected 50 days after seeding (DAS) of seeds. The data were recorded on 3 randomly selected plants in each replication for stem height (cm), root length (cm), leaf area plant^{-1} (cm^{2}), moisture %, Leaves dry weight, protein g /100g, Minerals (Ca, K, Mg, P, Fe, Mn, Cu, Zn, B, Mo), Ascorbic acid, Phytochemicals (total phenolic content, total flavonoids content) Bio-chemical substances (chlorophyll a, chlorophyll b, chlorophyll a+b, net Photosynthetic rate.

Chlorophyll content determination

Chlorophyll content was determined using Poora method (2002). Each treatment had a random sample of leaves collected, which were then weighed. One gram of fresh plant material was crushed in acetone using a pestle and mortar. In a volumetric flask, the mixture was made to a volume of 100 ml. An Atomic Absorption Spectrophotometer (AAS Model SP9) was used to measure the absorbance of chlorophyll a, chlorophyll b, and total chlorophyll at the appropriate wavelength. The absorbance was calculated using the formulas below.

Chlorophyll a (*µg/ml) = 12:25 (A663:6) - 2:55 (A646.6)*
Chlorophyll b (*µg/ml) = 20:31 (A646:6) - 20:31 (A663.6)*

Determination of Vitamin C (ascorbic acid)

10 g of the slurry sample was weighed into a 100 ml volumetric flask and diluted to 100 ml with a 3% meta-phosphoric acid solution (0.0033 M EDTA). The diluted samples were filtered using a Whatman filter paper No. 3. The filtrate (10 ml) was pipette into a small flask and titrated immediately with a standardized solution of 2.6 dichlorophenol-indophenol to a faint pink endpoint. The ascorbic acid content of the fruit was calculated from the relationship below

$$\frac{v \times t}{w \times 100} \; mg \; ascorbic \; acid \; per \; 100g \; sample$$

where: V = ml dye used for titration of the aliquot of diluted sample. T = ascorbic acid equivalent of dye solution expressed as mg per ml of dye. W = gram of sample in aliquot titrated.

Proximate composition

The proximate constituents of the crop were determined according to Tafadzwa (2021). All analyses were carried out in triplicate.

Determination of mineral content

Leaves *Amaranth viridis* was dried at 70 °C in a well-ventilated drying oven for 24 hours. Dried leaves stem amaranth (*A. viridis*) were ground finely in a mill and the milled powder passed through 841 microns' screen, then portions of the dried tissues were analyzed for the following macronutrients (Ca, Mg, K and P) and microelements (Fe, Mn, Cu, Zn, B. Mo). All macronutrients and microelements were extracted after dissolution of the *A. viridis* samples by nitric-perchloric acid digestion (Bader, 2011). According to Sarker and Oba (2018) nitric-perchloric acid digestion was performed by adding 0.5 g of the dried samples to 400 ml of nitric acid (65%) with 40 ml of perchloric acid (70%) and 10 ml of sulphuric acid (96%) in the presence of carborundum beads. After nitric-perchloric acid digestion, the solution was appropriately diluted and P analysis was performed in triplicate according to the Ascorbic Acid Method (Chakrabarty et al, 2018). In an acidic medium, orthophosphates formed a yellow-colored complex with molybdate ions and, after the addition of ascorbic acid and Sb, a blue-colored phosphomolybdenum complex was formed. Absorbance was taken according to the method described by Sarker and Oba (2018) in triplicate at wavelength of 422.7 nm (Ca), 285.2 nm (Mg), 766.5 nm (K),880 nm (P), 248.3 nm (Fe), 279.5 nm (Mn), 324.8 nm (Cu), 213.9 nm (Zn), 430 nm (B), 313.3 (Mo) by atomic absorption spectrophotometry (AAS) (Hitachi, Tokyo, Japan). For calibration, AAS standard solutions (1000 mg l-1 in 5% HNO3) were purchased from Merck, Germany. Finally, interferences were reduced by adding 0.1% lanthanum and caesium chloride to samples and standards.

Extraction of samples for TPC, TFC analysis

One gram of dried leaves from each cultivar was ground and suspended in 40 ml of 90% aqueous methanol in a tightly capped (100 ml) bottle, which was then placed in an 80 °C shaking water bath (Thomastant T-N22S, Thomas Kagaku Co., Ltd., Japan). After 1 h, the extract was cooled and filtered for further analytical assays of total polyphenol content, total antioxidant activity and total flavonoid content.

Determination of total polyphenols (TPC)

The total phenolic content of red amaranth was determined using the Folin-Ciocalteu reagent method described by Velioglu et al. (1998) with gallic acid as a standard phenolic compound. Briefly, 50 µl of the leaf extract solution was placed in a test tube along with 1 ml of Folin-Ciocalteu reagent (previously diluted 1:4, reagent: distilled water) and then mixed thoroughly. After 3 min, 1 ml of Na2CO3 (10%) was added, and the mixture was allowed to stand for 1 h in the dark. The absorbance was measured spectrophotometrically using a Hitachi U1800 instrument (Hitachi, Tokyo, Japan). The concentration of total phenolic compounds in the leaf extracts was determined using an equation obtained from a standard gallic acid graph, then putts gallic acid concentrations on the X-axis and their corresponding absorbance on the Y-axis. The results are expressed as µg gallic acid equivalent (GAE) g-1 dry weight (DW).

Determination of total flavonoid content (TFC)

The total flavonoid content in vegetable amaranth extract was determined using the aluminum chloride colorimetric method described by Chang et al. (2002). For this assay, 500 µl of leaf extract was transferred to a test tube along with 1.5 ml of methanol, 0.1 ml of 10%

aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After 30 min at room temperature, the absorbance of the reaction mixture was measured spectrophotometrically using a Hitachi U1800 instrument (Hitachi, Tokyo, Japan). Rutin was used as the standard compound, and TFC is expressed as μg rutin equivalent (RE) g-1 DW.

RESULTS AND DISCUSSION

Growth and Proximate composition

The main effects of farmyard manure and Humic acid on morphological (stem length, root length, leaf area) and proximate composition (moisture %, leaves DM, protein g 100 g^{-1}) of *A. viridis* in different regions i.e Matta (1,120m above sea level and Latitude: 34.93070 N, Longitude: 72.41690 E) in District Swat and Horticulture farm at The University of Haripur, Haripur District (520 m above sea level and *Latitude*: 33.99460 N, *Longitude:* 72.91060 E and District Peshawar (331m sea level and *Latitude*: 34.0151° N, *Longitude:* 71.5249° E) shows in Table 1. The mean values of morphological parameters increased significantly ($p<0.05$) with increased the rate of farmyard manure compared to that of Humic acid, while the lowest mean values of overall morphological parameters were recorded in the control treatment. Table 1 indicates that stem length, root length, leaf area recorded the highest trend in all three regions (Haripur, followed by Peshawar and Swat) when plants were treated with at FYM 15 t/ha, followed by 10 t/ha and 5 t/ha. This possibly explains why organic amendments promoted better growth of *A. viridis* than in the control. In case of Humic acid, when plants treated with 30kg/ha showed the highest growth and proximate compositions i.e stem length (53.65 ± 2.0 cm), root length (11.57 ± 0.5 cm), leaf area (25.67 ± 1.6 cm), moisture (90.54 ± 0.0 %), leaves DM (9.46 0.0 cm), protein (2.72 0.1 g 100g^{-1}) followed by 10 kg/ha and 5 kg/ha due to the availability of sufficient nutrients in the applied compost in all three mentioned regions (Haripur, followed by Peshawar, Swat). The proximate composition (moisture %, Leaves DM, Protein g 100 g^{-1}) as influenced by Humic acid. the maximum proximate composition was obtained with the application of 30kg/ha which were significantly ($p<0.05$) greater than those plants which were treated with Fym 5t /ha in all agro-ecological zones (Haripur followed by Peshawar, Swat In case of *ecological alterations,* highest stem length (56.25 6.3 cm), root length (11.69 1.0 cm), leaf area (24.71 ± 3.6 cm^2), moisture (90.47 ± 0.1 %), leaves DM (9.53 ± 0.1), protein (2.78 ± 0.4 g 100g^{-1}) were recorded in Haripur followed by Peshawar while the lowest cell numbers were recorded in Swat in the mentioned table 1.

Table 1: Effect of Farmyard manure and Humic acid of morphological (stem length, Root length, leaf area) and proximate composition (moisture %, Leaves DM, Protein (g 100 g^{-1}) on *A. viridis* in different regions.

Manures	Stem length	Root length	leaf area	moisture %	Leaves DM	Protein g 100 g-1
Control	35.24 ± 1.9	6.81 ± 0.3	9.61 ± 1.0	90.84 ± 0.0	9.16 ± 0.0	1.54 ± 0.1
FYM $_{5tone}$	51.60 ± 1.9	11.20 ± 0.6	23.78 ± 1.4	90.59 ± 0.0	9.41 ± 0.0	2.62 ± 0.1
FYM $_{10tone}$	64.22 ± 2.7	12.66 ± 0.5	30.44 ± 1.3	90.39 ± 0.0	9.61 ± 0.0	3.44 ± 0.1
FYM $_{15tone}$	81.65 ± 3.9	14.73 ± 0.7	37.00 ± 1.3	90.08 ± 0.1	9.92 ± 0.1	4.26 ± 0.2
HA $_{10kg}$	39.02 ± 1.6	8.69 ± 0.6	14.56 ± 1.1	90.66 ± 0.1	9.34 ± 0.1	1.73 ± 0.0
HA $_{20t\ kg}$	46.87 ± 2.1	10.28 ± 0.5	18.83 ± 0.8	90.64 ± 0.1	9.36 ± 0.1	1.99 ± 0.1
HA $_{30\ kg}$	53.65 ± 2.0	11.57 ± 0.5	25.67 ± 1.6	90.54 ± 0.0	9.46 ± 0.0	2.72 ± 0.1
LSD at P≤ 0.05	0.7462	0.2482	0.3468	6.989	6.989	0.0239
Swat	48.67 ± 5.4	9.86 ± 0.9	20.60 ± 3.4	90.61 ± 0.1	9.39 ± 0.1	2.42 ± 0.3
Peshawar	54.61 ± 6.2	11.00 ± 1.0	23.21 ± 3.6	90.52 ± 0.1	9.48 ± 0.1	2.64 ± 0.4
Haripur	56.25 ± 6.3	11.69 ± 1.0	24.71 ± 3.6	90.47 ± 0.1	9.53 ± 0.1	2.78 ± 0.4
LSD at P≤ 0.05	0.3882	0.2113	0.4843	6.827	6.827	0.0264

The average of three samples is used to compute the Mean ± SEM for each value in the table

Macro-mineral composition

The applications of various treatments of FYM and Humic acid in different agro-ecological zones (Haripur, Peshawar, Swat) significantly affected the mean values of mineral compositions of *A. viridis* in Tables 2, 3. The macro-mineral composition of the crop was appreciably influenced by organic manure application, the overall mineral compositions showed more prominent variations in the terms of FYM amendment than Humic acid. While the lowest trend was recorded in control treatments. The content of calcium (Ca) ranged from 1.92 ± 0.04 mg g^{-1} to 3.75 ± 0.08 mg g^{-1}. The highest Calcium content was obtained at 3.75±

0.08 mg g^{-1} when plants received 15 t/ha. In case of HA, high Ca content was noted at 3.12 ± 0.07 mg g^{-1} when plants were treated with 30 kg/ha compared to those that received 5 t/ha FYM. The lowest calcium content 1.92 ± 0.04 mg g^{-1} was noted in control treatment. Similarly, potassium, magnesium and phosphorus showed an increased trend when plants treated with 15 t/ha. In case of Humic acid, the increased trend of potassium, magnesium and phosphorus was noted when plants received humic acid at 30 kg/ha than those plants that received 5 t/ha FYM. In terms of ecological changes, Haripur had the highest calcium content (3.03 ± 0.25 mg g^{-1}), potassium (5.91± 0.48 mg g^{-1}), magnesium (3.03 ± 0.25 mg g^{-1}), and phosphorus (1.28 ± 0.18 mg g^{-1}), while Swat had the lowest macro-mineral content.

Table 2: Effect of Farmyard manure and Humic acid of macro-mineral compositions in *A. viridis* in different regions

Manures	Ca (mg g^{-1} DW)	K (Mg mg g^{-1})	Mg (mg g^{-1})	P (mg g^{-1})
Control	1.92 ± 0.04	3.11 ± 0.09	1.92 ± 0.04	0.51 ± 0.02
FYM $_{5tone}$	3.05 ± 0.06	5.94 ± 0.16	3.05 ± 0.06	1.25 ± 0.04
FYM $_{10tone}$	3.42 ± 0.09	6.26 ± 0.15	3.42 ± 0.09	1.55 ± 0.05
FYM $_{15tone}$	3.75 ± 0.08	6.79 ± 0.25	3.75 ± 0.08	1.86 ± 0.05
HA $_{10kg}$	2.31 ± 0.09	5.51 ± 0.13	2.31 ± 0.09	0.80 ± 0.06
HA $_{15t\ kg}$	2.81 ± 0.09	5.65 ± 0.16	2.81 ± 0.09	1.11 ± 0.06
HA $_{30\ kg}$	3.12 ± 0.07	6.17 ± 0.30	3.12 ± 0.07	1.33 ± 0.02
LSD at P≤ 0.05	0.0122	0.0975	0.0122	0.0126
Swat	2.78 ± 0.23	5.31 ± 0.42	2.78 ± 0.23	1.10 ± 0.17
Peshawar	2.93 ± 0.23	5.69 ± 0.45	2.93 ± 0.23	1.22 ± 0.17
Haripur	3.03 ± 0.25	5.91 ± 0.48	3.03 ± 0.25	1.28 ± 0.18
LSD at P≤ 0.05	0.0257	0.0904	0.0257	0.0240

The average of three samples is used to compute the Mean ± SEM for each value in the table

Micro-mineral composition

In case of micro-mineral composition of vegetable amaranth, irregular increasing trends were noted in terms of FYM and HA application (Table 3). The content of iron rangeg from 5.21 ± 0.31 to 49.69 ± 3.73 µg g^{-1} DW. The micro-mineral composition of the crop was also appreciably influenced by various organic manures. The significant differences in iron content was caused by the applications of organic fertilizer. The plants that received 15 t/ha FYM had a higher iron content of 49.69 ± 3.73 µg g-1 DW than those that received 5 t/ha or 10 t/ha. Whereas HA also showed an irregular trend. The 30 kg/ha humic acid treatment had a higher iron content of 15.35 ± 0.19 µg g^{-1} than the 10 kg/ha, 15 kg/ha, treatments. The increasing trend was also recoded in Mn (7.08 ± 0.23 µg g^{-1}), Cu (4.60 ± 0.15 µg g^{-1}), Zn (16.67 ± 0.88 µg g^{-1}), B (16.00 ± 0.66 µg g^{-1}), Mo (0.507 ± 0.02 µg g^{-1}) in those plants when treated with 15 t/ha followed by 10 t/ha and 5 t/ha. The overall decreasing trend was noted for all parameters in the control treatment. In terms of ecological changes, Haripur had the highest iron content (20.63 ± 6.55 µg g^{-1}), manganese (3.92 ± 0.80 µg g^{-1}), cupper (2.85 ± 0.43 µg g^{-1}), zinc (10.71 ± 1.57 µg g^{-1}), boron (11.75 ± 1.16 µg g^{-1}), molybdenum (0.33 ± 0.05 µg g^{-1}) while Swat had the lowest micro-mineral content.

Table 3: Effect of Farmyard manure and humic acid of micro-mineral compositions of *A. viridis* in different regions

Manures	Fe (µg g^{-1})	Mn (µg g^{-1})	Cu (µg g^{-1})	Zn (µg g^{-1})	B (µg g^{-1})	Mo (µg g^{-1})
Control	5.21 ± 0.31	1.46 ± 0.18	1.24 ± 0.13	4.77 ± 0.78	7.33 ± 0.41	0.144 ± 0.02
FYM $_{5tone}$	12.55 ± 0.87	3.46 ±0.29	2.52 ± 0.20	8.67 ± 0.88	10.99 ± 0.64	0.302 ± 0.02
FYM $_{10tone}$	31.38 ± 3.27	5.31 ± 0.35	3.42 ± 0.15	12.67 ± 0.88	12.93 ±0.67	0.407 ± 0.03
FYM $_{15tone}$	49.69 ± 3.73	7.08 ± 0.23	4.60 ± 0.15	16.67 ± 0.88	16.00 ± 0.66	0.507 ± 0.02
HA $_{10kg}$	7.91 ± 0.50	1.85 ± 0.08	1.55 ± 0.12	5.67 ± 0.88	8.24 ± 0.36	0.177 ± 0.01
HA $_{15t\ kg}$	11.26 0.73	2.33 ± 0.07	2.22 ± 0.15	7.72 ± 0.83	9.97 ± 0.64	0.247 ± 0.01

HA $_{30\ kg}$	15.35 ± 0.19	3.74 ± 0.25	2.75 ± 0.12	9.67 ± 0.88	11.59 ± 0.59	0.317 ± 0.02
LSD at P≤ 0.05	0.7225	0.0461	0.0170	0.1427	0.0354	0.1023
Swat	16.34 ± 4.98	3.22 ± 0.72	2.35 ± 0.42	7.78 ± 1.54	9.90 ± 1.03	0.27 ± 0.05
Peshawar	20.17 ± 6.56	3.66 ± 0.77	2.63 ± 0.43	9.71 ± 1.57	11.37 ± 1.12	0.30 ± 0.05
Haripur	20.63 ± 6.55	3.92 ± 0.80	2.85 ± 0.43	10.71 ± 1.57	11.75 ± 1.16	0.33 ± 0.05
LSD at P≤ 0.05	1.6540	0.1231	0.0339	0.0843	0.0814	0.0134

The average of three samples is used to compute the Mean ± SEM for each value in the table

Antioxidant leaf pigments

The composition of antioxidant leaf pigments of the crop was also appreciably influenced by various organic manures (Table 4). The chlorophyll a content in slender amaranth ranged from (251.23 ± 3.7 to 294.78 ± 2.7 µg g^{-1}). The highest chlorophyll *a* content (294.78 µg g^{-1} ± 2.7) was noted in those plants treated at 15 t/ha followed by 10 t/ha and 5 t/ha. The content of chlorophyll an in leaf pigment ranged from (266.22 ± 3.2 to 277.00 ± 2.6). The plants treated with humic acid (30 kg/ha) showed a higher increasing trend (277.00 µg g^{-1} ± 2.6) than those treated with FYM (5 t/ha) (271.56 ±3.6 µg g^{-1}). Similarly, the highest chlorophyll b (152.85 ± 3.5 µg g^{-1}), chlorophyll ab (447.63 ± 6.14 µg g^{-1}) and net Photosynthetic rate (43.40 ± 1.62 µmol) were recorded in those plants which received 15 t/ ha FYM followed by 10 t/ha and 5 t/ha. In case of Humic acid, the maximum chlorophyll b (143.80 ± 1.5 µg g^{-1}), chlorophyll ab (420.80 ± 2.69 µg g^{-1}), net Photosynthetic rate (26.47 ± 1.47 µmol) were noted when compared with those treated with 5 t/ha FYM. The overall decreasing trend was documented in the control treatment. It was observed that the chlorophyll content was gradually becoming superior from control treatment to increased does of every type of compost application. Haripur had the highest chlorophyll a (278.50 ± 4.6 µg g^{-1} FW), chlorophyll b (141.64 ± 4.91 µg g^{-1} FW), chlorophyll ab (420.14 ± 9.09 µg g^{-1} FW), and net Photosynthetic rate (27.58 ± 4.20 µmol (CO_2) m^{-2} s^{-1}) in terms of ecological alteration, while Swat had the lowest photosynthetic pigments.

Manures	Chlorophyll a (µg g-1 FW)	Chlorophyll b µg g^{-1} FW	Chlorophyll a+b (µg g-1 FW)	Net Photosynthetic rate
Control	251.23 ± 3.7	113.88 ± 1.2	365.11 ± 4.85	12.51 ± 0.94
FYM $_{5tone}$	271.56 ±3.6	138.59 ± 2.1	410.15 ± 5.68	24.70 ± 1.31
FYM $_{10tone}$	282.89 ± 3.5	143.92 ± 1.2	426.81 ± 4.71	36.09 ± 1.76
FYM $_{15tone}$	294.78 ± 2.7	152.85 ± 3.5	447.63 ± 6.14	43.40 ± 1.62
HA $_{10kg}$	266.22 ± 3.2	136.63 ± 4.5	402.86 ± 7.73	17.52 ± 1.43
HA $_{15t\ kg}$	270.00 ± 2.7	137.21 ± 1.9	407.21 ± 4.58	19.74 ± 1.20
HA $_{30\ kg}$	277.00 ± 2.6	143.80 ± 1.5	420.80 ± 2.69	26.47 ± 1.47
LSD at P≤ 0.05	1.7033	1.8348	4.3373	0.1978
Swat	267.94 ± 5.0	135.09 ± 4.58	403.03 ± 9.15	23.04 ± 3.91
Peshawar	273.71 ± 4.8	137.65 ± 4.48	411.36 ± 8.93	26.70 ± 4.13
Haripur	278.50 ± 4.6	141.64 ± 4.91	420.14 ± 9.09	27.58 ± 4.20
LSD at P≤ 0.05	2.1420	1.6180	1.4550	0.1472

The average of three samples is used to compute the Mean ± SEM for each value in the table

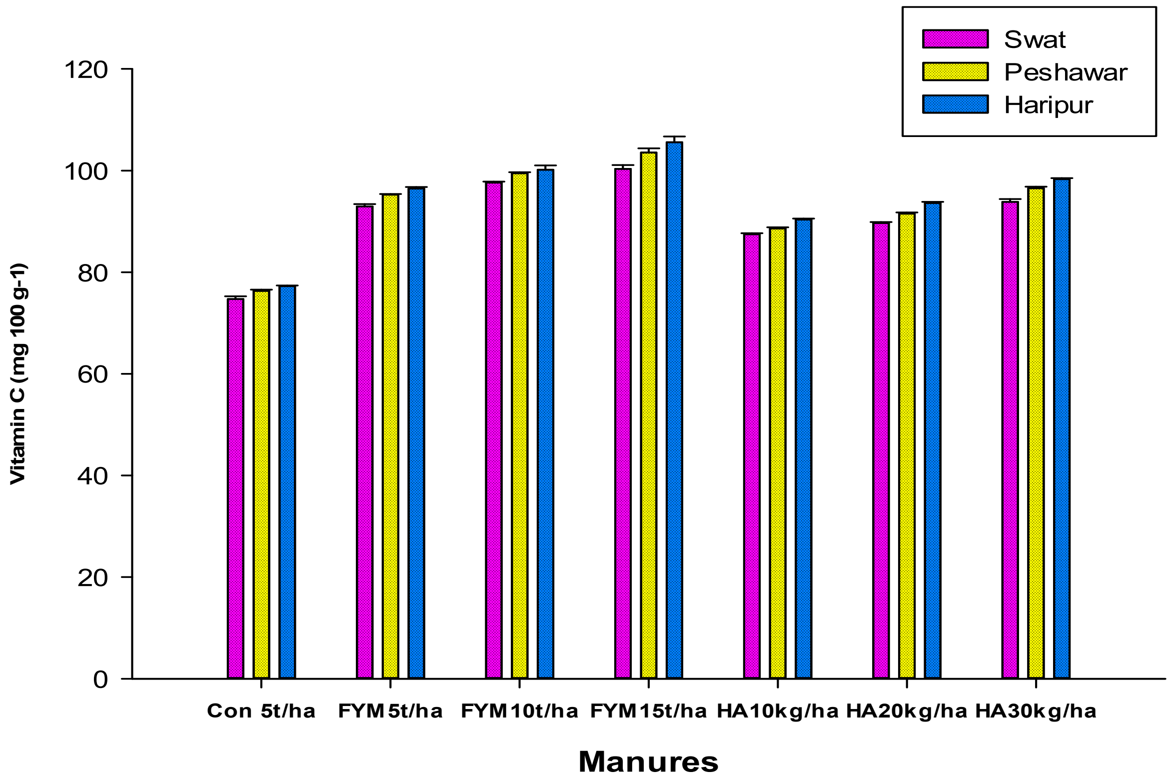
Table 4: Effect of Farmyard manure and humic acid on antioxidant leaf pigments of *A. viridis* in different regions.

Antioxidant of phytochemicals and Vitamin C:

The interactive values of vitamin C, total phenolic and flavonoid content (TPC and TFC) of slender amaranth were considerably affected by the use of various levels of FYM and

Humic acid in agro-ecological zones (Haripur, Peshawar, and Swat). (Fig 1) Vitamin C content significantly affected by various treatments of farmyard manure and Humic acid in selected locations i.e Matta (1,120 m above sea level and Latitude: 34.93070 N, Longitude: 72.41690 E) in District Swat and Horticulture farm at The University of Haripur, Haripur District (520 m above sea level and *Latitude*: 33.99460 N, *Longitude:* 72.91060 E and District Peshawar (331m sea level and *Latitude*: 34.0151° N, *Longitude:* 71.5249° E). The Vitamin C content (Fig-1) showed increasing trend as FYM doses increased from 5 t/ha to 15 t/ha. The higher Vitamin C content was documented (105.59, 103.55, 100.33) in Haripur, Peshawar, Swat locations in those experimental plots which received a single dose FYM 15 t/ha as compared FYM (5. 10 t/ha) and Humic acid (10. 20, 30 kg /ha). Vitamin C content also affect by Humic acid in those plants which received 30 kg/ha. The highest Vitamin C content (98.33, 96.54, 93.85) were examined in Haripur, Peshawar and Swat locations in those plants which treated with 30 kg/ha as compared those plots that treated with Humic acid (10, 20 kg/ha) and FYM (5 t/ha). The lowest Vitamin C content (77.26, 76.33, 74.72) were recorded in unfertilized plots in Swat, Peshawar and Haripur.

Fig 1. Interactive effects of FYM and HA on Vitamin C of *A. viridis* in different locations



In this study, it was observed that total phenolic and flavonoid content (TPC and TFC) were also affected by FYM and Humic acid in different regions (Haripur, Peshawar, Swat) in KPK province (Fig 2-3). The highest phenolic content (46.24 GAE µg g^{-1} FW) and flavonoid content (182.16 RE µg g^{-1} DW) were recorded in those plants when treated with 15 t/ha as compared FYM (5. 10 t/ha) and Humic acid (10. 20, 30 kg /ha). while highest phenolic content (37.48 GAE µg g^{-1} FW), flavonoid content (149.93 µg g^{-1} DW) were noted that received with 10 t/ha and phenolic content (42.377 GAE µg g-1 FW), flavonoid content (169.97 RE µg g-1 DW) were observed that received 5 t/ha. Whereas, Humic acid also showed irregular trend, the increasing trend was recorded phenolic content (43.307 GAE µg g-1 FW) and flavonoid content (169.97 RE µg g-1 DW) in those treated plants which received 30 kg/ha when compared with that received 5 t/ha.

Fig 2. Interactive effects of FYM and HA on TPC of *A. viridis* in different locations

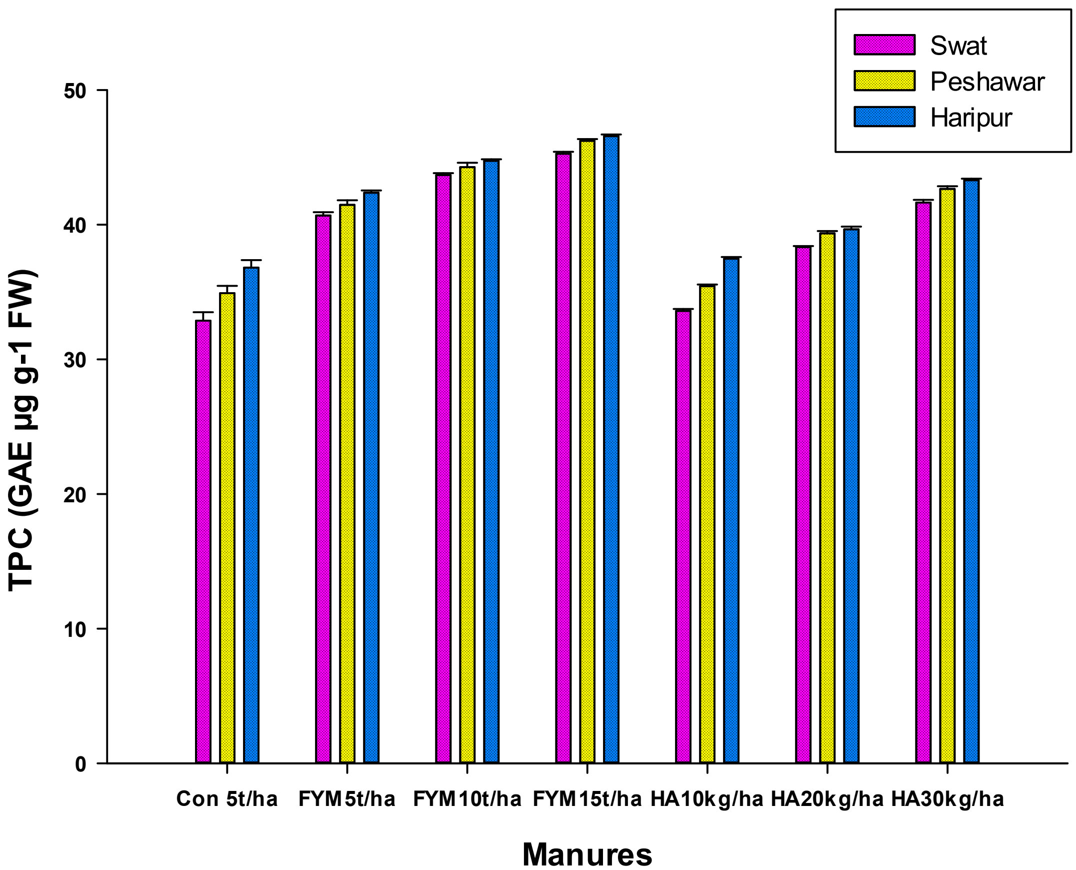
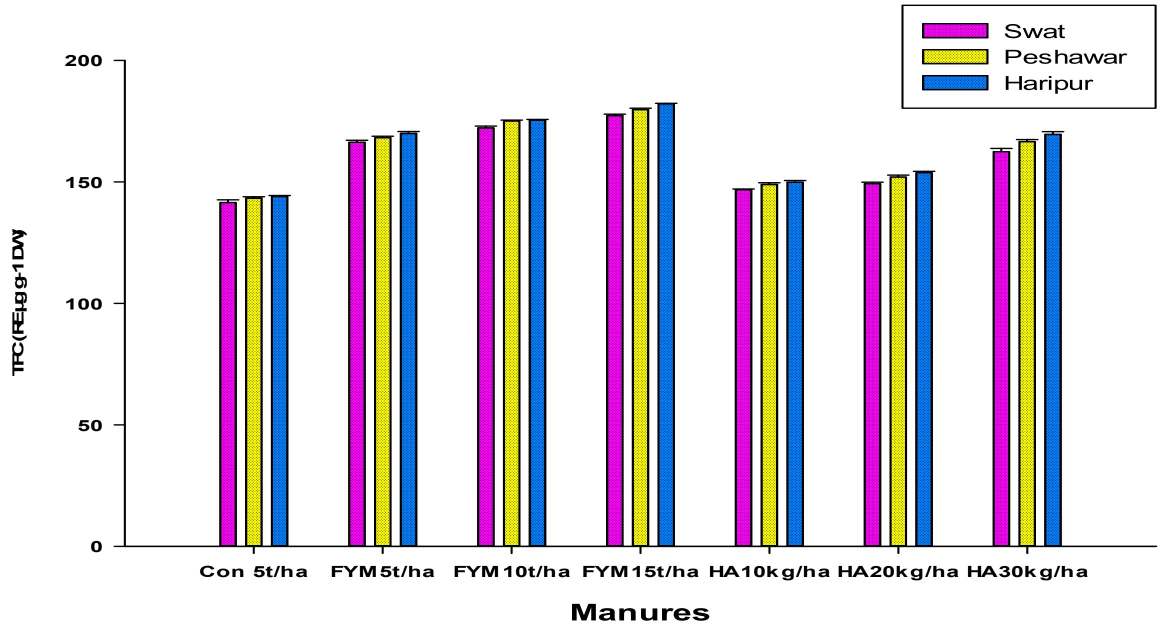


Fig 3. Interactive effects of FYM and HA on TFC of *A. viridis* in different locations



CONCLUSION

In this study, *Amaranthus viridis* cultivated three different locations with applied various level of FYM (5, 10, 15 ton/ha) and Humic Acid (15, 20, 30). Results from the single FYM (15 t/ha) trial promoted growth, root length, leaf area, moisture %, and dry matter yield of *A. viridis in all districts* as compared Humic acid application. Similarly, Significant changes in proximate composition, minerals, antioxidant leaf pigments, vitamin C, TPC, TFC were observed by FYM (15 t/ha). While in control treatment poor soil may not be a good option in *A. viridis* production as the outcome in this study showed. Therefore, this study recommends utilization of 15 t ha^{-1} FYM based compost in augmenting poor soil to enhance growth and quality of *Amaranthus viridis L.*

AUTHOR'S CONTRIBUTIONS:

Shah Masaud Khan supervised this Ph.D. work and review the manuscript, Waseem Ahmad did most statistical analysis.

STATEMENT: CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

Abbas, W., Hussain, W., Hussain, W., Badshah, L., Hussain, K., & Pieroni, A. (2020). Traditional wild vegetables gathered by four religious groups in Kurram District, Khyber Pakhtunkhwa, North-West Pakistan. *Genetic Resources and Crop Evolution, 67*(6), 1521-1536.

Assad, R., Reshi, Z. A., Jan, S., & Rashid, I. (2017). Biology of amaranths. *The botanical review, 83*(4), 382-436.

Bader, N. R. (2011). Sample preparation for flame atomic absorption spectroscopy: an overview. *Rasayan Journal of Chemistry, 4*(1), 49-55.

Banu, S. N., Sivakumar, A., & Subramanian, M. (2003). Effect of manures on biomass production and pharmacobiochemical properties of some greens. *Ancient Science of Life, 23*(2), 123.

Blanco-Canqui, H., Shapiro, C. A., Wortmann, C. S., Drijber, R. A., Mamo, M., Shaver, T. M., & Ferguson, R. B. (2013). Soil organic carbon: The value to soil properties. *Journal of Soil and Water Conservation, 68*(5), 129A-134A.

Chakrabarty, T., Sarker, U., Hasan, M., & Rahman, M. (2018). Variability in mineral compositions, yield and yield contributing traits of stem amaranth (Amaranthus lividus). *Genetika, 50*(3), 995-1010.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis, 10*(3).

Dada, O. A., Imade, F., & Anifowose, E. (2017). Growth and proximate composition of Amaranthus cruentus L. on poor soil amended with compost and arbuscular mycorrhiza fungi. *International Journal of Recycling of Organic Waste in Agriculture, 6*(3), 195-202.

Gill, S. S., Khan, N. A., Anjum, N. A., & Tuteja, N. (2011). Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects. *Plant Stress, 5*(1), 1-23.

Hazra, K., Swain, D., Bohra, A., Singh, S., Kumar, N., & Nath, C. (2018). Organic rice: Potential production strategies, challenges and prospects. *Organic agriculture, 8*(1), 39-56.

Jahan, R., POLASH, S., ARIF, M., KARIM, M., JUTHEE, S. A., FAKIR, S. A., & HOSSAIN, A. (2021). Extraction, characterization and biochemical analysis of betacyanins derived from beetroot (Beta vulgaris). *Research on Crops, 22*(1).

Jiménez-Aguilar, D. M., & Grusak, M. A. (2017). Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of Amaranthus leafy vegetables. *Journal of Food Composition and Analysis, 58*, 33-39.

Joshi, D. C., Sood, S., Hosahatti, R., Kant, L., Pattanayak, A., Kumar, A., . . . Stetter, M. G. (2018). From zero to hero: the past, present and future of grain amaranth breeding. *Theoretical and Applied Genetics, 131*(9), 1807-1823.

Kannan, R. L., Dhivya, M., Abinaya, D., Krishna, R. L., & Krishnakumar, S. (2013). Effect of integrated nutrient management on soil fertility and productivity in maize. *Bulletin of*

Environment, Pharmacology and Life Sciences, 2(8), 61-67.

Kassem, H., & Marzouk, H. (2002). Effect of organic and/or mineral nitrogen fertilization on the nutritional status, yield and fruit quality of Flame seedless grapevines grown in calcareous soils. *J. adv. Res, 7*(3), 117-126.

Khaled, H., & Fawy, H. A. (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil and Water Research, 6*(1), 21-29.

Kumari, R., & Singh, D. P. (2020). Nano-biofertilizer: An emerging eco-friendly approach for sustainable agriculture. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 90*(4), 733-741.

Lal, R. (2006). Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. *Land degradation & development, 17*(2), 197-209.

Lara, M. V., Offermann, S., Smith, M., Okita, T. W., Andreo, C. S., & Edwards, G. E. (2008). Leaf development in the single-cell C4 system in Bienertia sinuspersici: expression of genes and peptide levels for C4 metabolism in relation to chlorenchyma structure under different light conditions. *Plant physiology, 148*(1), 593-610.

Lodh, S., & Swamy, M. K. (2019). Phytochemical aspects of medicinal plants of Northeast India to improve the gynaecological disorders: an update *Natural bio-active compounds* (pp. 353-367): Springer.

Mostafa, M. R. (2011). Effects on growth, yield, and fruit quality in tomato (Lycopersicon esculentum Mill.) using a mixture of potassium humate and farmyard manure as an alternative to Mineral-N fertiliser. *The Journal of Horticultural Science and Biotechnology, 86*(3), 249-254.

Porra, R. J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls an and b. *Photosynthesis research, 73*(1), 149-156.

Premsekhar, M., & Rajashree, V. (2009). Influence of organic manures on growth, yield and quality of okra. *American-Eurasian Journal of Sustainable Agriculture, 3*(1), 6-8.

Ren, J., Liu, X., Yang, W., Yang, X., Li, W., Xia, Q., . . . Yang, Z. (2021). Rhizosphere soil properties, microbial community, and enzyme activities: Short-term responses to partial substitution of chemical fertilizer with organic manure. *Journal of Environmental Management, 299*, 113650.

Ruth, O. N., Unathi, K., Nomali, N., & Chinsamy, M. (2021). Underutilization Versus Nutritional-Nutraceutical Potential of the Amaranthus Food Plant: A Mini-Review. *Applied Sciences, 11*(15), 6879.

Sahoo, A. (2018). Silage for climate resilient small ruminant production. *Ruminants: The Husbandry, Economic and Health Aspects, 11*.

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2014). Genotypic variability for nutrient, antioxidant, yield and yield contributing traits in vegetable amaranth. *J. Food Agri. Environ, 12*, 168-174.

Sarker, U., & Oba, S. (2018). Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chemistry, 252*, 72-83.

Sarker, U., & Oba, S. (2018). Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chemistry, 252*, 72-83.

Sarker, U., & Oba, S. (2019). Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of Amaranthus spinosus and *Amaranthus viridis* weedy species. *Scientific Reports, 9*(1), 1-10.

Sarker, U., & Oba, S. (2020). Nutritional and bioactive constituents and scavenging capacity of radicals in Amaranthus hypochondriacus. *Scientific Reports, 10*(1), 1-10.

Shaheen, M., Abd ElWahab, S. M., El-Morsy, F., & Ahmed, A. (2013). Effect of organic and bio-fertilizers as a partial substitute for NPK mineral fertilizer on vegetative growth, leaf mineral content, yield and fruit quality of Superior grapevine. *Journal of Horticultural Science & Ornamental Plants, 5*(3), 151-159.

Tafadzwa, M. J., Zvamaziva, J. T., Charles, M., Amiel, M., Pepukai, M., & Shepherd, M. (2021). Proximate, physico-chemical, functional and sensory properties OF quinoa and amaranth flour AS potential binders in beef sausages. *Food Chemistry, 365*, 130619.

Tehranifar, A., & Ameri, A. (2012). *Effect of humic acid on nutrient uptake and physiological characteristics of Fragaria× ananassa'Camarosa'.* Paper presented at the VII International Strawberry Symposium 1049.

Türkmen, Ö., Dursun, A., Turan, M., & Erdinç, Ç. (2004). Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (Lycopersicon esculentum L.) seedlings under saline soil conditions. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 54*(3), 168-174.

Usharani, K., Roopashree, K., & Naik, D. (2019). Role of soil physical, chemical and biological properties for soil health improvement and sustainable agriculture. *Journal of Pharmacognosy and Phytochemistry, 8*(5), 1256-1267.

Verma, D. K., Kapri, M., Billoria, S., Mahato, D., & Prem, P. (2017). Effects of thermal processing on nutritional composition of green leafy vegetables: A review. *Engineering Interventions in Foods and Plants. As part of book series on "Innovations in Agricultural and Biological Engineering", Apple Academic Press, USA*.

Yunma, Y., Shichuan, X., Huiying, D., Yanqun, W., & Yingchun, L. (2004). The influence of humic acid fertilizer on the content of the flavonoids in the ginkgo biloba leaf. *Journal of Hebei Agricultural University, 27*(6), 22-25.

Zhang, X., Ervin, E., & Schmidt, R. (2003). Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. *Crop science, 43*(3), 952-956.

Effects of thermal processing on nutritional composition of green leafy vegetables: A review. *Engineering Interventions in Foods and Plants. As part of book series on "Innovations in Agricultural and Biological Engineering", Apple Academic Press, USA*.