RESPONSE OF MORPHOLOGY, PHYTOCHEMICAL AND STEM MICROMETRY OF SLENDER AMARANTH WITH DIFFERENT LEVELS OF ORGANIC MANURES IN AGRO-ECOLOGICAL REGIONS

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ABSTRACT

The present study was conducted to determine significant effects of manures (FYM and HA) and different locations on morphological, micrometry and phytochemical screening of *Amaranthus viridis.* A randomized complete block design having a split-plot arrangement with three replications was used in each location. The experiment had 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. The results showed that positive effects of FYM (15 t/ha) on plant height (63 cm), leaf area (35.57 cm^2), epidermis cell length (32.23 µm), Stem epidermis width (18.13 µm), cortex length (17.33 µm), cortex width (10.51 µm), pericycle length (22.87 µm), pericycle width (18.10 µm), Xylem Length (35.12 µm), Xylem width (20.84 µm) were recoded as compared to those plots of applied humic acid. Phloem length and width were also significantly higher (22.14 µm, 15.17 µm) in plots treated with 15 t/ha than in those plots treated with 5, 10 t/ha) and humic acid. The same trend was observed when plants were treated with 15 t/ ha i.e pith length (19.79 µm), pith width (16.08 µm), endodermis length (19.36 µm) and endodermis width (16.56 µm). The phytochemical components of stem and root extract were recorded after micro-chemical screening tests such as Flavonoids, Saponins, Phenolic, Hydrolysable tannins, Condensed tannins, Mucilage, Lignin, and Cellulose. In the case of ecological alterations, all means of stem anatomy also express high plasticity and can easily adapt to diverse agrological situations. Overall, maximum mean cell numbers were recorded in Haripur, followed by Peshawar, while the lowest cell numbers were recorded in Swat. Based on the results,

FYM (15t/ha) was found beneficial in ecological conditions of Haripur, Peshawar and Swat and can be recommended for *Amaranthus viridis* mentioned parameters.

Keywords: agro-ecological, manures, morphological**,** micrometry, location, phytochemical

INTRODUCTION

The Amaranth plants are annuals or short-lived perennials with over 103 species of flowering plants in the family *Amaranthaceae*, distributed nearly worldwide (McGaw et al., 2022). In the current study, Slender amaranth (Amaranthus viridis) also demonstrates remarkable diversity due to its extensive adaptability to diverse eco-geographic situations. The agreements also agree with (Jeelani et al., 2018; Jeelani et al., 2019; Kakabouki et al., 2021). which expresses high plasticity to environmental changes in selected regions (Hoang et al., 2020). The growth of a plant could be influenced by several environmental factors, such as biotic and abiotic stress, that damage the physiological characteristics of plants (Bhargava and Srivastava, 2020; Shabbir et al., 2022).

Studying morphological, phytochemical, and anatomical modifications in stem can help in understanding biological processes in plants regarding organic manures and ecological conditions (Shekhawat and Manokari, M., 2018; Revathi et al., 2019). Organic manures have positive effects on plant growth (Souri and Aslani, 2018) which has a great effect on plant growth due to its improvement of soil fertility, and to its enhancement of mineral nutrient absorption by plants (Chen et al., 2020; Hafez et al., 2021). Due to the decrease in the content of organic matter and native fertility of the soil around the world, international attention has focused on adopting appropriate and sustainable strategies such as using organic fertilizers from animals to make up for the lack of nutrients and its effect on plant growth and physiology to reduce the use of chemical fertilizers (Dhaliwal et al., 2019; Zhang et al., 2019; Voltr et al., 2021).

Furthermore, organic manures applied to soil as a plant nutrient have shown a great effect on anatomical diversity (Xu. et al., 2021). We expected that the organic manure amendment that is able to bring more mineral elements, could be useful for promoting plant growth and physiology. But, basic research on morphological, phytochemical and anatomical changes occurring in plants under organic manure application is scarce and needs further investigation.

Therefore, the objective of this article is to present the details of the morphological, phytochemical screening, and anatomical characters that will be used to show the rate of diversity among selected plants of *Amaranthus viridis* from selected regions among selected plants of *Amaranthus viridis* from selected regions.

MATERIAL AND METHODS

The fresh stems were taken from three different experimental regions 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 micrometry and phytochemical screening of *Amaranthus viridis* with different levels of FYM (5, 10 and 15 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 the 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 and 15 t/ha), and humic acid (10, 20 and 30 Kg/ha), which were randomly assigned to 1 x 1 m^2 sub plots with 25 rows 25 cm apart. Proper irrigation was provided to maintain the normal growth of the crop. Stem samples were collected 30 days after the sowing of the seed. Appropriate growing practises 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.

Data collection, plant material and sowing

Seeds were collected from different locations (Matta, Peochar, Batkhela, Thana, Peshawar, Tarnab, Haripur, Sarikot and Shangla) in Khyber Pakhtunkhwa province in August-September 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 (25ºC) until used in the experiments during the Phase I growing season, which runs from March to June 2020. Seeds 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 in the regions of Peochar and Matta. The collected seed was 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 to April, 2021. Data was collected 30 days after the seeding (DAS) of seeds. In each replication, data for stem height (cm), root length (cm), and leaf area per plant-1 (cm2) were collected on three randomly selected plants.

Phytochemical Screening

The micro chemical screening tests of *A. viridis* stem were determined to include saponins, flavonoids, phenolic, condensed tannins, hydrolysable tannins, mucilage, lignin, and cellulose. Qualitative standard operating procedures were followed (Baskaran et al., 2015; Sarker and Oba, 2019; Velioglu et al., 1998).

Micrometry

The method of micrometry was followed by Oluwasemire and Alabi (2004). In micrometry, objects are measured under a microscope. The magnification power of the microscope was 40. 10 was the power of the ocular lens and 4 was the power of the objective lens. The object to be measured was calibrated contrary to scales. The following sizes are measured for stems in control and experimental plants. Endodermis Length, Epidermis Width, Cortex Length, Cortex Width, Pericycle Length, Pericycle Width, Xylem Length, Xylem Width, Phloem Length, Phloem Width, Pith Length, Pith Width

Statistical analysis

Plant height and leaf area data analyzed by Statistix 8.1, while anatomical data are included for comparison purpose for comparison purpose.

RESULTS AND DISCUSSION

The data regarding plant height, leaf area showed **(**Fig. 1, 2**)** that interaction (manures and locations) remained highly statistically significant ($p \leq 0.05$) with the increased doses of farmyard manure when compared to that of humic acid in selected regions. This possibly explains why organic amendments promoted better growth of *A. viridis* than in the control. The finding also agrees with Bisikwa, et al., (2020), who reported that higher levels of Fym application produced significantly taller and broader Amaranthus plants. The plant height, leaf area showed an increasing trend in selected regions (Haripur, followed by Peshawar and Swat) as plants were treated with FYM at a dose of 15 t/ha, which showed maximum plant height (63 cm) in the Haripur location, followed by Peshawar (60.47 cm) and (56.50 cm) in the Swat location. Similarly, leaf area (cm2) was also affected as it increased the levels of FYM from 5t/ha up to 15t/ha. The maximum leaf area (35.57) was noted in the Haripur location, followed by Peshawar (33.50) and Swat (31.47) plots which received FYM of 15 t/ha. In the case of humic acid, the maximum plant height (40.87, 38.40, 36.63) and leaf area (23.33, 21.43, 18.30) were documented in those plants that received humic acid (30kg/ha) in Haripur, followed by the Peshawar and Swat regions. Kashaniet al., (2013) investigated that application of organic manures has more photosynthetic resulted in higher plant height, stem girth, leaf area index and chlorophyll content.

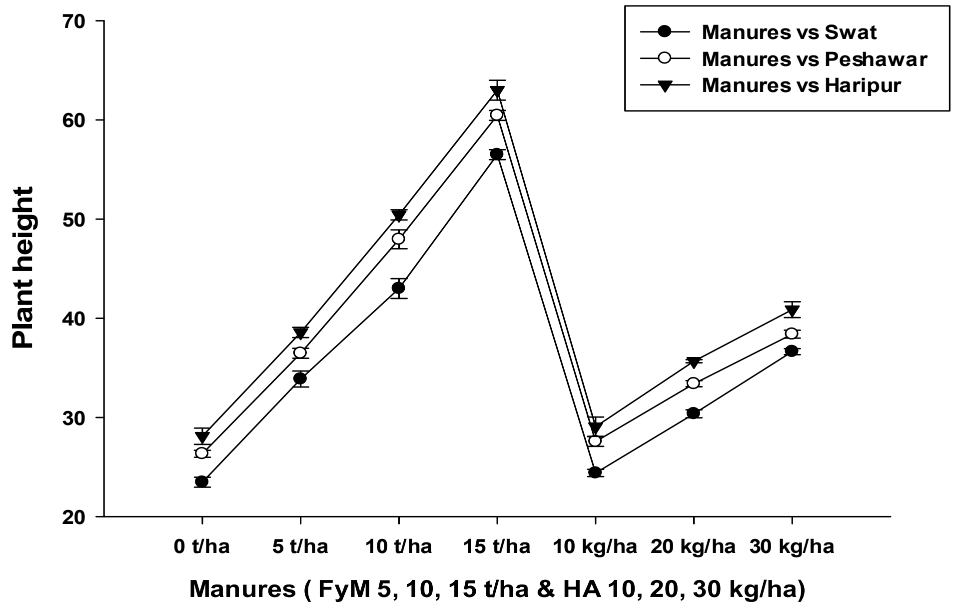

Fig 1. Plant height (cm) of *A. viridis* as affected by manures (FYM and HA) and locations

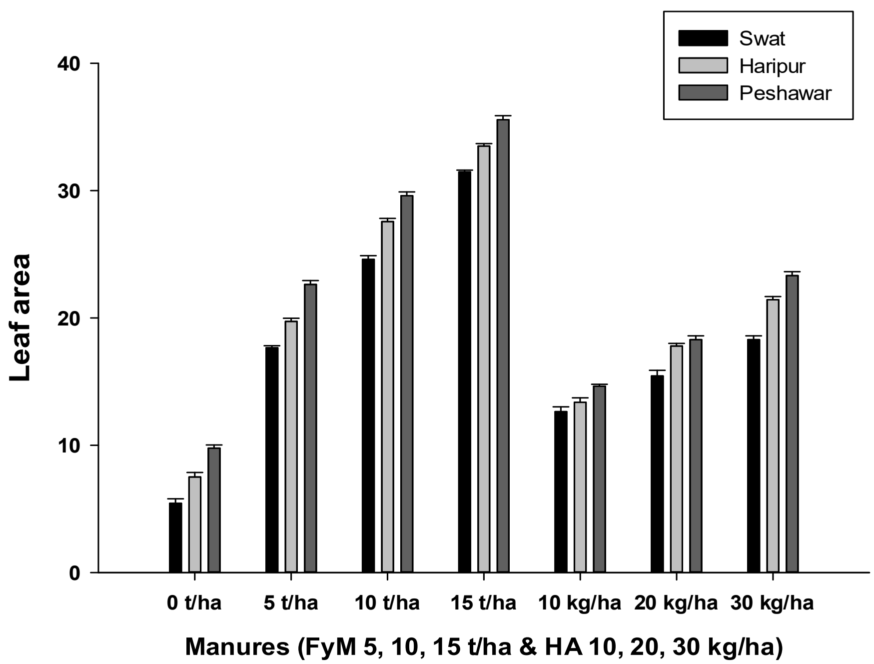

Fig 2. Leaf area (cm2) of A. viridis as affected by manures (FYM and HA) and locations

Stem

Farmyard manure and humic acid treatments resulted in greater changes in stem microscopy in three districts (Swat, Peshawar, and Haripur) when compared to the same factors in the control. The comparative effect of FYM applications and humic acid on the stem anatomy of *A. viridis* (Table 1). The overall mean factors increased with FYM levels from 5 to 15 t/ha. FYM (15 t/ha) had a positive effect on epidermis cell length (32.23 m), stem epidermis width (18.13 m), cortex length (17.33 m), cortex width (10.51 m), pericycle length (22.87 m), pericycle width (18.10 m), Xylem length (35.12 m), and Xylem width (20.84 m) when compared to humic acid plots. On the other hand, overall parameters also showed an increasing trend when plants were treated with Humic acid at 30 kg/ha i.e epidermis length **(**26.23 µm), epidermis width (15.17 µm), cortex length (14.30 µm), cortex width (8.56 µm), pericycle length (18.20 µm), pericycle width (15.16 µm), xylem length (30.12 µm), xylem width (16.18 µm) as compared to those of received 5 t/ha FYM). This might be due to farmyard manures containing micro and macro nutrients that result in the division and expansion of stem cells. This might be due to manures containing both macro and micro nutrients when plants receive the manures as a result of division and expansion of stem cell. The present results also agree with Nagel et al., 2001.

Manures	Epidermis Length (µm)	Epidermis width (µm) Stem	Cortex Length (µm)	Cortex width (µm)	Pericycle Length (µm)	Pericycle width (µm)	Xylem Length (µm)	Xylem width (µm)
Control	14.22 ± 0.89g	7.13 ± 0.38g	10.27 ± 0.30g	4.53 ± 0.15g	12.16 ± 0.50g	9.18 ± 0.48g	23.13 ± 0.43g	10.18 ± 0.48g
FYM $_{5tone}$	24.29 ± 0.93d	14.14 ± 0.34d	13.33 ± 0.29d	8.51 ± 0.17d	17.22 ± 0.44d	14.18 ± 0.48d	29.12 ± 0.44d	15.18 ± 0.48d
FYM $_{10tone}$	28.29 ± 0.86b	16.20 ± 0.32b	15.21 ± 0.27b	9.52 ± 0.15b	19.87 ± 0.79b	16.19 ± 0.48b	31.80 ± 0.75b	17.86 ± 0.80b
FYM $_{15tone}$	32.23 ± 0.89a	18.13 ± 0.37a	17.33 ± 0.29a	10.51 ± 0.17a	22.87 ± 0.79a	18.10 ± 0.43a	35.12 ± 1.0a	20.84 ± 0.81a
HA $_{10kg}$	18.30 ± 0.89f	11.17 ± 0.35f	11.21 ± 0.27f	6.56 ± 0.14f	14.20 ± 0.46f	11.13 ± 0.51f	25.13 ± 0.43f	12.19 ± 0.47f
HA $_{15t\ kg}$	22.24 ± 0.95e	13.13 ± 0.37e	12.27 ± 0.26e	7.49 ± 0.17e	16.20 ± 0.46e	13.18 ± 0.48e	27.12 ± 0.44e	14.18 ± 0.48e
HA $_{30\ kg}$	26.23 ± 0.91c	15.17 ± 0.35c	14.30 ± 0.32c	8.56 ± 0.14c	18.20 ± 0.46c	15.16 ± 0.50c	30.12 ± 0.44c	16.18 ± 0.48c
LSD at P≤ 0.0000	***0.0487***	***0.0197***	***0.0156***	***0.0368***	***0.0429***	***0.1119***	***0.0399***	***0.0156***

Locations

Swat	22.00 ± 2.31[a]	12.90 ± 1.37a	12.89 ± 0.92a	7.69 ± 0.74a	16.15 ± 1.26a	12.92 ± 1.15b	27.71 ± 1.41a	14.10 ± 1.24a
Peshawar	24.00 ± 2.29[b]	13.75 ± 1.37b	13.50 ± 0.91b	7.95 ± 0.75b	17.64 ± 1.39b	14.23 ± 1.15a	29.11 ± 1.55b	15.64 ± 1.39b
Haripur	25.07 ± 2.30[c]	14.10 ± 1.35c	13.87 ± 0.92c	8.23 ± 0.75c	17.94 ± 1.39c	14.47 ± 1.13a	29.56 ± 1.65c	15.94 ± 1.39c
LSD at P≤ 0.0001	***0.4805***	***0.2718***	***0.2483***	***0.2338***	***0.2689***	***0.3200***	***0.3434***	***0.2657***

Table 1. Stem anatomy of *A. viridis* as affected by manures (FYM and HA) and locations in Khyber Pakhtunkhwa

Table 2 shows the effect of farmyard manure and humic acid on stem microscopy in different agro-ecological zones (Swat, Peshawar, and Haripur). The mean cell numbers significantly increased as the levels of both FYM and HA increased when compared with control treatments. Phloem length and width were significantly higher (22.14 µm, 15.17 µm) in plots treated with Fym (15 t/ha) than in those plots treated with Fym (5 and10 t/ha). Humic acid also gave higher phloem length and phloem width (19.19 µm, 13.19 µm) when plants were treated with 30 kg/ha compared to those plots that received FYM 5 t/ha. The same trend was observed when plants were treated with 15 t/ha, i.e pith length (19.79 µm), Pith width (16.08 µm), endodermis length (19.36 µm), and endodermis width (16.56 µm). Pith length (15.13 m), pith width (13.13 m), endodermis length (14.13 m), and endodermis width (12.51 m) were greater in plots treated with 30 kg/ha than in plots treated with 5 t/ha FYM, i.e., pith length (14.12 m), pith width (12.12 m), endodermis length (13.14 m), and endodermis width This could be because plants received manures containing both macro-and micronutrients, which stimulated cell division and expansion. The agreements also agree with Matthews et al., 2010.

In the case of ecological alterations, all means of stem anatomy also express high plasticity and can easily adapt to diverse agrological situations. Overall, the highest cell number means were found in Haripur, followed by Peshawar, and the lowest cell number means were found in Swat (Tables 1-2). The findings are also consistent with Ozimede et al.'s (2019) findings from an anatomical diversity study on three Amaranthus species from Rivers State, namely A. *hybridus L.*, *A. viridis L.,* and *A. spinosus L.*

Table 2. Stem anatomy of *A. viridis* as affected by manures (FYM and HA) and locations in Khyber Pakhtunkhwa, Pakistan

Manures	Phloem length (µm)	Phloem width (µm)	Pith length (µm)	Pith width (µm)	Endodermis length (µm)	Endodermis width (µm)
Control	14.20 ± 0.46g	8.19 ± 0.47g	10.14 ± 0.43g	7.80 ± 0.78g	9.13 ± 0.38g	7.18 ± 0.48g
FYM 5tone	17.84 ± 0.81d	12.19 ± 0.47d	14.12 ± 0.42d	12.12 ± 0.44d	13.14 ± 0.38d	11.52 ± 0.16d
FYM 10tone	20.18 ± 0.44b	13.59 ± 0.14b	17.16 ± 0.42b	14.14 ± 0.44b	15.99 ± 0.48b	13.84 ± 0.48b
FYM 15tone	22.14 ± 0.46a	15.17 ± 0.46a	19.79 ± 0.74a	16.08 ± 0.47a	19.36 ± 0.82a	16.56 ± 1.08a
HA 10kg	15.19 ± 0.47f	9.18 ± 0.47f	11.13 ± 0.42f	9.11 ± 0.47f	10.48 ± 0.15f	9.19 ± 0.48f
HA 15tone	17.22 ± 0.49e	11.21 ± 0.45e	13.14 ± 0.42e	11.06 ± 0.49e	12.13 ± 0.38e	10.51 ± 0.16e
HA 30tone	19.19 ± 0.47c	13.19 ± 0.47c	15.13 ± 0.42c	13.13 ± 0.47c	14.13 ± 0.38c	12.51 ± 0.16c
LSD at P≤ 0.0000	***0.0461***	***0.0434***	***0.0453***	***0.1065***	***0.1230***	***0.0771***
Locations						
Swat	16.98 ± 0.99b	11.00 ± 0.97c	13.49 ± 1.20c	10.93 ± 1.15c	12.72 ± 1.20c	10.83 ± 1.08c
Peshawar	18.37 ± 0.99a	12.09 ± 0.93b	14.57 ± 1.30b	12.21 ± 1.06b	13.55 ± 1.28b	11.81 ± 1.22b
Haripur	18.64 ± 0.98a	12.36 ± 0.92a	15.07 ± 1.30a	12.62 ± 1.05a	14.18 ± 1.42a	12.20 ± 1.23a
LSD at P≤ 0.0001	***0.3009***	***0.1579***	***0.3838***	***0.2741***	***0.4704***	***0.3310***

Phytochemical analysis

A. viridis plants were treated with different levels of FYM and humic acid in different regions (Matta, Peshawar, Haripur). The phytochemical components of stem and root extract were recorded after micro chemical screening tests such that Flavonoids, Saponins, Phenolic, Hydrolysable tannins, Condensed tannins, Mucilage, Lignin Cellulose are given (**Table 3**).

Table 3. Phytochemical screening of *A. viridis* as affected by manures (FYM and HA) and locations in Khyber Pakhtunkhwa, Pakistan.

Manures	Phenolic	Flavonoids	Saponins	Hydrolysable tannins	Condensed tannins	Mucilage	Lignin	Cellulose
Control	+	+	+	+	+	+	+	+
FYM $_{5tone}$	+	+	+	+	+	+	+	+
FYM $_{10ton}$	+	+	+	+	+	+	+	+
FYM $_{15ton}$	+	+	+	+	+	+	+	+
HA $_{10kg}$	+	+	+	+	+	+	+	+
HA $_{15tone}$	+	+	+	+	+	+	+	+
HA $_{30tone}$	+	+	+	+	+	+	+	+
Locations								
Swat	+	+	+	+	+	+	+	+
Peshawar	+	+	+	+	+	+	+	+
Haripur	+	+	+	+	+	+	+	+

CONCLUSION

The study indicated that manures (FYM x Humic acid) and locations affected overall parameters of *Amaranthus viridis*. When the effect of manures (FYM x Humic acid) and locations on plant height, leaf area, micrometry of stem and phytochemical screening were evaluated. The FYM showed best result than Humic acid. In the case of locations, the overall parameters showed best results under Haripur ecological conditions followed by Peshawar and Swat. Based on the results, FYM (15t/ha) was found beneficial in ecological conditions Haripur, Peshawar and Swat and can be recommended for *Amaranthus viridis* cultivations.

AUTHOR CONTRIBUTIONS

Shah Masaud khan supervised the present Ph.D. works. The remaining authors analyzed the data and help me review the manuscript.

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