COMPARATIVE STUDY OF SECONDARY THICKENING IN AMARANTHACEAEN FAMILY MEMBERS

Project submitted

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Submitted by

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CERTIFICATE

This is to certify that this project work entitled **"Comparative Study of Secondary Thickening in Amaranthaceaen Family Members"** is a bonafide piece of project work done AISWARYA PRAKASH **(**Reg.no**:** 210021022642**)** & CILENA XAVIER (210021022648**)** in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

Place : Thrikkakara

Date :

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DECLARATION

I hereby declare that this project entitled **"Comparative study of Secondary Thickening in Amaranthaceae Family Members"** is the result of work carried out by me under the guidance of Dr. Lins Simon, Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

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CONTENTS

Chapter 1

INTRODUCTION

The Amaranthaceae family is often known as 'Amaranth family'. It is a well-known broad family consisting of annual or perennial herbs just few are trees, vine or shrubs (Blunden et al., 1999). Species belong to this family are primarily found worldwide especially in the tropic and sub–tropical regions. It consists of several varieties that are cultivated as ornamentals, vegetable or for grains (Flora of America, 2015), among which, some are weeds (Brenner et al., 2000). Amaranthus, collectively recognized as amaranth, which is a diverse genus including annual or short-cycled crops. Most of the Amaranthus species are annual weeds.

Generally, this Amaranthaceae family is found in moist to humid areas below 1400 m elevation with a few species reaching 2700 m. The family is represented in the Neotropics by ca. 490 species of which 25 are consistently reported as climbers that reach two or more m in length. Iresine, contain several species that are scandent herbs Chamissoa and Pleuropetalum a species each that are scandent shrubs; Gomphrena, Hebanthe, and Pedersenia have species that are twining lianas and sometimes scandent shrubs with elongated stems (Pedro Acevedo-Rodriguez 2018). In the absence of fertile material, climbing Amaranthaceae are sometimes confused with Asteraceae or Acanthaceae. However, woody Amaranthaceae, i.e., scandent shrubs and twining lianas, are easily identified by the cylindrical stems with successive rings of xylem and phloem and by the presence of swollen nodes. Anomalous secondary growth wood of the perennial stem, normal secondary growth is observed only in the sub-family Poly–cnemoideae leaves can be alternate, opposite, simple or entire, sometimes monoecious while at times dioecious (Stevens, 2001). The leaves are opposite or alternate depending on the genus, with entire margins, gland-less blades and petioles, and lack stipules. Fruits are circumscissile utricles.

The genus Amaranthus L. (Amaranthaceae Juss.) consists about 65–80 generas and more than 850 species in which inflorescences stretching from red, purple, through green and red to gold. However, due to the few studies on Amaranthus systematics the number still tentative. Also, the nomenclature of Amaranthus is critical since hundred names were published during the centuries and the nomenclatural disorder/misapplication of names create intricate problems in its taxonomy (Costea et al., 2001; lamonico,2014a, 2014b, 2016a, 2016b; Nestor, 2015). Furthermore, some Amaranthus species are aggressive annual weeds in the world, causing impacts on the natural floras and vegetation and on cultivated field Amaranthus presence can lower grain yield and quality as well as hinder mechanical harvest (Wax 1995). Some of this species also have been shown to possess allelopathic chemicals that reduce seedling vigor of several crops and weed species (Menges 1987,1988), while others may induce toxicosis and death in dairy cattle (Kerr and Keleh, 1998). The grain Amaranths are ancient crops with increasing prospects as potential food and feed resources because of their high grain protein and starch quality and high-nitrogen, highly digestible vegetative tissues (Cai et al., 1998a, b).

Some Amaranthus species have medicinal importance as *A. spinosus(Pal et al., 2013).* Plants from the Amaranthaceae are used in folk medicine for their nutritional qualities and for the treatment of various disorders such as gastrointestinal and respiratory problems, skin infections as well as some infectious diseases and as an abortifacient (Okeke, Ilodibia and Okoli 2019).

1.1 OVERVIEW OF AMARANTHACEAE FAMILY

General characters

1. **STEMS-** Amaranthaceae members have herbaceous stems usually are 5 mm or less in diameter, and up to 3 m in length, woody species are 1 to 3 cm in diameter and up to 15 m in length. Mostly cylindrical stem, herbaceous in Iresine or woody in Chamissoa, Gomphrena, Hebanthe, Pedersen ia, and Pleuropetalum. Stem have a large medulla, and successive rings of xylem and phloem are known to occur in woody taxa. Barks are rough, light colored, and with numerous rounds, light-colored lenticels. Taxa with opposite leaves usually have swollen nodes.
2. **LEAVES-** Leaves are mostly simple, exstipulate, alternate or opposite with entire crenate margins. Petioles are short to long, among which some species

have sessile leaves, and have no extrafloral nectaries or gland on leaves and blade.

3. **INFLORESCENCES-** Inflorescences usually dense head, loose, or spike, raceme or panicle, Cymose, bracteates, bracts white or coloured with one or more flowers.
4. **FLOWERS-** Flowers are Actinomorphic, hermaphrodite or bisexual, 3–5-merous, usually < 5 mm long. Tepals are light green, cream, or whitish which is subequal or the inner ones shorter, distinct or less often partly connate at base with long, straight or wooly trichomes at base. Stamens are many in number and opposing the tepals. filaments connate together to form a short cup at base, which is sometimes alternating with staminal appendages. Female part *ie* Gynoecium-superior, syncarpous, 2 or 3 carpellate, the style elongated or absent, the stigmas bilabiate, capitate or 2–3-branched; placentation basal, ovules solitary or several per carpel.
5. **FRUITS-**Small (hardly longer than the flower), of one to several-seeded circumscissile utricles.
6. **SEEDS-**Seeds lenticular, reniform or sub-rounded, exarillate, black and shiny or arillate in Chamissoa.
7. **CLIMBING MECHANISM-** There are leaning herbs Eg:Iresine, scandent shrubs ,Eg:Alternanthera, Chamissoa, Gomphrena, species of Pedersenia, and Pleuropetalum, or twining lianas Eg:species of Gomphrena, Hebanthe and Pedersenia.
8. **EXUDATES-**Exudates that are produced from Amaranthaceae family members are odorless and colorless.

1.2 TAXONOMIC CLASSIFICATION

Kingdom: Plantae

Class : Dicotyledons

Sub class : Monochlamydeae

Series : Curvembryeae

Order : Caryophyllales

Family : Amaranthaceae

1.3 DIVERSITY

The Amaranthaceae family indeed exhibits a wide distribution, ranging from tropical to cool temperate regions. While predominantly tropical, some members, like those formerly classified under Chenopodiaceae, thrive in dry temperate and warm temperate areas. Many species within the family are adapted to challenging environments such as salty soils or dry steppes, with some being halophytes. Vegetable amaranths, for instance, flourish in hot, humid regions like Africa, Southeast Asia, Southern China, and India. Although only a few genera are found in temperate regions, Amaranthus is a prominent one. The family encompasses about 175 genera and over 2500 species, primarily consisting of herbs and subshrubs, and is evenly distributed worldwide, with centers of diversity in Central and South America, tropical and South Africa, and Australia.

1.4 IMPORTANCE OF AMARANTHACEAE FAMILY

It seems like the Amaranthaceae family has a diverse range of uses, including food, medicinal, dye, and ornamental purposes. While it may not be as economically significant as some other plant families, it still plays a valuable role in various cultural practices and traditional uses around the world.

FOOD: Seeds of *Amaranthus caudatus* are edible. *Amaranthus cruentus* and *A. frumentacea* are raised as cereals by primitive tribes in Tropical Asia. The leaves of *Amaranthus viridis*, *A. spinosus* and *A. tricolor* are also used as vegetables.

MEDICINAL: Achyranthes aspera is diuretic and purgative. Decoction of *Aerua tomentosa* is used to remove swellings. The stem and leaves of Alternanthera are used in snake-bite. The flowers and seeds of Digera muricata (syn. D. arvensis) are given for urinary discharges.

<u>DYE</u>: Leaves of *Bosia amherstiana* yield a black dye. The fruit juice of Deeringia is a substitute for red ink.

<u>WEEDS</u>: Some genera are weeds e.g. Amaranthus, Celosia, Digera, Achyranthes, Gomphrena etc.

<u>ORNAMENTALS</u>: Celosia cristata (Cockscomb), *Gomphrena globosa* (Globe amaranthus) are cultivated in gardens

plant selected for study:-

1. Amaranthus spinosus

2. Amaranthus cruentus

3. Amaranthus viridis

1.5 ANATOMICAL STUDY IN PLANTS

MORPHOLOGY OF THE STEM- The stem characteristics of amaranths can vary significantly based on environmental factors. Factors such as light, temperature, water availability, and soil nutrients contribute to this variability. This flexibility allows amaranths to adapt their stem morphology to different growing conditions, resulting in differences in length, diameter, orientation, branching pattern, and even color.

THE STEM EPIDERMIS -The stem epidermis of this genus exhibits varying cell arrangements, with narrow zones containing stomata and wider zones lacking stomata. Stomatiferous zones feature isodiametric cells with chloroplasts and thin primary cell walls, typically surrounded by 2 to 8 neighboring epidermal cells, forming an anomocytic stomatal apparatus. These zones, ranging from 3 to 20 cells wide, may be sunken or not compared to areas without stomata. In contrast, zones lacking stomata have heterodiametric cells with thickened primary cell walls and lack chloroplasts. Trichomes are found exclusively in non-stomatiferous zones, resembling those on leaves, being multicellular and uniseriate or occasionally mixed multiseriate and papillate. Uniseriate trichomes often have a multicellular base, with the terminal cell swollen and larger than others. The main stem of Amaranthus displays numerous primary and secondary vascular bundles, with varying numbers along its length. In transverse section, the primary bundles are arranged in 2 or 3 concentric rings, while secondary bundles are discussed separately. The outer ring comprises minor axial and branch trace bundles, while the innermost ring consists of major axial anastomotic and leaf trace bundles. Each set of leaf traces, representing the leaf vascular supply, is bordered by two major axial anastomotic bundles on either side.

The eustele of seed plants comprises primary vascular tissue organized into continuous axial bundles, producing leaf trace complexes sequentially from these bundles. Each leaf trace originates from axial bundles, with the same principle applying to branch traces in early developmental stages. In dicotyledons, shoot vascular systems are classified as open or closed. Open systems have separate sympodia that do not anastomose, while closed systems feature regular connections between sympodia. Although interconnected, sympodia in closed systems align parallel to the stem axis, following phyllotactic spirals. According to Gibson (1994), the primary vascular system in amaranths is of the closed type, forming a network of anastomosing bundles throughout the stem (Dermer 1945; Beck et al 1982).

The count of primary bundles at a specific stem level is influenced by factors such as phyllotaxy, leaf vascular organization, axial bundle count, presence of branch traces, and the number of internodes traversed by both leaf and branch traces before exiting the stem. Phyllotaxy directly correlates with the arrangement of the primary vascular system, as the number of sympodia at a stem level matches the number of leafs orthistichies at that particular position.

1.6 Significance of the study

Comparative anatomical studies on three members of the Amaranthaceae family carry significant implications across various fields. Firstly, they contribute to taxonomy and phylogeny by unraveling the evolutionary relationships among different species through comparisons of anatomical features such as leaf morphology and vascular tissue arrangement. Secondly, these studies shed light on how plants within this family have adapted to diverse environmental conditions, aiding in conservation and agricultural practices, especially in the context of climate change. Additionally, anatomical characteristics serve as crucial diagnostic features for species identification and classification, facilitating accurate taxonomic delineation. Furthermore, insights gained from such studies can elucidate the ecological roles of different species within ecosystems, providing valuable information for understanding ecosystem dynamics. Lastly, the identification of bioactive compounds and medicinal properties within certain species of the Amaranthaceae family holds promise for biomedical and pharmaceutical applications. Overall, comparative anatomical studies offer a comprehensive approach to understanding plant diversity, evolution, adaptation, and ecological interactions, with broad implications for both basic and applied research.

1.7 Objectives

- Compare the anatomical features of leaves and stems among three selected species of the Amaranthaceae family
- Elucidate the potential adaptations of these species to different environmental conditions through anatomical analysis
- Contribute to the taxonomic understanding of the Amaranthaceae family by identifying key anatomical traits for species differentiation and classification

Chapter – 2

REVIEW OF LITERATURE

Ozimede et al., (2019) explains and delves into the morphological and anatomical characteristics of three Amaranthus species – *A. hybridus L., A. viridis L., and A. spinosus L*. Transection analysis of the blades uncovered features such as a uniseriate epidermis, angular collenchyma within the ground tissue, and slender parenchyma. Notably, vascular bundle shapes consistently exhibited a crescent pattern.

In addition, the study found three distinct patterns in the cross-sectional shape of the midrib: arc, rounded, and crescent bundles. Furthermore, the examination of petioles revealed novel characteristics specific to *A. viridis*, including variations in petiole shape, vascular bundle shape, number, and arrangement. However, observations of stem structure showed comparatively less variation. Notably, differences were observed in the number of vascular bundles in the midrib among different populations of Amaranthus hybridus, with the Rivers East population having 9 bundles, Rivers West 6 bundles, and Rivers South East 4 bundles. In the case of *A.viridis*, both Rivers East and Rivers South East populations exhibited six vascular bundles, while the Rivers West population had 5. Regarding *A. spinosus*, the Rivers East, Rivers West, and Rivers South East populations had 5, 4 and 6vascular bundles, respectively. Interestingly, only *A. hybridus* and *A. spinosus* from the Rivers South East population, with 4 and 6 vascular bundles, respectively, align with the findings of El-Ghamery et al. (2016). Additionally, the results obtained from the petiole anatomy of *A. spinosus* and *A. hybridus* are consistent with those reported by El-Ghamery et al. (2016).

Interestingly, while no study specifically focusing on *A. viridis* was found, trichomes were reported to be absent in all species studied except for Amaranthus hybridus from the Rivers East population, where they were present. Additionally, protuberances were observed in all nine species studied, indicating a shared characteristic across the examined Amaranthus species.

Manik et al., (2013) demonstrates that the stem of *Amaranthus spinosus* Linn are terete or obtusely angular, glabrous or slightly pubescent, green. The leaves alternate and are simple without stipules; petiole is approximately as long as the leaf blade. The epidermis was an outermost layer of barrel to rectangular cells. The cells were thickly cuticularized. A few stomata occurred in the epidermis and a few unicellular or multicultural hairs were also present. The cortex was multi-layered and differentiated into collenchyma and parenchyma. A few layered collenchymatous hypodermises follows epidermis. It was 3-5 layered deep. Parenchyma followed collenchymatous hypodermis and was few cells deep. The cells were spherical to oval. The cells might contain a few to many chloroplasts. In endodermis, a distinct endodermis with Casparian strip was present. A prominent starch sheath was present in its place. Pericycle was represented by a few sclerenchymatous cells in the old stem. A large zone of vascular tissue lied just below the starch sheath. Starch sheath was followed by a large amount of conjunctive tissue in which secondary vascular bundles were embedded. Secondary phloem was situated just below the starch sheath. It was found in small groups. Two- layered ring of cambium separated secondary phloem from secondary xylem. Secondary xylem of secondary vascular bundle lied below the cambium. This secondary xylem was embedded in conjunctive tissue that appeared as a complete ring below the primary 6 vascular bundles were called medullary bundles. The central part of the section had large parenchymatous pith. Cambial activity took place in these medullary bundles. Hence, a little amount of 7 secondary phloem and secondary xylem were also present.

Ekeke et al., (2019) describes about **t**he comparative morphological, anatomical, and phytochemical studies conducted on the two species of the genus Amaranthus L. (*A. hybridus* and *A. spinosus*) aimed to discern their distinctions, facilitating easier identification and identifying potential sources of raw materials for pharmaceutical use.

The presence of spines on *A. spinosus* sets it apart from *A. hybridus*, while the reddish-brown stem of *A. spinosus* contrasts with the light green stem of *A. hybridus*. Differences in the number of vascular bundles in the midrib, petiole, stem, and root offer additional means of differentiation. Quantitative phytochemical analysis revealed varying concentrations of constituents between the two species. *A. spinosus* typically exhibits a narrow cambial ring, whereas A. hybridus presents a more pronounced cambial ring. Additionally, the stem of *A. spinosus* typically contains 52 vascular bundles, while A. hybridus generally has fewer than 52. Moreover, the root of *A. spinosus* typically contains 16 vascular bundles, whereas A. hybridus tends to have 18 vascular bundles.

Vrbnicanin et al., (2009) explained about the comparative analysis of the anatomy of two populations of Red-Root Amaranth (*Amaranthus retroflexus* L.) stems, within the family Amaranthaceae, offers valuable insights into their structural characteristics. Stem anatomy, particularly focusing on the secondary xylem, was examined by Rajput (2002) across 70 species within 9 genera of the Amaranthaceae family. Meanwhile, Costea and DeMason (2001) delved into the taxonomic significance of stem morphology and anatomy specifically within the genus Amaranthus.

It's noted that secondary thickening of the stem in woody representatives of Amaranthaceae is unusual, distinguishing their secondary stem anatomy from many other dicotyledonous species. These studies likely shed light on the unique features and variations within the stem anatomy of Red-Root Amaranth and other members of the Amaranthaceae family. Representatives of the Amaranthaceae family exhibit intriguing characteristics related to the formation of successive cambium and its resulting products. In the case of the weed species *A. retroflexus*, its stem cross-section reveals a typical anatomy found in herbaceous dicots, comprising three distinct zones: the stem epidermis, cortex, and central cylinder.

The stem cortex of *A. retroflexus* Is composed of collenchyma and cortex parenchyma cells. The collenchyma layer, situated beneath the epidermis, consists of several cellular layers with thickened tangential walls. This structural arrangement likely plays a significant role in providing mechanical support to the stem, which is essential for the plant’s upright growth and overall stability.

Okeke et al., (2020) conducted comparative morphological study on *A. spinosus*, Celosia argentea, and Gomphrena celosioides aimed to delineate their taxonomic morphological characters within the family Amaranthaceae. Using hand lenses and visual observations of various plant parts, the study revealed several key similarities and differences among the species.

Results indicated similarities among the three species, including possession of simple leaves, small flowers, spike-like inflorescences, herbaceous nature with a woody base, pentamerous perianths and stamens, absence of stipules, and presence of pinkish pigment in some plant parts. However, they differed in certain characteristics such as the presence of spines in *A. spinosus* and the presence of trichomes in *Gomphrena celosioides*. These distinctions help delineate each species within the family Amaranthaceae, contributing to their taxonomic classification.

Abbas et al, (2016) provided anatomical features of mature leaves and stems of 12 Amaranthus taxa within the Family Amaranthaceae revealed significant variation among them, introducing new characters to aid in their differentiation. By evaluating internal structures, the research aimed to address taxonomic complexity and identification challenges within the genus. Observations of blade transections indicated several key findings like, epidermis was uniseriate, suggesting a single layer of cells, ground tissue comprised angular collenchyma and thin parenchyma. Collenchyma cells provide structural support due to their thickened cell walls, while parenchyma cells serve various metabolic functions. These anatomical features contribute valuable insights into the taxonomy and identification of Amaranthus species, shedding light on their structural diversity and aiding in their classification. The vascular bundles in the leaves of the studied Amaranthus taxa exhibit three main shapes: crescent, ring, and ovate. Additionally, these bundles may either be united or separated within the leaf structure. The midrib shape in cross-section displays two patterns: U-shaped, cordate, or crescent bundles.

All leaves in the taxa under examination are petiolate, meaning they have a stalk or petiole connecting the leaf blade to the stem. The examination of petioles revealed new and varied characters, including petiole shape in cross-section and characteristics of vascular bundles such as their shape, number, and arrangement.

Interestingly, the characters resulting from the observation of stem structure showed less variation compared to those observed in leaf anatomy. Overall, the study identified nineteen qualitative characters with 38-character states arising from leaf anatomy, providing valuable insights into the taxonomic diversity and identification of Amaranthus species.

Oksana et al, (2019) described that, four common species of amaranth (Amaranthus spp.): *A. cruentus L., A. caudatus L*., *A. hybridus L*., and *A. spinosus L*. These species are known for their wide variation in morphological and biological features, both in cultivated and wild environments. The research paid particular attention to observing the morphological and biological characteristics of these plants across different growth stages throughout the growing season. Additionally, the study investigated the yield of green mass and grain production over a three-year period, providing insights into the productivity and performance of these species under varying environmental conditions. By comprehensively analyzing the growth patterns and yield potential of these amaranth species, the research aims to contribute valuable information for agricultural practices and the cultivation of these plants for various purposes.

The experiment demonstrated the feasibility of cultivating heat-loving amaranth species in the climatic conditions of the Chuvash Republic. It identified adaptive species suitable for producing green mass (*A. cruentus* and *A. caudatus*) as well as for grain production (*A. cruentus*).The growth stages of amaranth from sowing to mature seeds were categorized as follows: seedlings, vegetative state, inflorescence formation, flowering, and seed maturation. Each growth stage was determined based on observation in 75% of the control plants, as outlined by Dmitrieva (2018c).

Amaranthus spp. Exhibit two extended growth stages: the vegetative state, lasting 35-45 days, and flowering, lasting 28-45 days. These findings provide valuable insights into the growth patterns and developmental stages of amaranth, essential for effective cultivation and management practices. The comparison of species during the vegetative state, when plants develop root systems and stems with leaves, revealed that *A. cruentus* completes this period 8-16 days faster than other species. This accelerated growth rate is a positive indicator, particularly in temperate climatic conditions.

Similarly, during flowering, *A. cruentus* exhibited an earlier blooming time, occurring 6-17 days ahead of other species. This early flowering pattern enabled the cultivation of *A. cruentus* for grain production in these conditions throughout the three-year experiment. These findings highlight the adaptability and suitability of *A. cruentus* for cultivation in the specified climatic conditions, emphasizing its potential as a reliable crop for grain production in the region.

The climatic conditions of the Chuvash Republic generally support the cultivation of all amaranth species for fresh green biomass. However, due to the early onset of frost, it is preferable to grow species with a shorter growing season, such as *A. cruentus* and *A. caudatus*. The main physiological indicator monitored in the experiment was the growth of plants, specifically changes in linear growth (height) and stem diameter of amaranth species. The species exhibited significant differences in height, leading to their classification into three groups: long-stemmed (*A. cruentus, A. caudatus*), medium-stemmed (*A. hybridus*), and short-stemmed (*A. spinosus*). This categorization provides valuable insights for agricultural planning, allowing growers to select species best suited to the local climate and growing conditions. By choosing species with appropriate growth characteristics, farmers can optimize yield and ensure successful cultivation of amaranth crops in the region.

Ekeke et al, (2019) explained two species of the genus Amaranthus, *A. hybridus* and *A. spinosus,* aimed to elucidate their morphological, phytochemical, and anatomical characteristics. Macro-morphological evaluation revealed that both species are monoecious erect herbs, typically reaching heights of 1-2 meters. They share major similarities in leaf arrangement, root colour, leaf type, and leaf shape. Additionally, both species have glaborous leaves, contributing to their smooth texture. However, a significant distinguishing feature between the two species lies in the presence of spines. *A. spinosus* exhibits spines on the stem at the base of the petiole, whereas A. hybridus lacks these spines. This morphological difference serves as a key characteristic for differentiation between the two species. The further investigation into phytochemical and anatomical characteristics of Amaranthus species aligns with previous morphological studies, enhancing our understanding of these plants. Within the Amaranthaceae family, most members are amphistomatic, possessing various types of stomata. For example, *A. brasiliensis* exhibits anomocytic and diacytic stomata, while *A. spinosus* and A. hybridus have anisocytic, paracytic, and anomocytic stomata.

In terms of epidermal characteristics, both *A. hybridus* and *A. spinosus* are amphistomatic and share similar epidermal cell shape and wall patterns. However, differences were observed on the adaxial surface of A. hybridus, which had anisocytic, tetracytic, and contiguous stomata, while *A. spinosus* exhibited anisocytic and isotricytic stomata. On the abaxial surfaces of both species, anisocytic and tetracytic stomata with irregular shape and undulating walls were observed.These findings contribute to our understanding of the stomatal characteristics within the Amaranthaceae family, although there may be discrepancies in reports regarding the anticlinal cell wall pattern. Overall, the comprehensive investigation of phytochemical and anatomical features provides valuable insights into the differences and similarities among Amaranthus species.

Chapter – 3

Materials and Methods

3.1 Collection of plant material

For undergoing an investigation on the comparative analysis of leaf and stem anatomy of Amaranthacean members, three well known species of Amaranthus were selected. Specimens collected were *Amaranthus spinosus* L. (spine amaranthus), *Amaranthus cruentus* (red amaranthus), and *Amaranthus viridis* L. (green amaranthus). All the materials were collected freshly and authenticated according to Gamble (1928). For ensuring their freshness and viability, they were preserved in fresh water and washed in 70% alcohol-water solution as per Dogan et al., (2008).

Fig. 3.1: Habit of *Amaranthacae viridis*

Fig. 3.2: Habit of *Amaranthacae spinosus*

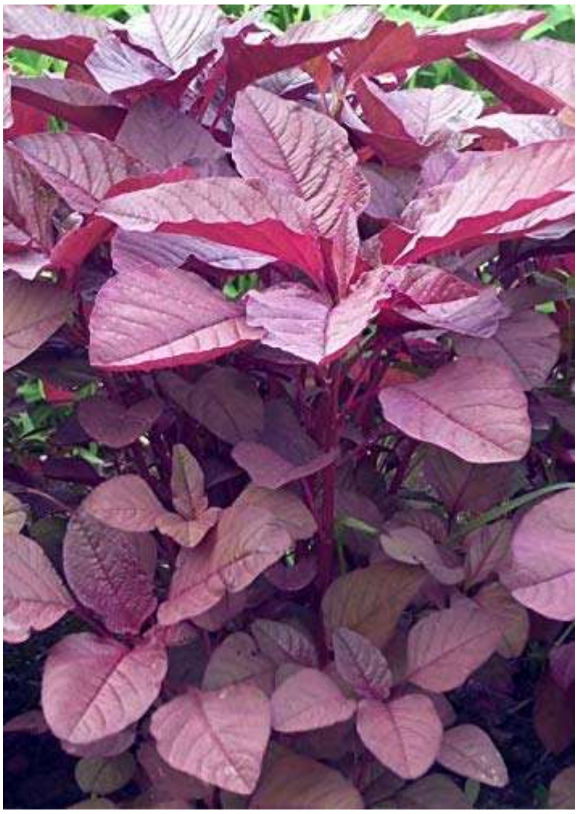

Fig. 3.3: Habit of *Amaranthacae cruentes*

3.2 Sectioning of the plant materials

The preserved specimens were taken to extract fine delicate cross sections of leaflets and stem in order to investigate their anatomical characters in laboratory conditions. For this sharp blade were used and fine, small, uniform sections were taken and best ones have been selected for further analysis. The selected sections were firstly stained with saffranin solution for easy visualization. Then it was washed to remove the excess stain and to prevent dehydration, the stained sections were mounted on a glass slide with glycerin and visualized in a microscope (Weswox Microscope with Achromat objective, Digital camera and visualization tablet PC). The clear images were taken using a camera apparatus attached to the microscope.

Fig. 3.4: Visualization of cross sections taken using Microscope apparatus with camera

Chapter- 4

RESULTS & DISCUSSION

When the anatomical observations among stem and leaflet of three Amaranthus species studied, there shows some significant variations between them. Anatomical study among the three species shows high homogeneity and similarity. However, there are some variations shown interspecifically. These anatomical characters are found to be useful and valuable taxonomically especially in delineating the three species, further aid the identification of species.

4.1 Anatomical study on leaflet and stem of *Amaranthus viridis*

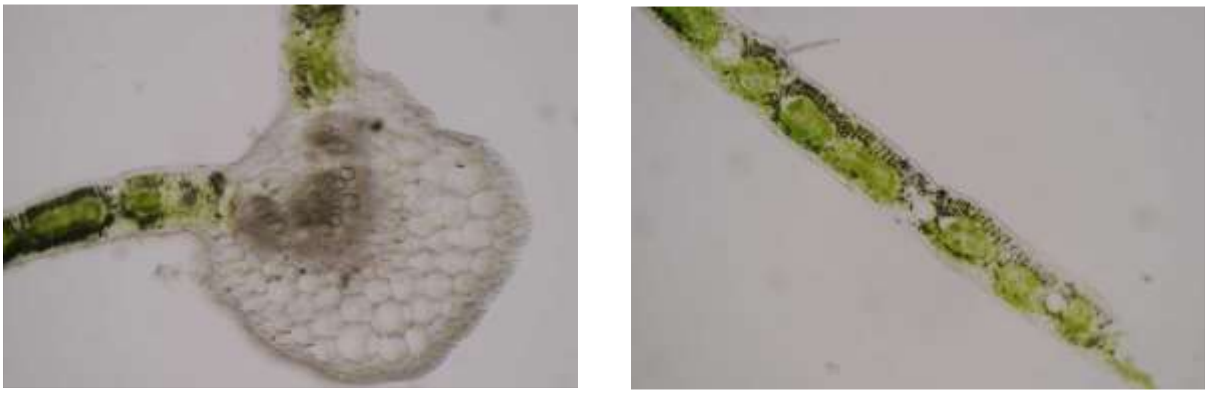

Fig. 4.1: Anatomical study on leaflets of *Amaranthus viridis*

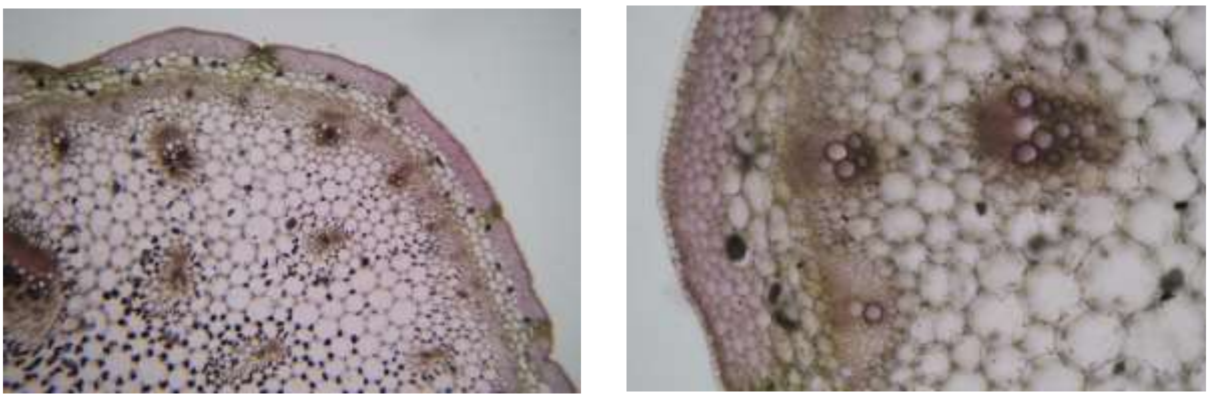

Fig. 4.2: Anatomical study on stem of *Amaranthus viridis*

The T.S. of *A. viridis* leaf shows single layered epidermis covered by cuticle. The midrib portion is characterised with thick-walled epidermis, while towards the arms, it becomes thin walled. The outer epidermis is represented with parenchyma cells with thick cuticular region with an irregular margin. Next to the epidermis, the hypodermis is composed of parenchymatous cells with oil ducts. Vascular bundles in the midrib region are arranged in a U-shaped manner. Towards the arm region of the leaf, the epidermal cells become thin and the hypodermis is designated with elongated palisade parenchyma cells. Inner to the parenchyma, prominent vascular bundles could be seen.

The transverse section of *A. viridis* stem shows a wavy outline structure. The outer region is made up of thin-walled cells which are arranged compactly to form a single layer. This is followed by 4-5 layer of isodiametric cells exhibiting wall thickening at corners. Beneath to that layer, it lies two types of cells, which are less stained and more stained cells. This conspicuous layer is followed by endodermis, which is very protective in nature. The central portion of the stem TS shows multiple number of vascular bundles, which are randomly arranged and it consists of primary xylem and phloem. The central region is also showing the presence of interfascicular cambium which implies the mechanism of secondary growth in the stem. Due to the abnormal position of the vascular cambium, the stem of *A. viridis* shows anomalous secondary growth and the TS resembles the secondary stem structure of *Boerhaavia diffusa*.

4.2 Anatomical study on leaflet and stem of *Amaranthus cruentus*

Fig. 4.3: Anatomical study on leaflets of *Amaranthus creuntus*

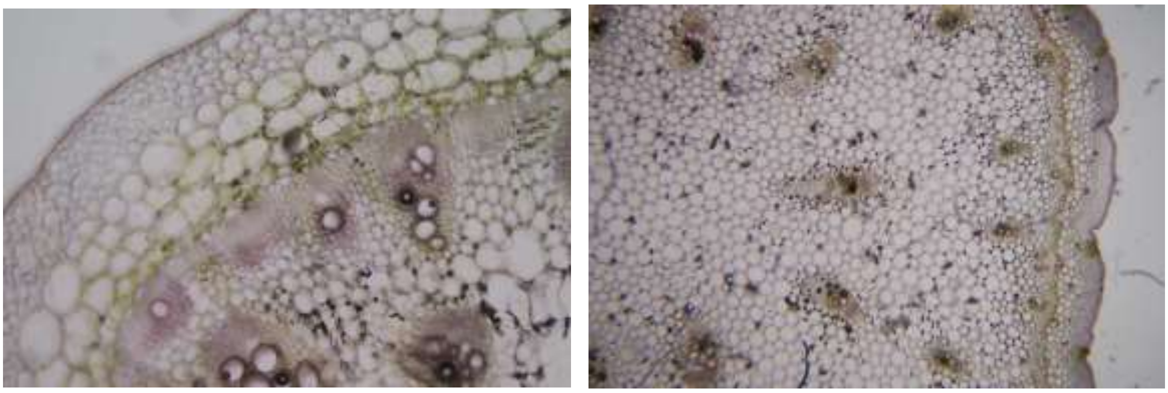

Fig. 4.4: Anatomical study on leaflets of *Amaranthus creuntus*

The T.S. of *A. creuntus* shows a similar outline when compared to the transverse section of *A. viridis*. The midrib portion of the leaf is designated with a single layer of parenchyma cells which are arranged compactly over the surface. Inner to the epidermis, there lies multilayered hypodermis which are of two types of cells; smaller and larger, which are arranged in the order of size. Few among the larger cells show prominent thickenings at the corner. At the centre of the midrib, there are vascular bundles situated in a horseshoe manner. The arms of the leaf show similarity to the arms of *A. viridis.*

The transverse section of *A. creuntus* stem also shows a wavy outline structure as in the case of *A. viridis*. The outer region is made up of thin-walled cells which are arranged compactly to form a uniseriate, thick, broad layer. This is followed by 6-7 layer of isodiametric cells which are lightly stained. Beneath to that layer, it lies multilayer of cells which are randomly shaped with minute intercellular spaces. This layer is immediately followed by endodermis, which is functioning as a protective layer of tissue. Inner to the endodermis, there is a single layered pericycle, which provides structure, support and protection for the inner lying vascular tissues. The central region is designated with the presence of vascular bundles which are really anomalous in nature. However, *A. creuntus* shows a structure which is very similar to dicot stem, the position of vascular bundle makes it anomalous in structure.

4.3 Anatomical study on leaflet and stem of *Amaranthus spinosus*

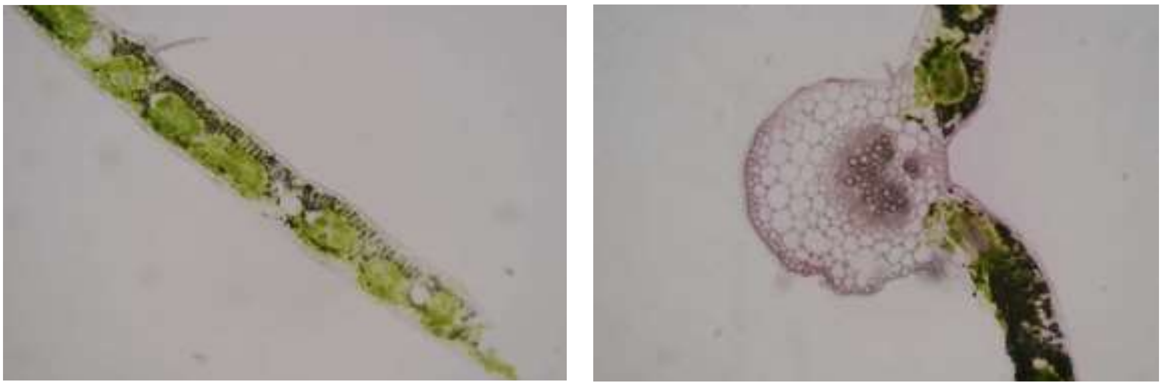

Fig. 4.5: Anatomical study on leaflets of *Amaranthus spinosus*

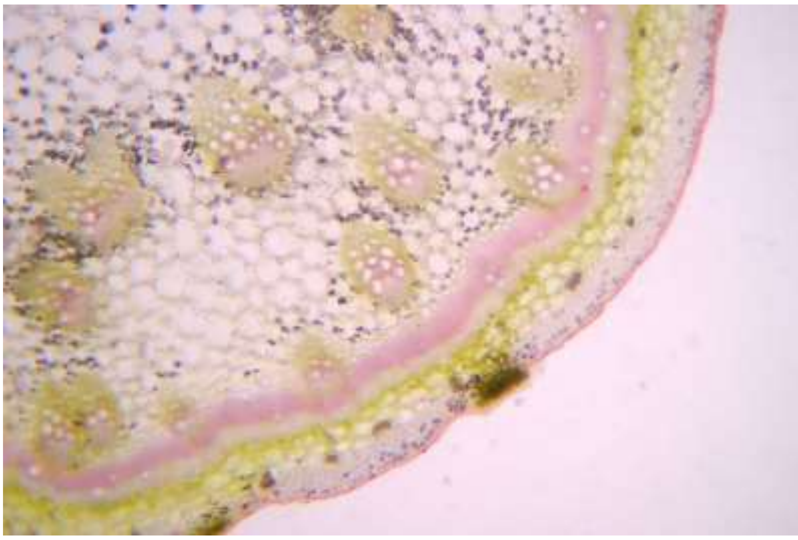

Fig. 4.6: Anatomical study on leaflets of *Amaranthus spinosus*

The T.S. of *A. spinosus* shows a similar outline when compared to the transverse section of other two Amaranthus species compared in the study. The outer region of cells is represented by a single layer of epidermis which is of parenchymatous in nature. The epidermis is covered by a thick cuticle which is of protective in nature. Beneath to the epidermis, there lies a multiple layer of hypodermis, which is specialised with slight thickenings at the corner. The centrally placed vascular bundles are of conjoint, collateral and closed type. Apart from the other two species of Amaranthus compared, the vascular bundles are not exactly arranged in U-shape manner.

Transverse section of *A. spinosus* shows a clear picture of anomalous secondary growth in few members of Nyctaginaceae family. The outer region is made up of thin-walled cells which are arranged compactly to form a uniseriate, thick, broad layer. This is followed by 6-7 layer of isodiametric cells which are lightly stained. Inner to this layer, there lies a single layer of chlorenchyma which is arranged very compactly. This layer appears as a lush green in colour. This layer is immediately followed by endodermis, which is functioning as a protective layer of tissue. Inner to the endodermis, there is a single layered pericycle, which provides structure, support and protection for the inner lying vascular tissues. The central region is designated with the presence of vascular bundles which are really anomalous in nature. However, *A. spinosus* also shows a structure which is very similar to dicot stem, the position of vascular bundle makes it anomalous in structure.

Chapter – 5

SUMMARY AND CONCLUSION

The study presents a comparative analysis of leaf and stem anatomy among three species of Amaranthus, namely Amaranthus tricolor, Amaranthus viridis, and Amaranthus spinosus. These species are widely distributed across various regions and are economically significant due to their nutritional and medicinal properties. The research aimed to elucidate the anatomical characteristics of their leaves and stems, with a focus on identifying key differences and similarities that could contribute to understanding their ecological adaptations and phylogenetic relationships.

Using light microscopy and various staining techniques, the anatomical structures of the leaves and stems were examined. The study revealed distinct variations in the anatomy of both organs among the three species. In terms of leaf anatomy, differences were observed in the arrangement of tissues such as epidermis, mesophyll, and vascular bundles, as well as in the presence of specialized structures like trichomes and stomata. Similarly, the analysis of stem anatomy revealed variations in the organization of tissues, including the arrangement of vascular bundles, sclerenchyma cells, and the presence of specialized structures like collenchyma.

Furthermore, the comparative analysis highlighted adaptive features related to environmental conditions and ecological niches occupied by each species. For instance, differences in leaf thickness, stomatal density, and trichome distribution may reflect adaptations to varying levels of sunlight, humidity, and herbivore pressure in their respective habitats. Similarly, variations in stem anatomy, such as the presence of spines in Amaranthus spinosus, could be linked to defense mechanisms against herbivory or environmental stress.

The findings of this study contribute to the understanding of the morphological diversity within the genus Amaranthus and provide insights into their ecological roles and evolutionary relationships. By elucidating the anatomical adaptations of these species, the research lays the groundwork for future investigations into their physiological responses to environmental changes and their potential applications in agriculture, medicine, and biotechnology.

In conclusion, the comparative analysis of leaf and stem anatomy among Amaranthus tricolor, Amaranthus viridis, and Amaranthus spinosus has revealed significant variations in their anatomical features. These differences reflect adaptive responses to diverse environmental conditions and ecological pressures experienced by each species. The distinct anatomical characteristics observed in leaves and stems contribute to our understanding of the ecological niche occupied by each species and shed light on their evolutionary history within the genus Amaranthus.

Moving forward, further research is warranted to explore the physiological implications of these anatomical adaptations and their functional significance in the context of plant ecology and evolution. Investigating the biochemical and molecular mechanisms underlying these adaptations could provide valuable insights into the resilience of Amaranthus species to environmental stressors and their potential for agricultural and pharmaceutical applications.

Overall, the comparative analysis presented in this study enhances our knowledge of the morphological diversity within the genus Amaranthus and underscores the importance of anatomical studies in elucidating plant adaptations and evolutionary relationships. By bridging the gap between morphology, ecology, and phylogenetics, this research contributes to the broader field of plant biology and paves the way for future interdisciplinary investigations into the functional significance of anatomical traits in Amaranthus species and beyond.