Anatomical, histochemical, phytochemical and allelopathic effects of two invasive species from asteraceae on agricultural crops

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DECLARATION BY STUDENT

I hereby declare that the data presented in the Dissertation / Internship report entitled, "**Anatomical, Histochemical, Phytochemical and Allelopathic effects of two invasive species from Asteraceae on agricultural crops**" is based on the results of investigations carried out by me in the **Botany** at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. S. Krishanan and the same has not been submitted elsewhere for the award of a degree or diploma by me Further, I understand that Goa Univerity or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Anatomical, Histochemical, Phytochemical and Allelopathic Effects of two invasive species from Asteraceae on agricultural crops**" is a bonafide work carried out by **Ms. Sanketa Ulhas Gawande**, under my supervision/mentorship in partial fulfilment for the requirements of the award of the degree of M.Sc. in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

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(Ms Sanketa Ulhas Gawande)

LIST OF PLATES

LIST OF TABLES

CONTENTS

CHAPTER 1: INTRODUCTION

INTRODUCTION

During the 16th century, humans began travelling the world, which significantly accelerated the intercontinental mobility of the species to a greater extent. After habitat alteration, the second most concerning threat to biodiversity is the invasion of plants. Foreign plants were introduced to provide various ecological functions such as feed, fuel-wood, medicines, fruits, shade, and aesthetic appeal. But, despite providing these services, some plant species tend to encroach into natural and agricultural regions, disrupting ecosystem functioning, decreasing native biodiversity, and harming the local economy and human well-being.

1.1. Invasive Plants

Invasion is a global threat to our biodiversity (Bellard *et al*., 2017). The term "Invasive plants" denotes the non-native plants introduced to the new region, which can be either deliberately or unintentionally (Kunwar, 2003). These plants have established self-reproducing populations and have caused significant changes to the ecosystem (Richardson, 1998). Plant invasion on land has caused a decrease in species richness and diversity (Hejda *et al*., 2009), altered ecosystem processes (Ehrenfeld, 2010), species extinction, crop failure, and reduced water yield (Harrington and Wingfield, 1998). Most non-native species behave ecologically more or less like resident species and occur at low to mid frequencies. Invasive plants are likely taller than native plants and can exhibit more prominence in growth, competitive abilities, or fecundity.

Invasion causes numerous ecological, financial, and societal issues (Dhanya, 2015). The alien plant species are considered to be one of the most significant threats to the earth's

ecosystems, by dominating the local ecosystem and competing with native species. These weeds typically occupy vacant spaces like barren lands, highway medians, and open fields (MacDougall and Turkington, 2005). By removing the allelopathic impact of invasive species or increasing the allelopathic effect of native species, exotic plant invasion can be controlled (Chen *et al*., 2017). Allelopathy plays a role in the invasiveness of exotic invasive plants, the resistance of native communities, the management of invasive plants, and the restoration of invaded environments **(Fig.1)**.

Studies on plant invasion and allelopathy will help in understanding the mechanism of invasions and their effects on global biodiversity and ecosystem functioning because the entire biosphere is struggling with the invasion of various weed species.

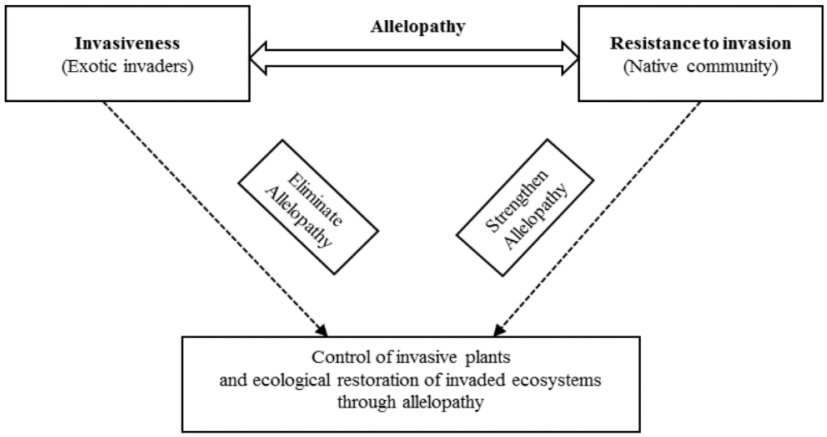


Fig.1. Role of Allelopathy in the invasiveness and the resistance of native communities. (Patel, 2016)

1.2. Allelopathy

Allelopathy is considered a 'novel weapon' that successfully helps exotic plants invade other ecosystems. The word Allelopathy originated from Greek, *Allelon* means 'of each other' or 'mutual harm,' and pathos means 'to suffering' or 'feeling.' Allelopathy is when a plant produces one or more biochemicals affecting one plant. The term 'Allelopathy' was coined by

the Austrian plant physiologist Hans Molisch in 1937, referring to both beneficial and detrimental biochemical interactions among all classes of plants, including microorganisms. Based on this, Elroy L. Rice (1984) defined allelopathy: as 'any direct or indirect, harmful or beneficial effect by one plant on another plant through the production of chemical compounds that escape into the environment' that means the chemicals which inhibit the growth of some of the species at specific concentrations can also stimulate the growth at lower concentrations.

The effect of allelopathy is widely studied in different plant groups, such as algae, lichens, and crops, including annual and perennial weeds (Rice, 1984; Lawrey, 1993; Horsley, 1991). The compounds or chemicals released in the environment by the plants that inhibit other plants' growth are called Allelochemicals. Many weeds influence crop plants by releasing allelochemicals through different modes **(Fig.2),** like leaching from the leaves or other parts of the plant, decomposing residues, root exudation, and volatilization. (Ghayal and Nirgundikar, 2020; Pan *et al*., 2015; Scavo *et al*., 2018).

Allelochemicals are secondary metabolites that are synthesized by the higher plants, which mediate plant-to-plant eco-physiological interaction and the mechanism of action of these compounds at the specific site of action at the molecular level. These chemical compounds do not hamper the primary metabolism essential for the host plants to survive in the environment (Shahena, 2021). The production of allelochemicals increases when the plant is under stress (Uyama, 2007). A plant with allelopathic potential is termed the "donor plant," while the plant in the area affected by the allelopathic compounds from the donor plant is referred to as the "receiver plant" (Simms, 1992; Karban and Baldwin, 1997).

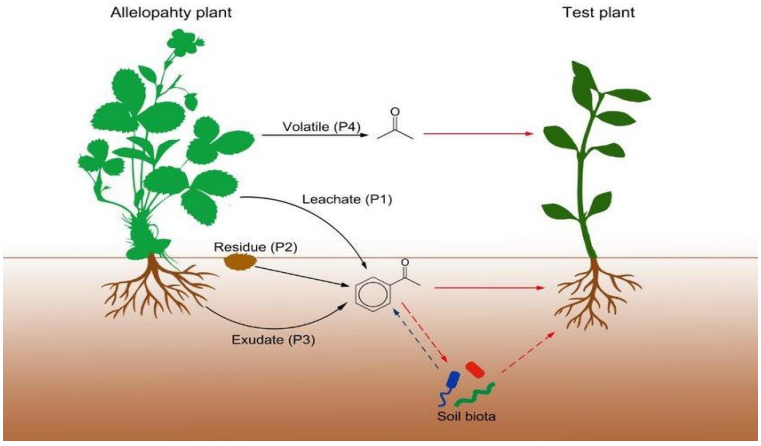


Fig.2. Different modes of allelochemicals released by plants and their influence on the crop.

(allelopathy - Bing images)

1.2.1. Effects of Allelopathy on Agriculture Crops

Developing nations in the tropics have major weed infestation on their agricultural land (Akodundu, 1992). According to the reports, many weed species are phytotoxic and interfere with the development and production of crops (Qasem, 2001). Due to their potential for allelopathy, they affect the growth of crops, and Asian farmers are currently facing a difficult task with their management. Because of the country's diverse climatic and environmental conditions, biotic invasion is presently on the rise in India, particularly in the agricultural sector (Akodunda, 1992).

Agroecosystem invasion by weeds like *Chromolaena odorata* (L.) R.M. King & H.Rob and *Sphagneticola trilobata* (L.) Pruski could result in significant losses and severe threats to the sustainability of global crop production (Ghayal and Nirgundikar, 2020). Previous studies showed that *C. odorata* produces a variety of allelochemicals, including flavonoids, terpenoids, and alkaloids; derivatives like cerylalcholol, eupatol (sesquiterpene alcohol), lupeol, and B-amyrin (terpene), salvigenin, isosakuranetin, flavan, 4,5-dihydroxy 3,7-dimethoxy flavone (flavanones),odoratin (chalcone) and P-anisic acid are present in

various parts of the plant (Ambika, 2002). Many reports revealed that the different concentrations of the plant *C. odorata* extracts significantly affected the seed germination of the crops.

Allelopathic effects are found by conducting various bioassays. Allelochemicals present in the plant *C. odorata* revealed suppression effects on different crops like cowpea, soybean, groundnut, maize, okra, and rice (Kumar *et al*., 2007; Masum *et al*., 2012; Muzzo *et al*., 2018) reddish, chickpea significantly reduced the germination and overall seedling growth, while it showed less suppression on black grams, cucumber and mustard (Hoque, 2003).

Different concentrations of aqueous leaf extract and leachate imparted a strong inhibitory effect and also hampered the dehydrogenase and catalase activity along with the decline in protein, DNA, and RNA contents (Parthapratim *et al*., 2013; Hamidi *et al.*,2014) Some researchers have found a positive effect of *C. odorata* on crops like Ragi (Ambika and Poornima, 2004). It is demonstrated that the plant has the potential to produce ample mineral nutrients which could be used as organic manure in okra cultivation. It is also used as green manure to increase the growth and yield of broccoli (Hafifah, 2016; Oluwafemi, 2012). *Sphagneticola trilobata* also showed a similar allelopathic effect on chickpeas, cowpea, and green gram. The inhibitory effect increases at the higher concentration of the extracts. The plant has Osthol, a coumarin derivative, diterpenes, sesquiterpenes, and other various metabolites, and the combined effect of these compounds could be the reason for the allelopathic development (Shahena, 2021).

1.3. Family: Asteraceae

The word 'aster' means star in Greek, and the family has an inflorescence that resembles a star-like appearance. What looks like one single flower is many small flowers attached to a specialized part of the stalk called the receptacle. The Asteraceae family consists of approximately 1500 genera, which comprise over 25,000 species of flowering plants. It is the world's most prominent family of flowering plants, commonly known to have high medicinal value (Bessada *et al*., 2015; Araujo *et al*., 2021; Jones and Luchsinger, 1987; Koc *et al*., 2014). A few members of the family are bushes, vines, or trees, but the majorities are annual or perennial herbs (Easmin *et al*., 2021). Due to essential oil production, this family contains species that are very significant in nutrition, pharmacy, and cosmetics (Milan *et al*., 2006). Asteraceae is an economically important family because it produces goods like cooking oils, sunflower seeds, lettuce, artichokes, sweeteners, substitutes for coffee, and herbal beverages. It includes grindelia, yarrow, and many other plants and is also significant in herbal therapy.

Many members of the family are utilized in the cultivation of flowers for ornamental purposes, and some of them are significant ornamental crops for the flowers industry, like chrysanthemum, gerbera, dahlia, zinnia, and many more (Easmin *et al*., 2021)

1.3.1 *Chromolaena odorata* (L.) R.M. King & H.Rob

The taxonomic hierarchy of *Chromolaena odorata* (Zahara, 2022).

Scientific Classification:

Kingdom : Plantae

Class : Magnoliopsida

Order : Asterales

Family : Asteraceae

Genus : *Chromolaena*

Species : *Chromolaena odorata*

Habitat

Chromolaena odorata formerly known as *Eupatorium odoratum* (L.) (Zahara, 2022; Ghayal and Nirgundikar, 2020). It is a weedy herb native to Central and South America (Ambika, 2002; Rouw, 1991; Panda *et al.*, 2010). It is commonly called siam weed, devil weed, and bitter bush (Sirinthipaporn and Jiraungkoorskul, 2017; Zahara, 2022). The species was introduced to Asia in the 1840s through the Botanical Garden in Kolkata, India, as an ornamental plant (Zachariades, 2009; Noguchi and Kato, 2023). Additionally, the species was introduced, expanded quickly, and naturally occurred in other nations in eastern and southern Asia, Austria, and southern and western Africa (Hu and Zhang, 2013). *C. odorata* is included in the top 100 worst invasive weeds list. There are numerous initiatives underway to eradicate this plant. However, none has succeeded significantly (Vaisakh and Pandey, 2012).

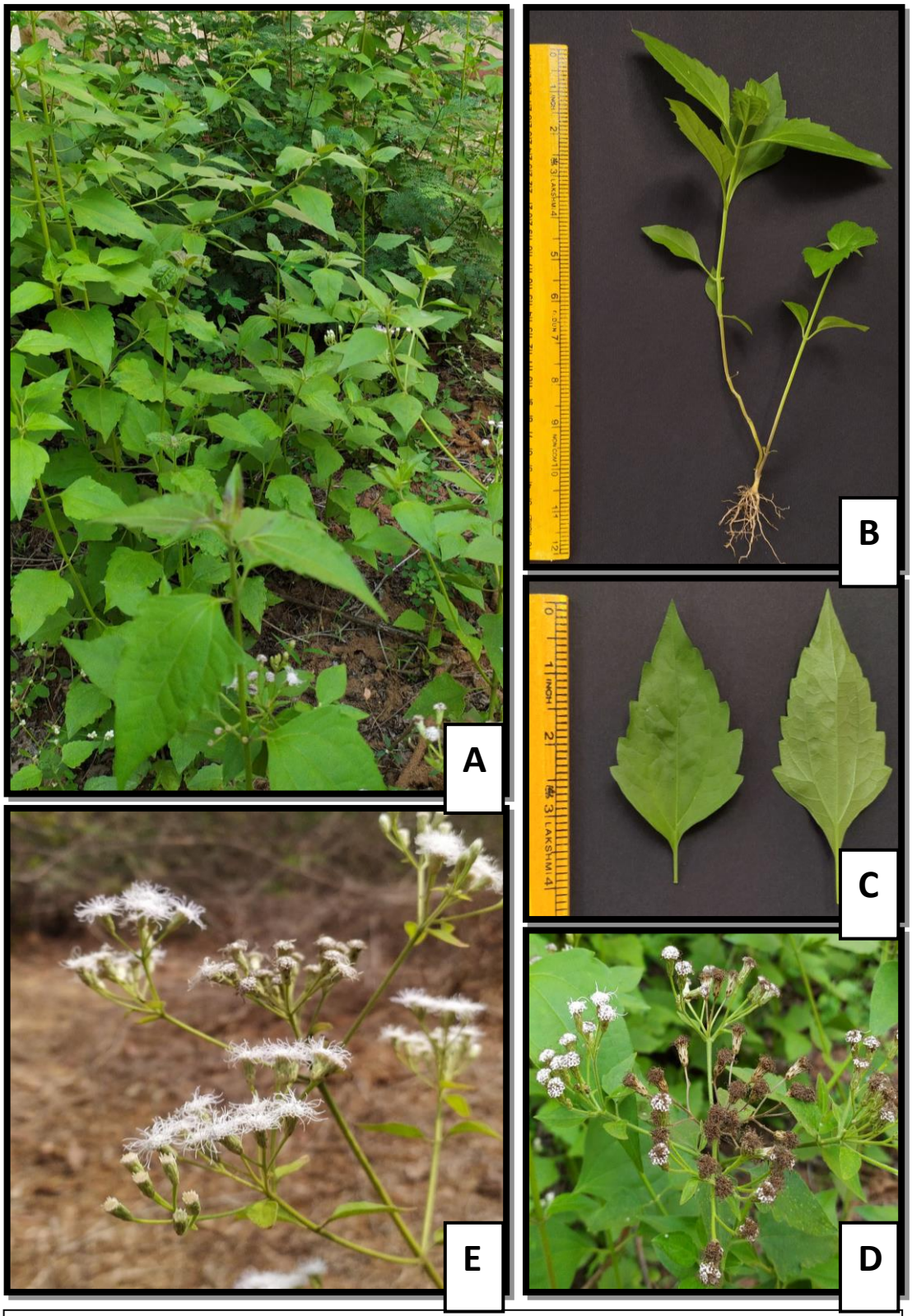


Plate 1: ***Chromolaena odorata*****: (A) Habit, (B) Uprooted plant, (C) Abaxial and adaxial leaf, (D) & (E) Inflorescence.**

Morphology

Chromolaena odorata (L.) R.M.King & H.Rob. is herbaceous to woody, perennial with a bushy habit. It forms a very dense thicket about 2-3 m in height; in almost pure stands, it can reach up to 5 to 10m with support by other vegetation (Zachariades *et al*., 2009; Pasiecznik, 2022; Penny and Yang, 1998; Gautier, 1992). When they reach a height of about 120cm, the isolated individuals start to form branches. After a year of growth, the plant develops a strong, woody underground storage organ which reaches a diameter of 20cm (Pasiecznik, 2022); stems are terete, striate, covered with pubescence shaves, especially young parts, brownish glandular punctate, opposite-decussate, often branch in pairs from the axillary buds (Penny and Yang, 1998, Pasiecznik, 2022); leaves are simple, opposite,decussate, the middle one larger, deltoid to the rhombic ovate blade, 7-15 cm long, 3.5-8 cm wide, acute to acuminate apex, obtuse to broadly cuneate or truncate base, pilosulose on both surfaces, brownish glandular beneath, prominently 3-nerved, sparsely dentate margins, subentire towards the apex, 1-3 cm long petiole, the upper leaves are gradually smaller towards the inflorescence. Internode elongated 5-9 cm long (Penny and Yang, 1998; Holm *et al*., 1977), as the species name 'odorata' suggests, the leaves on crushed emit a pungent odour (Noguchi and Kato, 2023); Capitula are borne in panicles at the end of the twigs(terminal) and are devoid of ray florets, grouped in 1-5 convex trichotomic corymbs, 5-10 cm in diameter. Heads discoid, 10-11mm long, 3-5mm wide, it has 25-35 florets; peduncle 8-16mm long, densely pilosulose; cylindrical involucre, 7-10mm long, 3-4mm broad; bracts are 4-seriate, distinctly imbricate, has 3 prominent, parallel green nerves and has scarious margins, tips are slightly flaring outward, outer bracts are ovate to ovate-oblong, 1.8-2mm long, 1.2mm wide, inner bracts are narrowly oblong, apex acute, base obtuse, 7-8mm long, 1.4mm wide, sparsely pilosulose. Receptacles are convex and glabrous. Corolla 5-lobed, 5mm long, colour ranges from pale-lilac to white; anthers brownish, 2mm long,

membranous appendages at apex, filaments 2.5mm long; style purplish, 10-11mm long, exerted; pappus capillary, brownish subequal to the corolla. Achenes are blackish brown, narrowly oblong, 4-5 mm long, 0.4-0.5 mm across, 5 ribbed, and ribs glabrous (Penny and Yang, 1998; Pasiecznik, 2022).

1.3.2 *Sphagneticola trilobata* (L.) Pruski

The taxonomic hierarchy of *Sphagneticola trilobata* (Hossain *et al*., 2005).

Scientific Classification:

Kingdom : Plantae

Class : Magnoliopsida

Order : Asterales

Family : Asteraceae

Genus : *Sphagneticola*

Species : *Sphagneticola trilobata*

Habitat

Sphagneticola trilobata (L.) Pruski is commonly referred to by its former name, *Wedelia trilobata* (Shushma, 2019; Shrestha *et al*., 2021). This species is native to Mexico and Central America (Shushma, 2019; Hossain *et al*., 2005). This species has been found as an ornamental herb grown in city streets and private gardens. It is usually seen escaping the garden and flourishing rapidly within city areas (Hossain *et al*., 2005). Genus *Sphagneticola* has about 70 species with tropical and subtropical distribution. *S. trilobata* has been listed

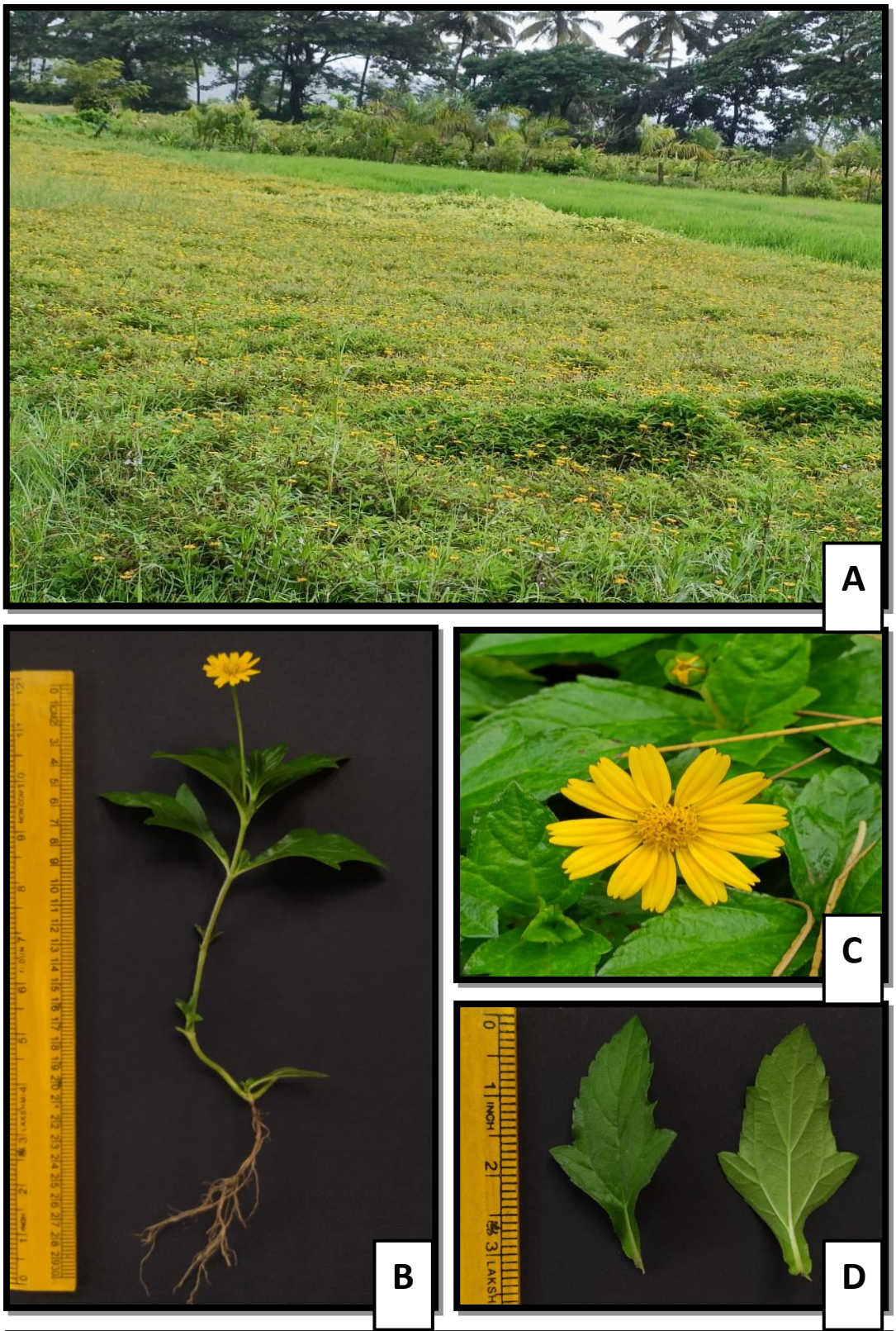


Plate 2: *Sphagneticola trilobata*: (A) Habit, (B) Uprooted plant (C) Flower (D) Abaxial and adaxial leaf.

among the world's 100 worst invasive species by IUCN. The plant is usually introduced as an ornamental or ground cover because of its beautiful bright yellow flowers. It reproduces vegetatively and rapidly forms a dense ground cover, preventing other plant species from regenerating (Shushma, 2019).

Morphology

Sphagneticola trilobata (L.) Pruski is the perennial procumbent herb with stems rooting at the nodes, cylindrical, much-branched, terete, glabrous or sparsely pubescent, green or reddish green, reaching up to 30cm or more. Leaves simple, opposite-decussate, shortly petiolate up to 3mm long; lamina lanceolate to elliptic, 3- lobed, base cuneate, pinnate venation, margins ovate-dentate, irregularly toothed or serrate, glabrous to puberulent, 4-7cm long and 1.5-2.5cm wide, dark green above and paler below. Capitulum terminal, radiate, heterogamous; involucre campanulate, 1-1.2 cm long and 0.4- 0.5 cm broad, hemispherical; bracts 2-seriate; peduncles 5.5-16cm long. Ray florets 8-13 per capitulum, pistillate, ligulate, Golden yellow corolla with 2-3 fid limb. Disc florets many, tubular, bisexual, with the 5-fid limb. Anthers are appendaged. Outer florets elongated with stylar arms; tips acute. Cyselas of outer florets 3-angled, tuberculate. Pappus a crown of short fimbriate scales (Shrestha *et al*., 2021; Hossain and Hassan, 2005; Rashid *et al*., 2022; Pharmacognosy and Gudlavalleru, 2018).

1.4. Phytochemistry

Phytochemicals are the chemical compounds naturally present in plants attributing to positive or adverse health effects (Shaikh and Patil, 2020; Silva *et al*., 2017). These substances appear non-essential to the plant that produces them, but they play a crucial role in the plant's survival by mediating ecological interactions with competitors, shielding it from

diseases, pollution, stress, and UV rays. They also contribute to the plant's colour, aroma, and flavour. People can use plants' metabolites to protect themselves from biotic and abiotic stresses to create medicines to treat various diseases (Njoku, 2009; Kocabas, 2017). Phytochemicals can be separated from the plant using different extraction techniques and solvents. The extraction depends on the extraction method used and the type of solvent used during the process.

Bioactive phytochemicals released into the environment can have beneficial or adverse effects on plant growth and development (Santana, 2009). The studies on the phytochemical analysis of *Chromolaena odorata* have shown the presence of Alkaloids, Saponins, Tannins, Phlobatannins, Anthraquinones, Steroids and sterols, Terpenoids, Flavonoids, Cardiac glycosides, Phytosterols, Coumarin, Saponins, Quinone, Phenolic compounds, Carbohydrates, Gums and Mucilages, Proteins and Amino acids (Akinmoladun *et al*., 2007; Muricken and Jofeena, 2019; Panda *et al*., 2010). Phytochemical screening of *Sphagneticola trilobata* showed the presence of Tannins, Steroids, Cardiac glycosides, Flavonoids, Terpenoids, Alkaloids, Phenols, Saponins, Anthraquinones, Phlobatannins, Oxalate, Amino acids and Carbohydrates (Govindappa *et al*., 2011; Shushma 2019; Pharmacognosy and Gudlavalleru, 2018).

1.5. Anatomy

Taxonomy is most important in studying the diversity of living things (Mauseth, 1998). Plant taxonomy can start with anatomical structure as the source of early data. Compared to the morphology of the reproductive organs, the anatomy of the vegetative organ is typically used more as a taxonomic feature. Plant anatomy is frequently investigated at the cellular level and involves sectioning and microscopy. It prevents beneficial characteristics that make phylogenesis analysis easier. Considering anatomical characteristics are stable and conserved the anatomical structure can be used as starting data source in plant taxonomy.

More than the anatomy of the reproductive organ, the anatomy of the vegetative organ is leaves, stems, and roots. Leaves exhibit anatomical structures, such as epidermis stomata, mesophyll, and vascular bundles. Also, there will be the presence of some specialized and unique tissues (Chatri *et al*., 2020). *Chromolaena odorata* has the presence of crystal druses in areoles and has higher areolar areas (Mabel, 2014). Vessels are oval, many trichomes are present, and medullary rays are prominent (Folorunso and Awosode, 2013). In *Sphagneticola trilobata*, vascular bundles are arc-shaped mucilage cells and multicellular and peltate glandular trichomes (Rashid *et al*., 2022).

1.6. Histochemistry

Histochemistry is the branch that combines biochemistry and histochemistry, a subfield of histology that deals with identifying the chemical components of cells and organs (Kadam 1999, Oyejide 2017). It helps visualize biological substances; histochemistry is involved in identifying and distributing chemical compounds using different types of stains and indicators and microscopy (Wick 2012). Active cell constituents such as starch, proteins, lipids, nucleic acids, and various elements are detected and localized in the cells (Badria and Aboelmaaty 2019). The histochemical analysis in S.trilobata showed the presence of lipids in secretory glands, starch, cellulose, lignin, mucilage, tannins, protein, lipids, calcium-oxalate crystals, alkaloids, and pectin in the plant.

CHAPTER 2:
REVIEW OF LITERATURE

REVIEW OF LITERATURE

Invasive plant species can dramatically alter the structure and dynamics of native plant communities and ecosystem function, resulting in strong competition. This can lead to a decline in abundance and can cause the extinction of certain native species. Certain populations of native plant species can co-exist with invasive plant species.

The relevant literature on the present study has been reviewed to get information and understand the various parameters of the study done on the mentioned objective.

Ghayal and Nirgundikar (2020) experimented to understand the influence of the *C. odorata* plant on various crops. They found that *C. odorata* releases allelochemicals through different plant parts that inhibited root, shoot length, plant growth, and crop yield of maize, tomato, groundnut, mungbean, and rice. They also reported that these allelochemicals help increase crop production, such as Ragi. It can produce considerable biomass used as compost manure in Okra and broccoli production. It is reported that elevated temperature and CO_2 enrichment have increased invasive plants' survival rate and allelobiogenetic performance. Hence research efforts should be focused on evaluating the potential of *C. odorata* in changing local climatic conditions to achieve sustainability in crop yields and economic stability.

Hoque *et al*. (2003) tested the allelopathic effect of different concentrations of *C. odorata* leaf water extract on the growth behaviour and germination of six crops, namely *Cicer arientinum*, *Brassica juncea*, *Cucumis sativus*, *Phaseolus mungo*, *Raphanus sativus* and *Vigna unguiculata* as bioassay material. They carried out experiments in sterile Petri dishes with a photoperiod of 24h approximate temperature of 28.5^{o}C. The effect of the different concentrations of the extracts was compared to the control (distilled water). The result revealed that different concentrations of leaf extract cause a suppressive effect on

germination shoo, and root elongation. It indicated that the inhibitory effect of the extract was proportional to the leaf extract concentration. The higher concentration has a more inhibitory effect than the lower one.

Hu and Zhang (2013) studied the Allelopathic effect of *C. odorata* on five native and five non-native invasive herbs. They took the aqueous extract of leaves and roots of *C. odorata* and made different concentrations like 1%, 5%, and 10% and examined the effect on seed germination and seedling growth of 10 herbaceous species which were common in most habitats of Southern China by using the petri dish bioassay. They found out that leaf extracts showed more inhibitory effect than root extract, and the suppressive effect increased with an increase in the extract concentrations and there was more inhibitory effect on native species compared to non-native species. Their result indicated that allelopathy contributes to the ability of the *C. odorata* plant to become dominant in invaded plant communities of the area studied.

Madane and Patil (2007) aimed to discover the allelopathic effect of *C. odorata* on amylase activity in seeds of *Cajanus cajan* and *Cicer arietinum*. In this study, they made the leaf extract of *C. odorata* and prepared different concentrations like 1%, 10%, 20%, and 30% seeds were soaked for different time durations, and then a petri dish bioassay was carried out, and then seeds were subjected to check amylase activity by preparing crude enzyme extract. They reported that the increase in concentration decreases the amylase activity in the case of *C. arietinum*. In the case of *C. cajan* increase in the concentration and seed soaking period decreases the amylase activity. They concluded that *C. odorata* works as a plant growth inhibitor.

Shahena *et al*. (2021) evaluated the allelochemical activity of *Sphagneticola trilobata* against the germination seeds and seedling growth of *Cicer arietinum, Vigna unguiculata,* and *Vigna radiata* using leaf extract by petri dish bioassay. They also noted the inhibition in

root and shoot growth and tested the dry and wet weight by one-way analysis of variance. It showed inhibition with increasing concentration. They also conducted growth inhibition by the organic solvent extract. The best solvent was taken as petroleum ether, the seed germination bioassay was analysed by HR LCMS-MSMS-QTOF analysis, and allelochemicals were identified.

Akinmoladun *et al*. (2007) carried out the phytochemical analysis of an aqueous and methanolic extract of *C. odorata*. Both methanolic and water extract showed positive results for tannins, phlobatannis, steroids, terpenoids, flavonoids, and cardiac glycosides. Alkaloids were detected only in the methanolic extract and not in the water extract. Using methanolic extract, they evaluated the antioxidant potential, and from the results, they suggested that a low value of antioxidant indices may not imply a low medicinal value.

Muricken and Jofeena (2015) conducted a study on the dried powder of C. odorata flowers wherein methanolic extract was used for phytochemical and antibacterial studies and used an aqueous extract of *C. odorata* for larvicidal and insecticidal studies. As a result, they found that the flowers of this plant have insecticidal and larvicidal activity and a slight range of antibacterial activity. Through phytochemical studies, they found the presence of tannins, phytosterols, coumarin, quinone, cardiac glycosides, terpenoids, anthraquinone, steroids, acids, phenol, and flavonoid in the flowers.

Ngozi *et al*. (2009) investigated the proximate amino acid profile of proteins, antinutrient composition, and phytochemical composition of *C. odorata*. They observed a high level of carbohydrates (20.58%WW and 50.82% DW), crude fibres (10.76%WW and 26.57% DW), and protein (6.56% WW and 16.20% DW) content. They also observed it as protein-rich amino acid with a protein score of 88.24%, with methionine as the limiting amino acid. The phytochemical studies revealed the presence of alkaloids, cyanogenic glycosides, flavonoids (aurone, chalcone, flavone, flavonol), phytates, saponins, and tannins.

Results suggested that *C. odorata* is a source of high-quality protein which could serve as a potential source of protein supplements.

Panda *et al*. (2010) carried out the phytochemical analysis of the *C. odorata* plant by using dried leaf powder in various solvents (petroleum ether, ethyl acetate, and methanol) using the Soxhlet as extraction process and fluorescence characteristics of different solvents were recorded using UV light at 360nm. They also noted the ash value of leaves. Anthelmintic and wound healing activities (using animals) were also carried out, revealing that the methanolic extract shows the best result. Compared to other solvent extracts, the methanol extract was endowed with potent anthelmintic properties and wound-healing properties. The activity revealed the concentration-dependent nature of different solvents. The potency of the extracts was found to be inversely proportional to wound healing.

Govindappa *et al*. (2011) evaluated phytochemical screening, antimicrobial, antioxidant, and anti-inflammatory activity using dry and fresh parts of the leaf, stem, and flower from water extract of *S. trilobata*. The phytochemical analysis showed the presence of tannins, cardiac glycoside, terpenoids, flavonoids, and saponins in all fresh and dried leaf, stem, and flower extracts. The steroids, alkaloids, and anthraquinones were absent in all the extracts. The antimicrobial activity gave different zone of inhibition on the organism tested. The fresh leaf water extract inhibited the growth of bacteria isolated, but the same extract did not show a similar effect on the fungal isolates.

Shushma (2019) explored the preliminary phytochemical analysis of the plant *S. trilobata* which is responsible for its pharmacological properties. Qualitative phytochemical screening of *S. trilobata* was studied, and the extract of powdered leaves stems, and roots were obtained using four different solvents such as petroleum ether, ethanol, chloroform, and distilled water. Results showed that out of thirteen phytochemicals screened, ten were present in various solvent extracts. They were flavonoids, alkaloids, saponins, terpenoids, glycosides,

steroids, tannins, proteins, amino acids, and carbohydrates. Compared with all the solvent extracts, more phytochemicals were present in the ethanol extract. Likewise, quinone, phlobatannins, and oxalates were absent in all the extracts of different parts of the plant.

Prasanth and Rao (2018) used the roots of *S. trilobata* to evaluate pharmacognostic properties and phytochemicals to ensure the purity and safety of the plant as medicinal. The fresh and dried root samples were evaluated for microscopic and macroscopic characteristics. Utilizing WHO-recommended criteria, physical and chemical parameters were performed. To identify and standardize the root of *S. trilobata*, a preliminary phytochemical and fluorescent study of the root sample was carried out. The roots and powdered root material were observed for their colour, shape, size, odour, and surface characteristics. Cross sections of the root and the powdered root under a light electron microscope showed the existence of cork cells, lignified spiral vessels, and parenchymatous cells. Flavonoids, tannins, phenols, saponins, steroids, carbohydrates, and glycosides were found during phytochemical analysis. Physiochemical characteristics of the root powder, including its moisture content, ash value, extractive value, and fluorescent activity, were identified. These variables can be used to distinguish between various powdered drug materials.

Silva *et al*. (2017) described the different extraction methods used traditionally and still in use: maceration, percolation, Soxhlet extraction, supercritical fluid extraction, microwave-assisted extraction, and accelerated solvent extraction. They also gave qualitative techniques for determining phytochemicals like alkaloids, carbohydrates, saponins, proteins, amino acids, phytosteroids, flavonoids, phenols, tannins, diterpenes, and different methods used under each test.

Mabel *et al*. (2014) described the foliar anatomy of 12 species of the family Asteraceae. It was observed that in *C. odorata, the* venation is actinodromous, areole is polygonal, rectangular, and triangular. Veinlet endings are divided singly and bifurcated,

ranging from 1 to 3. Crystal druses are present in areoles and are prominent. Lamina has a uniseriate epidermis and rectangular cells. Cuticles on both surfaces are not prominent, but where they are seen, they are non-striated. Palisade mesophyll tissue is one layer thick, consisting of tightly packed cylindrical elongated parenchyma cells. Spongy mesophyll tissue consists of parenchyma cells that are largely irregular in shape and irregularly arranged with intercellular spaces. Simple uniseriate, bicellular, and multicellular trichomes are present. Midrib has a uniseriate epidermis, and rare epidermis cells vary in size and arrangement. The vascular bundle is one amphicribal, shield-shaped. Trichomes are simple uniseriate and multicellular.

Folorunso and Awosode (2013) conducted a comparative anatomical study on two invasive species (*Chromolaena odorata* and *Tithonia diversifolia*) and two non-invasive species (*Ageratum conyzoides* and *Aspilia africana*) in the family Asteraceae. The objective was to compare the anatomical traits of invasive and non-invasive species, identify the traits in invasive species that contribute to invasiveness, and link these traits with their roles in invasive species. The light microscope was used to investigate the foliar and stem micromorphology of invasive and non-invasive species. It was observed that an essential part of the skeletal system in invasive species is the presence of vessels in support of the dense sclerenchyma tissues. The distinctive parenchymatous cell is for the effective transport of nutrients and water. Various vessel types are short and long, together with vast and narrow vessels for the reduced vulnerability of stem cavitation and water conservation. Long but coiled trichomes for adequate light piping were observed. The stomata have the low stomatal index to reduce excessive evaporation, which might lead to desiccation and severe disruption of photosynthetic function. These characters are reporas for the invasive species and responsible for their aggressive nature.

Mable *et al*. (2013) described the petiole anatomy of 12 species of the family Asteraceae, including *C. odorata*. The transverse section of the petiole was taken using Reichert Sledge Microtome. The sections were stained using 1% Safranin O for 5min, washed and counterstained in 1% Alcian blue solution for 5 minutes, and cleared in xylene. The section was mounted in DPX. Petiole shapes, variations in the number, arrangement, and shape of vascular bundles, and different types of trichomes were observed.

Ekeke and Mensah (2015) studied the comparative anatomy of 17 species from 14 genera of the family Asteraceae. The midrib was hand sectioned and stained with 1% safranin or alcian, then mounted on a slide, observed under a microscope, and a microphotograph was taken. The result showed that the 12 species, including *C. odorata* have secretory ducts; in others, it was absent. It was observed that the number of adaxial and abaxial parenchymatous cells, the midrib shapes, and the number and arrangement of the vascular bundle varied from one species or genera to the other.

Rashid *et al*. (2022) studied the leaf anatomy and micromorphology of *S. trilobata* in the Asteraceae family. The transverse section of the petiole, midrib, lamina and margin was taken using a sliding microtome, and epidermal peels were observed under the light microscope. The micromorphological study of the leaf was conducted using a scanning electron microscope. The vascular bundles were observed to be arc-shaped, anomocytic stomata, crusts, flakes, and granules of waxes, simple multicellular, capitate, and peltate glandular trichomes. The study revealed that this observation of *S. trilobata* can be used in taxonomical identification, differentiation, and classification at the species level.

Sutar and Parveen (2020) studied the anatomical characteristics of the *S. trilobata* leaf. They also carried out a phytochemical study revealing that starch, protein and alkaloids are present in the root and tannins, saponins and fats are present in the stem and leaves while absent in the glycosides are present in the leaf and absent in stem and root. The transverse

section of nodes, petiole, and leaf lamina was taken by free hand. Fresh materials were used for the nodal anatomy. The epidermal peel was taken and stained in 1% safranin and mounted in glycerine, and semi-permanent slides were made. The leaves were cleared in 20% KOH solution, stained with camel pad ink, and washed with water. The observation for anatomical studies was noted for the petiole, node, and leaf. The anatomical studies revealed that the petiole is triangular in outline, the leaf is dorsiventral and amphistomatic, and the stomata are anomocytic type.

Ferraro *et al*. (2017) studied the seasonally-dry environments of the neotropics. They described the underground system and leaf anatomy of three species of the family Asteraceae from the portion of Chaco. The three plants they studied are *Pterocaulon purpurascens, Wedelia trichostephia,* and *Pectis gardneri.* They used conventional plant anatomical methods and characterize their structural and adaptive characteristics. *W. trichostephia* showed slightly thickened xylopodia with gemmiferous character, a self-grafted stem shoot. The senescent trichomes were found present in the periderm. Leaves of the three species are perennial and amphistomatic, and showed aquifer cells in various tissues.

Khandekar and Gopalkrishnan (2022) conducted a study on *Sphagneticola calendulacea,* and pharmacognosy was carried out to standardize this plant. They tested the leaves for the parameters like macroscopy, microscopy, and histochemical and powder study. The leaves were also investigated for fluorescence, physicochemical and phytochemical analysis. They carried out the powder study, which revealed the presence of anisocytic stomata, cells filled with tannins, palisade tissue, calcium oxalate crystals, starch grains, oil globules, and different types of trichomes and the same results were shown by the microscopy of the leaves and physicochemical. The Phytochemical analysis revealed the presence of phytoconstituents like saponins, tannins, flavonoids, anthraquinones, glycosides, starch, alkaloids, terpenoids, amino acids, and proteins. Through histochemistry, they found

the localization of starch, cellulose, lignin, mucilage, tannins, protein, lipids, calcium-oxalate crystals, alkaloids, and pectin in the plant.

Beatriz *et al*. (2008) investigated the presence of secretory structures on thickened and non-thickened subterranean organs, and they conducted their study on seven different species of Asteraceae from three other tribes eupatorieae, vernonieae, pulcheeae. For the first time, they described the presence of secretory idioblasts in the *Chromolaena sphaerocephala*. The histochemical test showed that lipids are present in all secretory structures. Arunachalam *et al*. (2020) studied three different species of Asteraceae that is *Wedelia chinesis*, *Wedelia trilobata,* and *Eclipta prostrata*

CHAPTER 3: OBJECTIVE

OBJECTIVES

Invasive species are known to exhibit allelopathic effects that contribute to competitive impacts on native vegetation for which control measures are limited. Allelopathic interactions involve plants producing chemicals that negatively affect the performance of other plants. Control of allelopathic invasive plants represents a global problem with conservation and policy implications.

Invasive weeds have strong allelopathic capabilities against resident plants. Thus, invasive plants *Chromolaena odorata* and *Sphagneticola trilobata* were chosen to investigate the allelopathic potential to confirm their ecological impacts on chosen agricultural crops. Also, anatomical and histochemical localization was carried out along with the phytochemical extraction to understand the presence of chemical constituents. The *Chromolaena odorata* is known to be invasive but it was introduced as an ornamental plant without knowing its impact on the ecosystem. The *Sphagneticola trilobata* is introduced and cultivated as an ornamental plant because of its beautiful flowers This study can be useful to know the harmful effect and despite that, it can be used for betterment.

Specific objectives are:

1) To study the Anatomical characteristics of the leaf, petiole, and stem of two selected invasive Plants of the family Asteraceae (*Chromolaena odorata, Sphagneticola trilobata*).
2) To localize tissue-specific primary and secondary metabolites in different tissues by histochemical staining procedures.
3) To determine the allelopathic potential of the selected plants on the seed germination and early seedling growth of 3 selected agricultural crops.

4) To compare the solvent best for the extraction of active components from plant parts (leaves) through preliminary phytochemical analysis and the Soxhlet Extraction and Maceration method was carried out to know the best method for extraction of plant material in four different solvents.

CHAPTER 4: MATERIALS AND METHODS

MATERIALS AND METHODS

4.1. Collection of plant materials

Plant samples of *Chromolaena odorata* (L.) R.M. King & H. Rob and *Sphagneticola trilobata* (L.) Pruski was collected from the located sites of Goa University during the study period 2022-2023. Mature and healthy parts of the plant were collected in separate zip-lock polythene bags and brought to the laboratory for further studies.

Table 1. Collection site of the selected invasive plants.

Sr No.	Name of the plant	Location	Coordinates
1.	*Chromolaena odorata*	Taleigao-Goa	$15^0$27’32.3”N, $73^0$50’02.7”E
2.	*Sphagneticola trilobata*	Taleigao-Goa	$15^0$27’34.6”N,$73^0$49’48.6”E

4.2. Study of Anatomical Characterization

Systematic anatomical characterization of the collected plants' leaves, petiole, and stem was done. The thin free-hand sections of the fresh leaf, petiole, and stem were taken with a sharp new razor blade. The sections were stained with 0.1% safranin for 2-3 minutes, then rinsed with distilled water to remove excess stain, mounted on the clean slide with 10% glycerin, then examined the stained and unstained sections under a bright-field microscope **(Nikon Eclipse E200)**, and images were captured using TC-capture software.

Preparation of 0.1% safranin stain: 0.1g of safranin was dissolved in 100mL of distilled water and filtered the stain and stored in a reagent bottle for further use.

4.3. Study of Histochemical Localization

Histochemical tests were performed on the fresh thin hand sections of leaf, petiole, and stem sections of *C. odorata* and *S. trilobata*. The thin hand sections were placed in specific stains for the histochemical localization of secondary metabolites.

4.3.1. Detection of Alkaloids (Wagner's test) (Krishnamurthy 1998)

Preparation Wagner's reagent: - 1.27g of iodine and 2g of potassium iodide were dissolved in 5 mL of distilled water and the total volume is made up to 100 mL with distilled water.

Sample preparation:- Thin hand sections of the material were taken. Put the sections in Wagner's reagent for 10min. then the sections were washed with distilled water for 30sec. mount the sections in 10% dilute glycerin.

The alkaloids will stain Brown in colour.

4.3.2. Detection of Phenolics (Ferric Chloride Staining) (Badria and Aboelmaaty 2019)

Preparation of stain: - 10g of ferric chloride was dissolved in 90 mL of distilled water. The stain was filtered using Whatman No.1 filter paper and stored in a reagent bottle for further use.

Preparation of sample: - Thin hand sections of the material were taken. Put the sections in 10% ferric chloride solution for 30min. the sections were washed twice in distilled water to remove excess stains. Mount the section in 10% of dilute glycerin and observe under a bright field microscope.

The phenolics will produce dark colour usually black, sometimes brown,

4.4. Preparation of plant extract

The fresh and mature leaves of *C. odorata* and *S. trilobata* were collected from the Goa University campus, Taleigao-Goa. The leaves were washed thoroughly with tap water to remove dust particles and were cleansed using a cloth. The leaves were utterly shade dried, and the place of drying was kept moisture free. After two weeks, the leaves are ground in the mixer grinder and made into a fine powder. The powder was transferred to an amber-coloured bottle and stored for further phytochemical analysis. Two methods were carried out for the extraction of the leaves viz: Soxhlet extraction and maceration method by using four solvents with different polarities, namely Methanol, Chloroform, Ethyl Acetate, and Distilled Water.

For Soxhlet extraction, 10g of dried leaf powder was subjected to extraction with 150 mL of one solvent at a time, and extraction was carried out at 35°C for 12h. The extracts were filtered with Whatman No.1 filter paper.

For maceration,10g of leaf powder was added to the 100 mL of solvent and kept in an orbital shaker for three days. After three days, the extracts were filtered using Whatman No.1 filter paper. The extracts were then concentrated using a Rotary Evaporator bath temperature of 40^0C and rotation of 50rpm for 37 minutes. The solvent-free extracts were kept in vials and stored at 4^0C for further use.

4.4.1 Phytochemical screening

Different qualitative chemical tests were performed to detect the presence of phytochemicals present in plant extract from different solvents. The following tests were performed on the leaf extracts for the detection of various phytoconstituents present in the plant, which was carried out according to the methods described by Raaman (2006).

4.4.2. Test for Alkaloids (Evans, 1997)

Solvent-free extract (50mg) was stirred with a few mL of diluted hydrochloric acid and then filtered. The filtrate was then tested with various alkaloidal reagents as follows:

1. **Mayer's test (Evans, 1997)**

Take 1 mL of filtrate, and two drops of Mayer's reagent were added (to the side of the test tube.)

A white precipitate confirmed the test positive.

Mayer's Reagent: Mercuric chloride (1.358 g) was dissolved in 60 mL of water, and potassium iodide (5.0 g) was dissolved in 10 mL of water. Then both solutions were mixed and made up to 100 mL with water.

A) Wagner's test (Wagner, 1993)

Take 1 mL of filtrate, and a few drops of Wagner's reagent were added (to the side of the test tube.)

A reddish-brown precipitate confirmed the test positive.

Wagner's Reagent: Iodine (1.27g) and potassium iodide (2g) were dissolved in 5 mL of water and made up to 100 mL with distilled water.

B) Hager's test (Wagner. *et al*., 1996)

To 1 mL of filtrate, 2 mL of Hager's reagent was added.

A prominent yellow precipitate confirmed the test positive.

4.4.3. Test for Carbohydrates

The 100mg of solvent-free extract was dissolved in 5mL of distilled water and then it was filtered. The filtrate was subjected to the following tests.

A) Molish test

To 2mL of filtrate, two drops of alcoholic solution of α-naphthol were added and shaken well. Then 1mL of concentrated sulphuric acid was added slowly along the sides of the test tube and was allowed to stand.

A violet ring confirmed the presence of carbohydrates.

B) Barfoed's test

To 1mL of filtrate, 1mL of Barfoed's reagent was added and was heated in a boiling water bath for 2 min.

Red precipitate confirmed the presence of sugar.

Barfoed's reagent: 30.5 g Copper acetate is dissolved in 1.8 mL of glacial acetic acid.

C) Benedict's test

To 0.5mL of filtrate, 0.5mL of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2min.

A characteristic-coloured precipitate confirmed the presence of sugar.

Benedict's reagent: 173 g of sodium citrate and 100 g of sodium carbonate are dissolved in 800 mL of distilled water and boiled till the solution became clear 17.3g of copper sulphate is dissolved in 100 mL of distilled water and added to the first solution.

4.4.5. Test for Glycosides

50mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours in a water bath, filtered and the hydrolysate was subjected to the following tests.

A) Borntrager's test (Evans, 1997)

To 2mL of filtrated hydrolysate, 3 mL of chloroform was added and shaken well, the chloroform layer was separated and 10% ammonia solution was added to it.

The pink colour confirms the presence of glycosides.

B) Legal's test

50 mg of the extract was dissolved in pyridine, and sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide.

The pink colour confirmed the presence of glycosides.

4.4.6. Test for Protein

100mg of extract was dissolved in 10 mL of distilled water and filtrate. The filtrate was used for the following test.

a) Biuret test

An aliquot of 2mL of the filtrate was treated with one drop of 2% copper sulphate solution, to this 1mL of 95% ethanol was added, and the excess of potassium hydroxide pellets was added.

The pink layer in the ethanolic layer confirms the presence of protein.

4.4.7. Test for Amino acids

100mg of extract was dissolved in 10 mL of distilled water and filtered. The filtrate was used for the following test.

a) Ninhydrin test

Two drops of ninhydrin solution were added to 2mL of aqueous filtrate.

The characteristic purple colour confirms the presence of amino acids.

4.4.8. Test for Phyto-steroids

a) Liebermann Burchard's Test

50mg of the extract is dissolved in 2mL of acetic anhydride then two drops of concentrated sulphuric acid are added slowly along the side of the test tube.

An array of colour changes confirms the presence of phytosteroids.

4.4.9. Test for Terpenoids

In 1mL of filtrate, 2 mL of chloroform and 3mL of conc H_2SO_4 were added.

The appearance of the blue-green ring at the junction confirmed the presence of Terpenoids.

4.4.10. Test for Fixed oil & Fats

a) Saponification Test

A few drops of 0.5N alcoholic potassium hydroxide solution were added to the few quantities of extract and a drop of phenolphthalein. The mixture was then heated in the water bath for 2 hours.

The formation of soap confirms the presence of fixed oils and fats

4.4.11. Test for Phenolic Compounds

A) Ferric Chloride Test

50mg of extract was dissolved in 5mL of distilled water then a few drops of neutral 5% ferric chloride solution were added.

A dark green colour confirmed the presence of the phenolic compound.

B) Gelatin Test

The 50mg of extract was dissolved in 5mL of distilled water and then 2mL of 1% solution of gelatin containing 10% sodium chloride was added to it.

White precipitate confirmed the presence of the phenolic compound.

C) Lead acetate Test

The 50mg of extract was dissolved in distilled water, and 3 mL of 10% lead acetate solution was added.

A bulky white precipitate confirms the presence of phenolic compounds.

4.4.12. Test for Quinones

In 1mL of extract, 1mL of H_2SO_4 is added.

The formation of red colour confirmed the presence of Quinones.

4.4.13. Test for Saponins (Kokate,1999)

50mg of extract was dissolved in distilled water and made the final volume to 20 mL, this suspension was shaken well in a graduated cylinder for 15 minutes.

A two cm layer of foam confirms the presence of saponins.

4.5 Allelopathic study

4.5.1. Collection of Donor plant

The weed *Chromolaena odorata* and *Shagneticola trilobata* are considered donor plants. The fresh plant leaves were collected from the Goa University campus, Taleigao -Goa.

4.5.2. Receptor plant

Brassica nigra L. (Black Mustard), *Vigna mungo* (L.) Hepper (Black gram) and *Vigna radiata*(L.) R. Wilczek (Green gram) was selected as the receptor plant. The seeds were sourced from the Goa Bagayatdar Bazar.

4.5.3. Preparation of Plant Extract

4.5.3.1. Water extract of leaf for checking the allelopathic effect

The aqueous extract was prepared from the fresh leaves of the donor plant and was used for the present analysis. Fresh leaves from the donor plants were collected and washed thoroughly with distilled water. Then 100g of leaves fresh leaves were hand crushed and soaked in 100mL of distilled water. This was kept at room temperature for 24h, the extract was then filtered and it was considered as 100% stock solution for further analysis. The serial dilution was made using this stock solution by using distilled water, concentrations of 10%, 25%, 50% and 75% were prepared. As a control, distilled water without plant extract was used (Shahena *et al.*, 2021; Hoque *et al*., 2003).

4.5.3.2 Preparation of donor seedlings

The seeds were washed thoroughly with distilled water and soaked overnight at room temperature. Seeds which showed signs of germination were used for the analysis.

4.5.3.3. Germination by Petri-dish Bioassay

Sterile Petri plates were used for the bioassay. Blotting paper was kept on the petri dish and 10 seeds were placed on the paper. Moistened with plant extract according to the concentrations. One plate is moistened with distilled water and kept as a control. Care was taken that the seeds should not dry up. They were kept moist by adding the extract with a respected concentration to get favourable conditions for the growth and germination of the seed.

4.5.3.4 Treatments

T_0= Receptor agricultural plant seeds are grown in distilled water as a control

T_1 = Receptor agricultural plant seeds are grown in 10% concentration

T_2 = Receptor agricultural plant seeds are grown in 25% concentration

T_3 = Receptor agricultural plant seeds are grown in 50% concentration

T_4 = Receptor agricultural plant seeds are grown in 75% concentration

4.5.3.5 Germination and growth records

A seed is recorded as germinated when radicals emerged from seeds and three replicas were maintained for observation

4.6.3.6 Germination Percentage

The germination Percentage was calculated as follows:

$$\textbf{crosses } \frac{G1}{G2} \times 100$$

Where G is germination percentage, G1 is No. of seeds germinated and G2 is the total no. of seeds that were sown.

CHAPTER 5:
RESULTS
AND
DISCUSSION

RESULTS AND DISCUSSION

5.1. Anatomical studies

The study on the transverse section of the leaf, petiole and stem of *Chromolaena odorata* revealed the following features:

5.1.2. T.S. of Leaf

T.S. of the leaf showed the presence of a thick layer of cuticle on the epidermis with two types of trichomes; one being simple, uniseriate, multicellular; the other was glandular trichome. The abaxial side had more trichomes compared to the adaxial side. The upper as well as lower epidermis was uniseriate, polygonal to irregular in shape. Palisade tissue was 1-2 layered thick consisting of tightly-packed elongated parenchyma cells, whereas spongy tissue was made up of irregularly arranged parenchyma cells with intercellular spaces. Midrib showed 5 vascular bundles (3 separate traces and 2 medullary) with 4-5 layers of parenchyma on the adaxial side and 3-4 layers of parenchyma on the abaxial side **(Plate 3).**

5.1.3. T.S. of Petiole

The petiole of *C. odorata* was boat shape, slightly concave at the adaxial and convex at the abaxial side. The epidermis was covered by a thin layer of cuticle, with simple, uniseriate, multicellular trichomes. The cortex region consisted of thick collenchyma cells interspaced with chlorenchymatous patches followed by 4-5 layers of thin-walled parenchyma cells. The vascular bundles were arranged in the form of an arc **(Plate 4).**

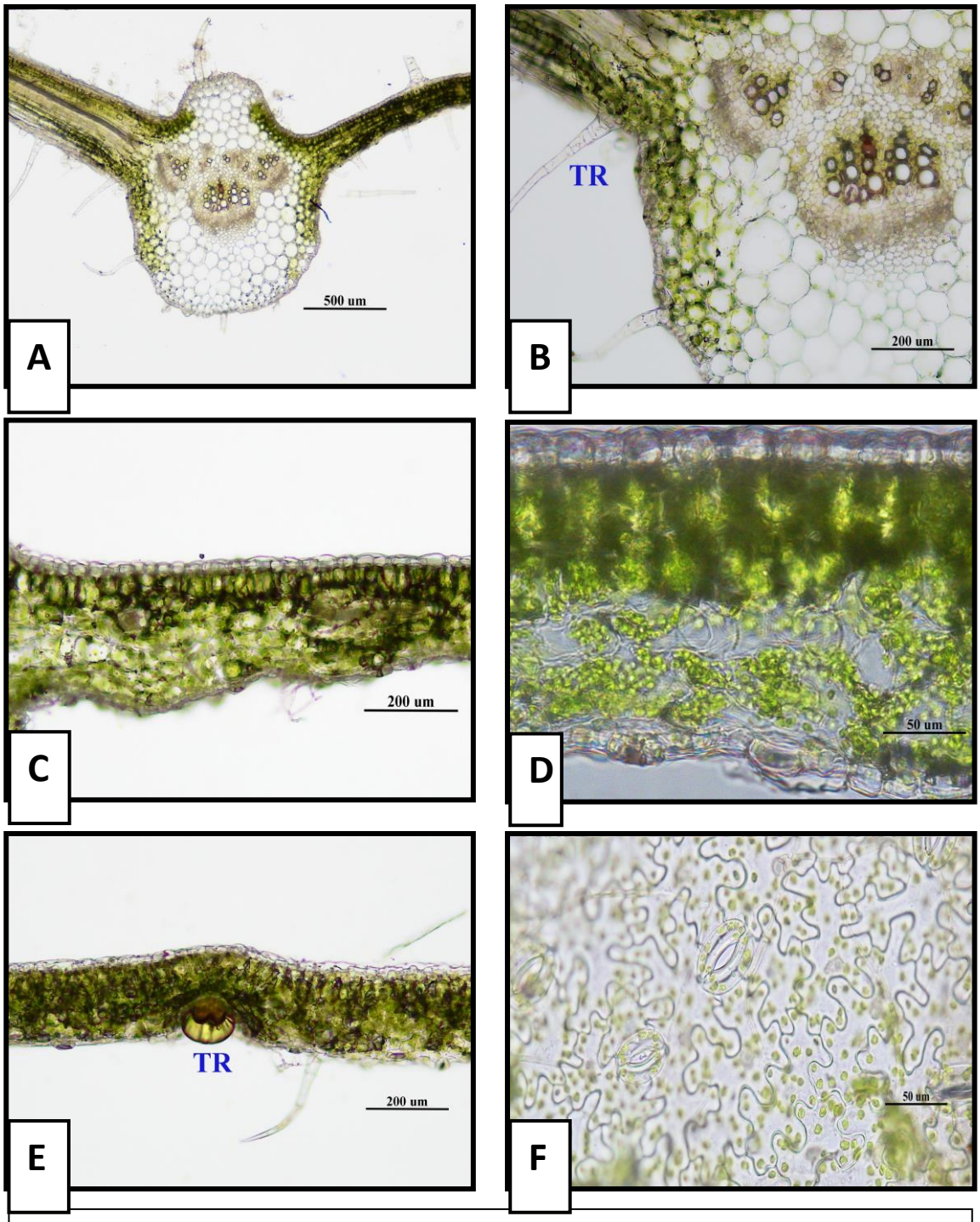


Plate 3: T.S of leaf of *Chromolaena odorata*: (A) Overview of leaf showing midrib region (4X), (B) A portion of midrib showing trichome and vascular bundle (10X), (C) Overview of lamina showing vascular bundle in a veinlet (10X), (D) Portion of lamina showing palisade and spongy tissue (40X), (E) Leaf showing glandular trichome and simple uniseriate multicellular trichome (10X), (F) Lower epidermal peel showing anomocytic stomata (10X). TR= Trichome.

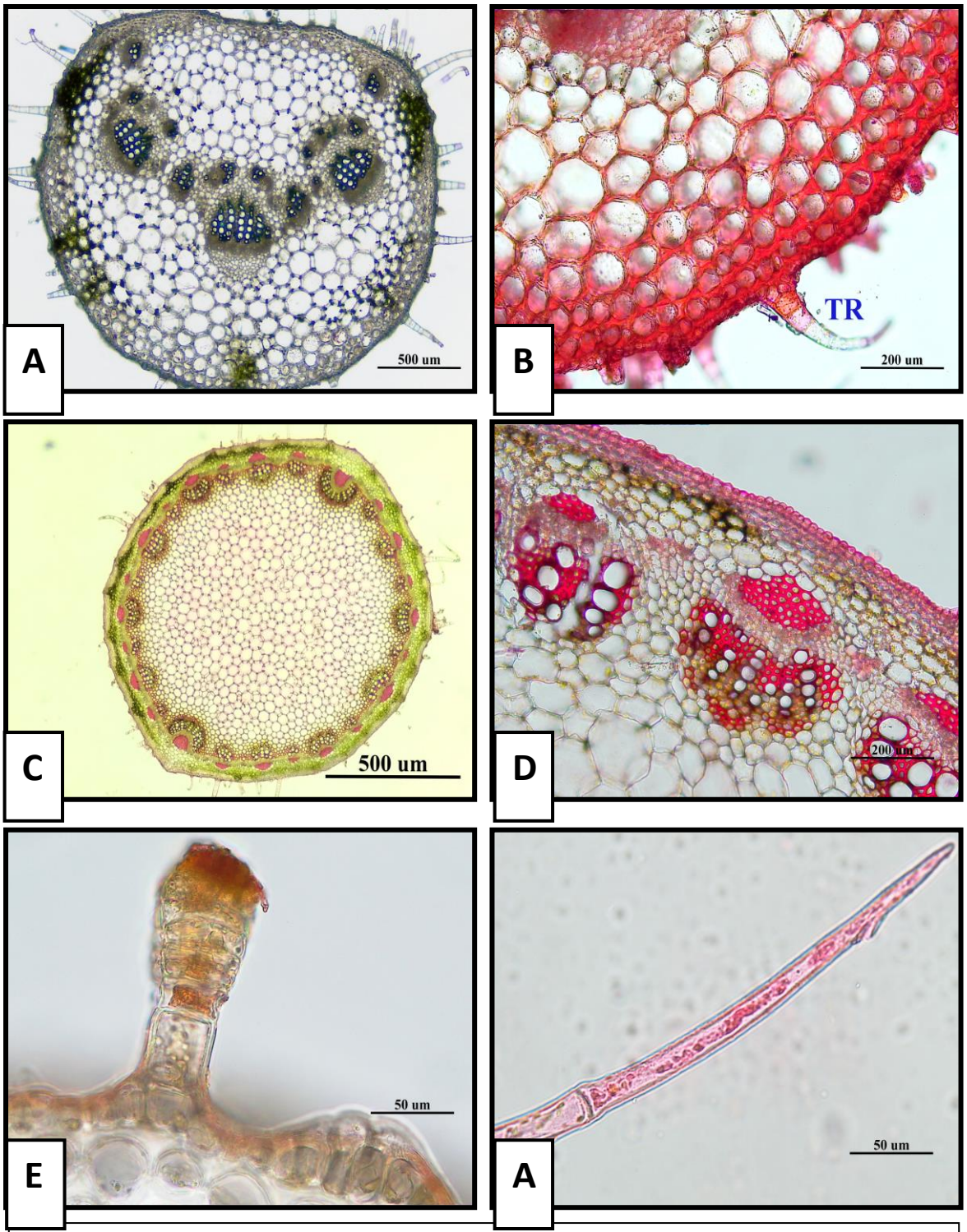


Plate 4: T.S of petiole and stem of *Chromolaena odorata* (A) Overview of petiole (4X), (B) A portion of petiole showing trichome (10X), (C) Overview of stem (4X), (D) A portion of stem showing vascular bundle (10X), (E) Glandular trichome on stem (40X), (F) uniseriate multicellular trichome on stem (40X). TR= Trichome.

5.1.4. T.S. of Stem

The stem was circular in outline with a thick cuticle showing the presence of simple multicellular trichomes and glandular trichomes. The epidermis was single-layered with compactly arranged thin-walled cells. The cortex is comprised of 2-3 layers of collenchyma cells with thickened corners mixed with chlorenchymatous patches followed by parenchyma cells with intercellular spaces. Vascular bundles were arranged in the form of a ring wherein sclerenchymatous patches were observed above each vascular bundle forming a cap-like structure. The vascular bundle was conjoint, collateral and open, with an endarch xylem (metaxylem towards the outer side and protoxylem towards the inner side). Cambium was seen in between the xylem and phloem. Medullary rays were present between the vascular bundles and pith made up of parenchyma cells **(Plate 4).**

The study on the transverse section of the leaf, petiole, and stem of *Sphagneticola trilobata* revealed the following features:

5.1.5. T.S. of Leaf

The thin layer of cuticle was present on the epidermis with two types of trichomes; one being simple, multicellular and the other simple, unicellular with enchinate ornamentation. The epidermal cells were rectangular to irregular in shape. Below the epidermis, a single layer of palisade tissue was seen with 4-5 layers of spongy mesophyll. Near the vascular region, there were 2-3 layers of chlorenchyma cells followed by 6-7 layers of parenchymatous cells wherein secretary canals were found embedded. The vascular bundle was arc-shaped **(Plate 5).**

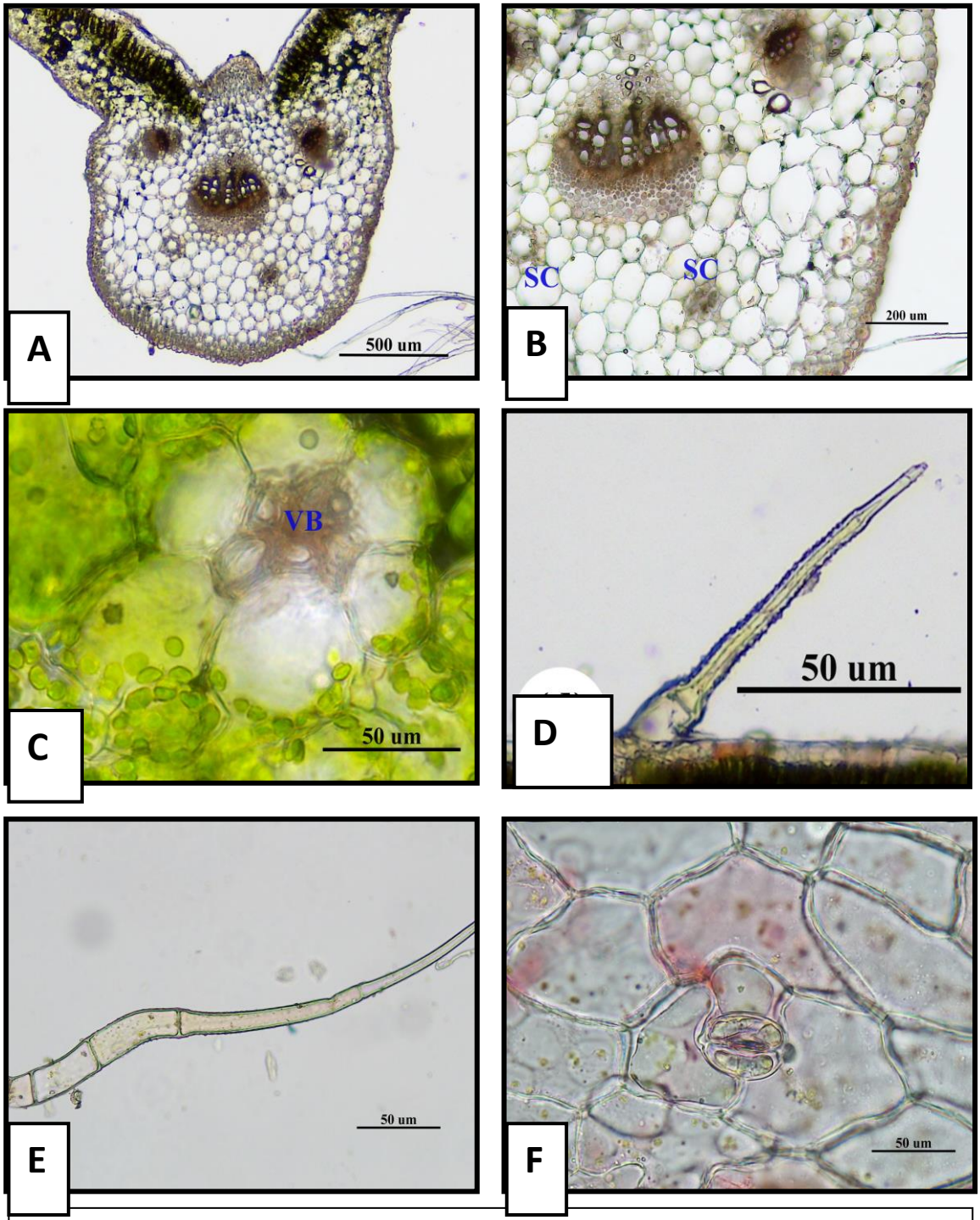


Plate 5:T.S of leaf *Sphagneticola trilobata* **(**A) Overview of midrib (4X), (B) A portion of midrib showing secretory canals (10X), (C) Leaf lamina showing a vascular bundle (40X), (D) Unicellular trichome on leaf surface (40X), (E) Multicellular trichome on leaf surface (40X), (F) Lower epidermal peel showing presence of anisocytic stomata (40X), SC= Secretory Cavity, VB= Vascular Bundle.

5.1.6. T.S. of Petiole

The outline of the petiole was a winged shape. Epidermis showed the presence of a thin layer of cuticle with glandular trichomes. The epidermal cells were circular, followed by 2-3 layers of chlorenchyma cells below which 4-5 layers of parenchyma cells were observed. Few secretory canals were present in the parenchyma layer. Three vascular bundles were present in an arc shape with sclerenchymatous patches surrounding them **(Plate 6).**

5.1.7. T.S. of Stem

The stem was circular, covered with a thin layer of cuticle, showing the presence of two types of trichomes, one type being uniseriate, multicellular and the other glandular. The epidermal cells were rectangular in shape. Chlorenchyma cells were seen below the epidermis in 2-3 layers followed by 5-6 layers of parenchyma cells with intercellular spaces. The presence of secretory canals was noted in the parenchyma layer in 1-2 rings surrounding the vasculature. Vascular bundles were arranged in the form of a ring, surrounded by patches of sclerenchyma cells in 2-3 layers. Vascular bundles were conjoint, collateral and open type with endarch xylem (phloem lies towards the outer side). Pith cells were parenchymatous **(Plate 6).**

There are many similar findings which had given the characteristics of invasive plants. For both plants earlier study has documented the invasive features, Folorunso and Awosode (2013), Mabel *et al*. (2014), Johnson *et al*. (2013), Ekeke (2015), Milan (2006), Mensah (2012), Rashid *et al*. (2022), Sutar and Parveen (2020) studied and documented the similar characteristics which are found in present documentation for both the plants *C. odorata*. and *S.trilobata.* Folorunso *et al*. (2013) revealed the characteristics present in invasive and non-invasive plants. The result they had given matches the findings of others who carried out anatomical studies for both the plants which are mentioned above and also the present study

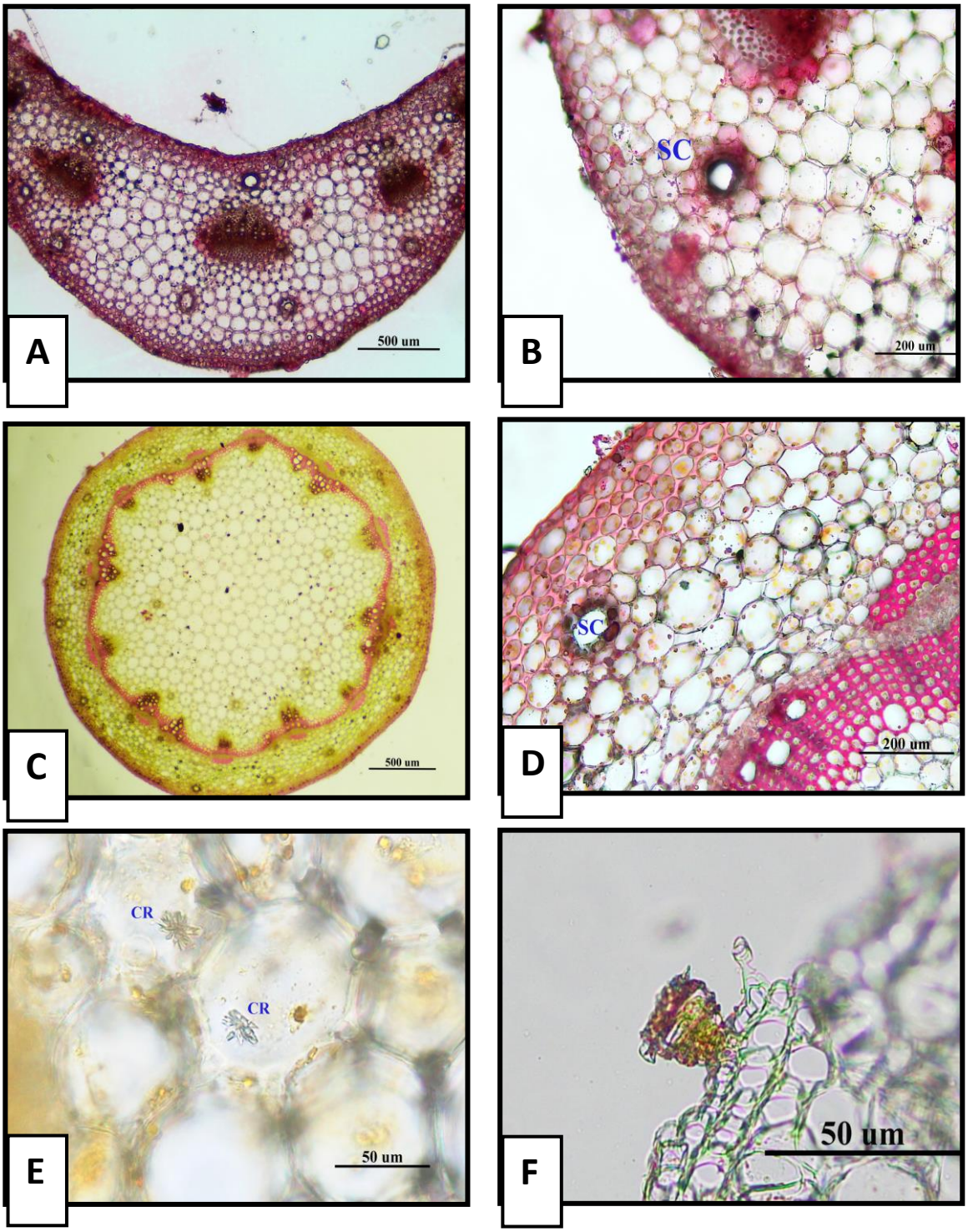


Plate 6:T.S of petiole and stem *Sphagneticola trilobata.* (A) Overview of petiole, (B) A portion of petiole showing presence of a secretory canal, (C) Overview of stem, (D) A portion of stem showing presence of secretory cavities and vascular bundle, (E) Crystals present in parenchyma cells of stem, (F) Presence of glandular trichome on stem. SC- Secretory Cavity, CR- Crystal.

which was carried out. They mentioned the invasive plant characteristics which are sclerenchyma cells above the vascular bundles, compactly arranged parenchyma cells, xylem with wider pores, presence of narrow and short vessels, and presence of long trichome which are coiled at the end, presence of many trichrome. Medullary rays are prominent in the. The presence of crystals was also observed.

5.2. Histochemical studies

5.2.1. Alkaloids

1) **Localization of alkaloids in the leaf**

   The epidermal layer, palisade tissue, mesophyll and phloem region showed the presence of alkaloids in the leaf of both plants. *S. trilobata* showed more concentration of alkaloids as compared to *C. odorata* and almost the same concentration was found in the midrib region **(Plate 7).**

2) **Localization of alkaloids in petiole**

   The section of petiole showed the presence of alkaloids in the secretory cavities and parenchyma cells whereas in *C. odorata* alkaloids were observed in the vascular bundle **(Plate 7).**

3) **Localization of alkaloids in stem**

   In the stem section of *S. trilobata* alkaloids were observed in the epidermal layer, collenchyma cells, parenchyma cells and the pith region of *S. trilobata* whereas in *C. odorata* alkaloids were found in the parenchyma and medullary rays. As compared to *S. trilobata* the concentration of alkaloids was less in *C. odorata* **(Plate 7).**

5.2.2. Phenolics

1) **Localization of phenolics in leaf**

Phenolics were found present in epidermal cells, palisade tissue and mesophyll tissue of both species. Phenolic content was more in *C. odorata* than in *S. trilobata* **(Plate 8).**

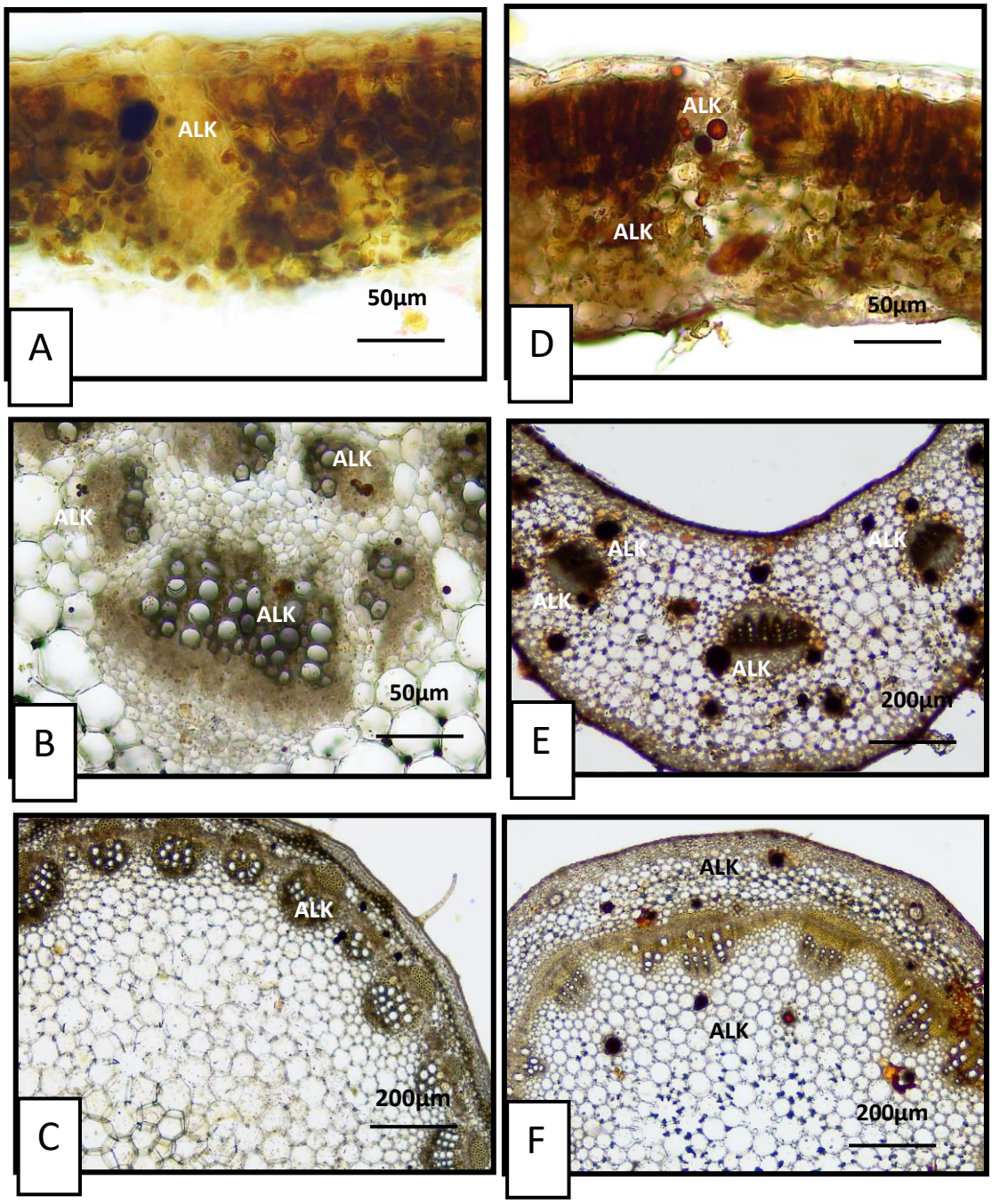


Plate 7: The T.S of leaf, petiole and stem showing presence of alkaloids:

(A-C) T.S of *C. odorata*: (A) Leaf (40X), (B)Petiole (40X), (C)Stem (10X),

(D-F) T.S of *S. trilobata*:(A) Leaf (40X), (B)Petiole (10X), (C)Stem (10X). ALK -Alkaloids.

1) **Localization of phenolics in petiole**

In *C. odorata* the phenolics were present in vascular bundles especially in the xylem tissue and in *S. trilobata* they were observed in epidermal cells. *C. odorata* had lesser phenolics than that *S. trilobata* **(Plate 8).**

2) **Localization of phenolics in stem**

In *C. odorata*, phenolics were found in between the vascular bundle whereas in *S. trilobata* they were observed in the epidermal layer and were comparatively higher in *C. odorata* than in *S. trilobata* **(Plate 8).**

Khandekar (2023) studied the histochemistry of the sphagneticola genus, he conducted the histochemical test for the alkaloids and reported that it is found in the vascular bundle and hypodermis, and phenolics were noted present in the vascular bundle and midrib region, the current study gave the similar results for alkaloids and phenolics tests.

5.3. Extraction values with the different solvents

The extracts of the leaves of two different plants, *C. odorata* and *S. trilobata were* obtained using two extraction methods that are Soxhlet extraction method and the maceration method. Methanol, Chloroform, Ethyl acetate and Water were the four different solvents used. The extracts were made concentrated with the use of a Rotary Evaporator machine. the samples were weighed using a weighing machine and yield was taken. The extract yield was calculated in the formula (formula 1.). It was found that the highest extraction yield was obtained in the Soxhlet extraction method than the maceration method. It was also observed that the yield varies for different solvents. The distilled water gave a more yield than the other three then the then and then ethyl acetate. The chloroform gave a lesser yield than the other three solvents as shown in **Table 2**)

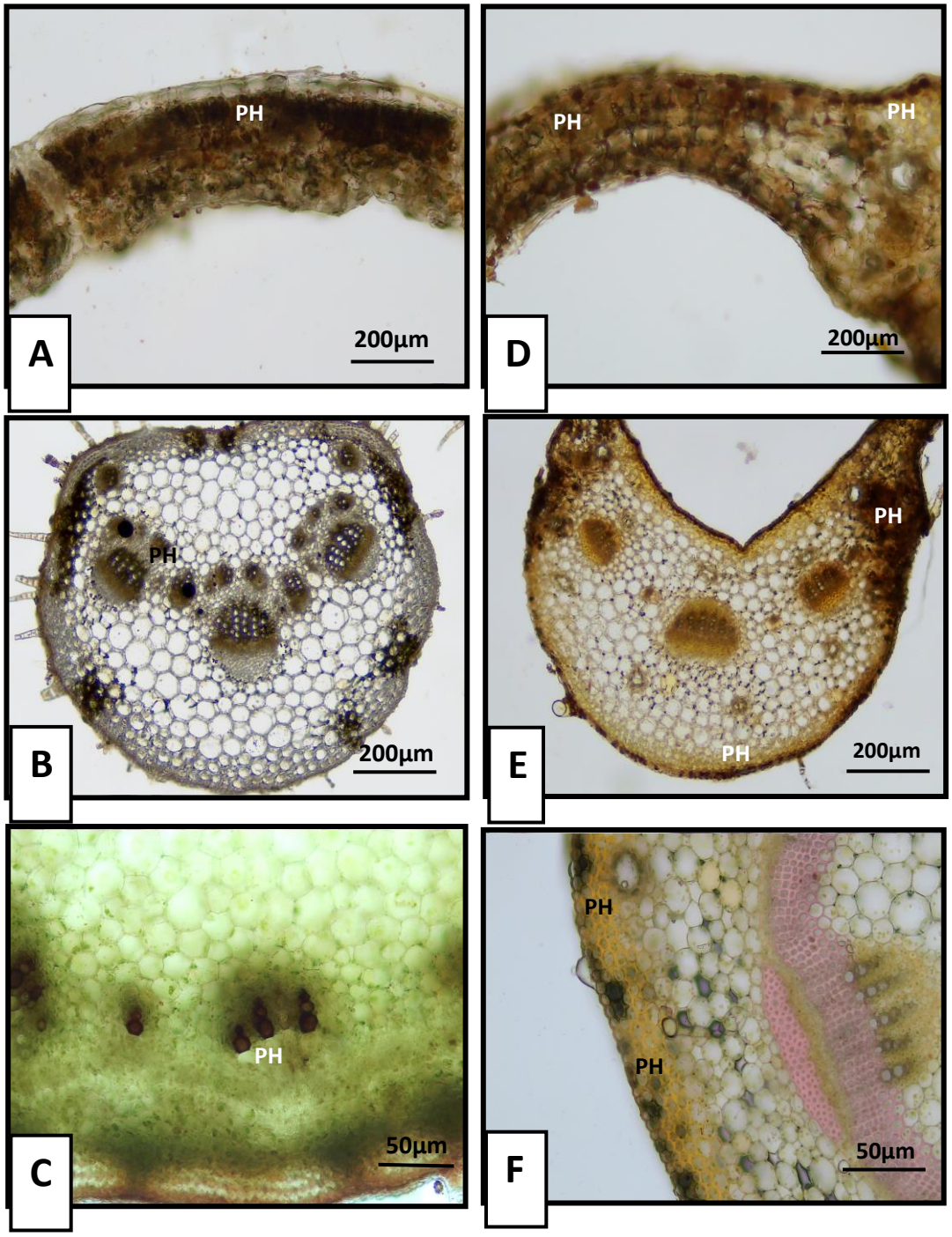


Plate 8: The T.S. of leaf, petiole and stem showing the presence of Phenolics

(A-C) T.S. of *C.odorata* (A) Leaf (10X), (B)Petiole (10X), (C)Stem (40X);

(D-F) T.S. of *S.trilobata* (A) Leaf (10X), (B)Petiole (10X), (C)Stem (40X),

ALK - Alkaloids

Table 2. Extraction yield of *C.odorata* and *S.trilobata* in percentage

Solvents used for extraction	The plant used in extraction with the type of extraction method			
	Chromolaena odorata		***Sphagneticola trilobata***	
	Soxhlet extraction	**Maceration**	**Soxhlet extraction**	**Maceration**
Methanol	17.05%	11.763%	16.72%	10.87%
Chloroform	5.96%	4.91%	6.61%	5.6%
Ethyl acetate	6.432%	5.149%	7.073%	6.18%
Distilled water	19.053%	13.62%	18.603%	12.14%

5.4. Preliminary quantitative phytochemical analysis of selected plants

The dried leaf powder of *C.odorata* and *S.trilobata* were processed with Soxhlet and maceration extraction process using various solvents; methanol, chloroform, ethyl acetate and water, which resulted in the separation of constituents of different polarity ranges in different solvent extracts.

In *C. odorata* for methanolic extract alkaloids, carbohydrates, glycosides, proteins, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins are found to be present and amino acid was absent in the Soxhlet method whereas in maceration alkaloids and amino acids were unable to detect. In chloroform extract, only two tests showed positive results are fixed oils and fats and phytosteroids and in other tests alkaloids, carbohydrates, glycosides, proteins, terpenoids, phenolic compounds, quinone and saponins and amino acids were found to be absent in both the extract. In ethyl acetate extract for the Soxhlet method alkaloids, carbohydrates, glycosides, phytosteriods, terpenoids, fixed oils and fats and quinone showed positive results and saponins, proteins, amino acids and phenolic compounds were found to be absent whereas maceration method showed the presence of carbohydrates, phytosteriods, fixed oils and fats and other tests showed the negative results.

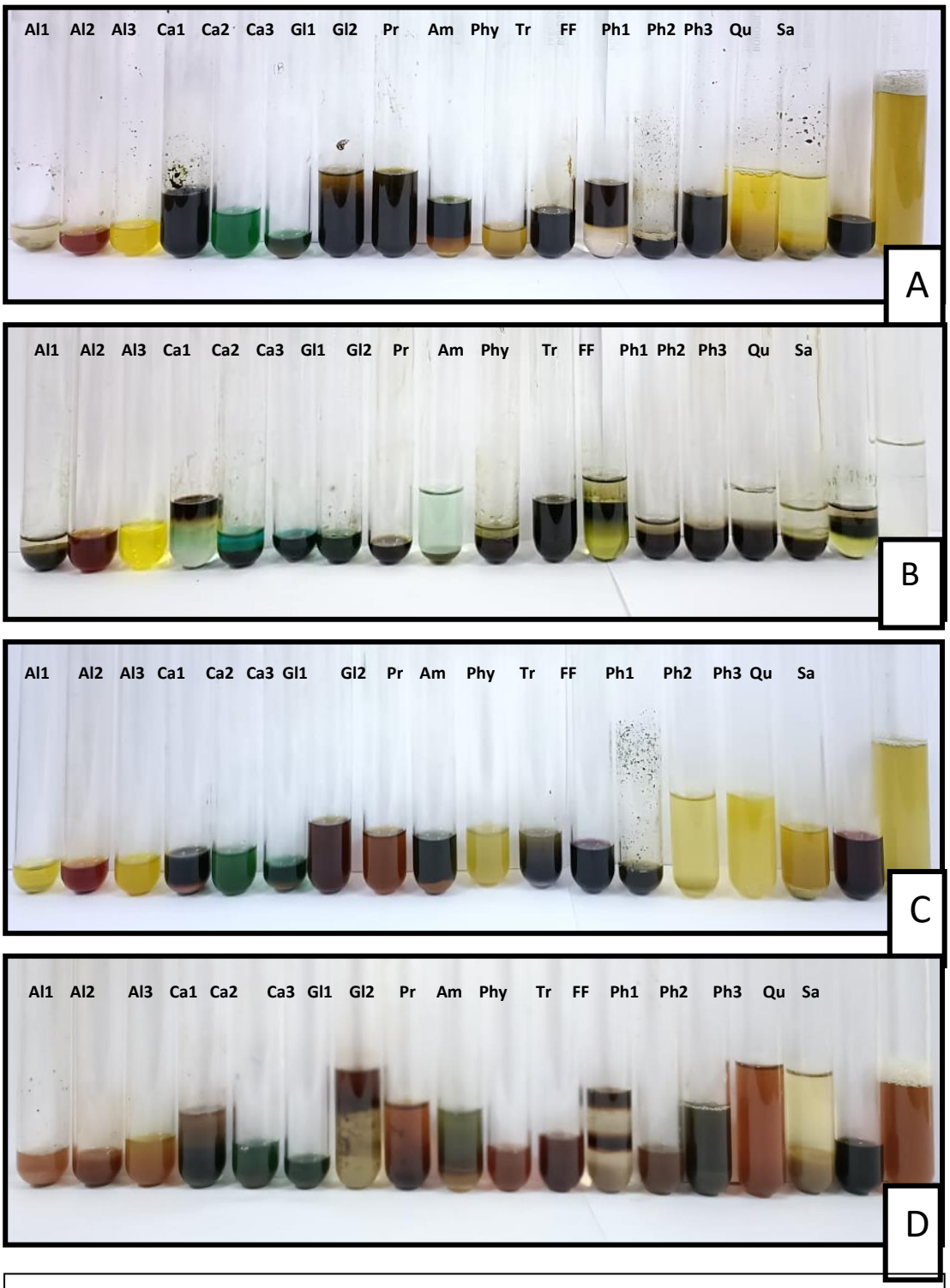


Plate 9: Preliminary qualitative phytochemical analysis of *C. odorata* through Soxhlet extraction method using different solvents. (A) Methanol (B) Chloroform (C) Ethyl acetate (D) Distilled water.

(Al1–Al3) = Alkaloids, (Ca1-Ca3) = Carbohydrates, (Gl1-Gl2) = Glycosides, (Pr) = Proteins, (Am) = Amino acids, (Phy) = Phytosteroids, (Tr) = Terpanoids, (FF) = Fixed oils and Fats, (Ph1–Ph3) = Phenolic compounds, (Qu) = Quinones, (Sa) = Saponin

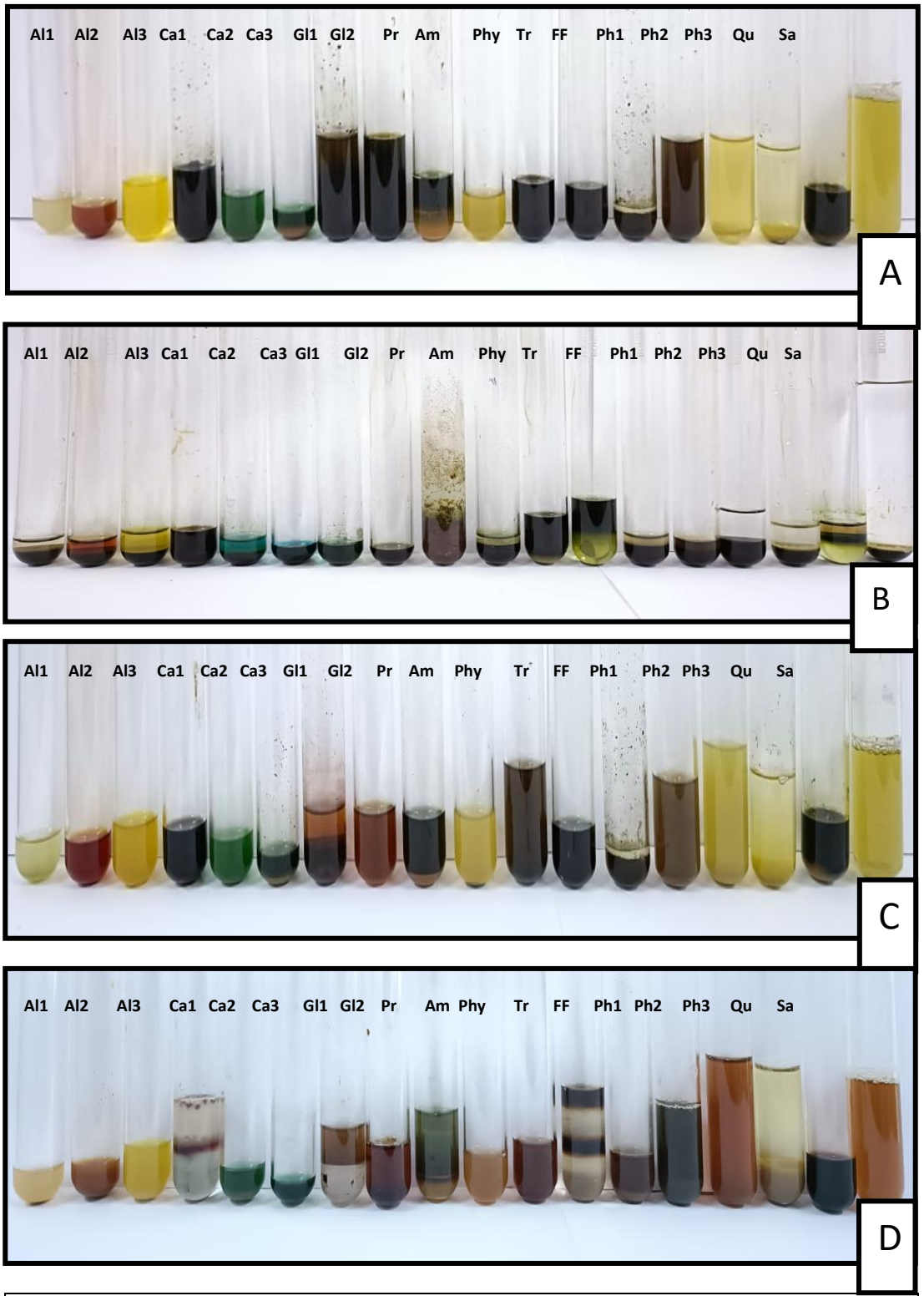


Plate 10: Preliminary qualitative phytochemical analysis of *C. odorata* through Maceration method using different solvents. (A) Methanol (B) Chloroform (C) Ethyl acetate (D) Distilled water.

(Al1–Al3) = Alkaloids, (Ca1-Ca3) = Carbohydrates, (Gl1-Gl2) = Glycosides, (Pr) = Proteins, (Am) = Amino acids, (Phy) = Phytosteroids, (Tr) = Terpanoids, (FF) = Fixed oils and Fats, (Ph1–Ph3) = Phenolic compounds, (Qu) = Quinones, (Sa) = Saponin

In water extract alkaloids, carbohydrates, glycosides, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins were found present it almost showed the same results as in methanol. Whereas in maceration only fats and oils, carbohydrates and saponins were found to be present and other tests showed negative results (Table 3) (Plate 9 & 10).

In *S. trilobata* alkaloids, carbohydrates, glycosides, proteins, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins were found to be present in the methanolic extract of Soxhlet extraction method and only amino acid was found to be absent. Whereas in the maceration method for methanol alkaloids, carbohydrates, proteins, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds and quinone were detected. For chloroform extract, only two tests showed positive results namely, phytosteroids and fixed oils and fats and all other tests were found to be absent for maceration, only phytosteroids and fixed oils were present these results were the same as for *C. odorata* extract in maceration. For the ethyl acetate extract in the Soxhlet extractor alkaloids, carbohydrates, glycosides, phytosteroids, terpenoids, quinones, fixed oils and fats, showed positive results and the rest of the other tests were negative and in maceration alkaloids, carbohydrates, phytosteroids, phenolic compounds and fats and oils were present and other test showed negative results. For distilled water alkaloids, carbohydrates, glycosides, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins are found present and in maceration alkaloids glycosides, proteins and terpenoids were found absent **(Table 4) (Plate 11 & 12).**

The result obtained for the qualitative screening of phytochemicals of leaves *C. odorata* is presented in Table 3, and the results of *S. trilobata* are presented in Table 4. For *C. odorata* and *S. trilobata*, eleven phytochemicals were screened and out of the eleven, ten were found

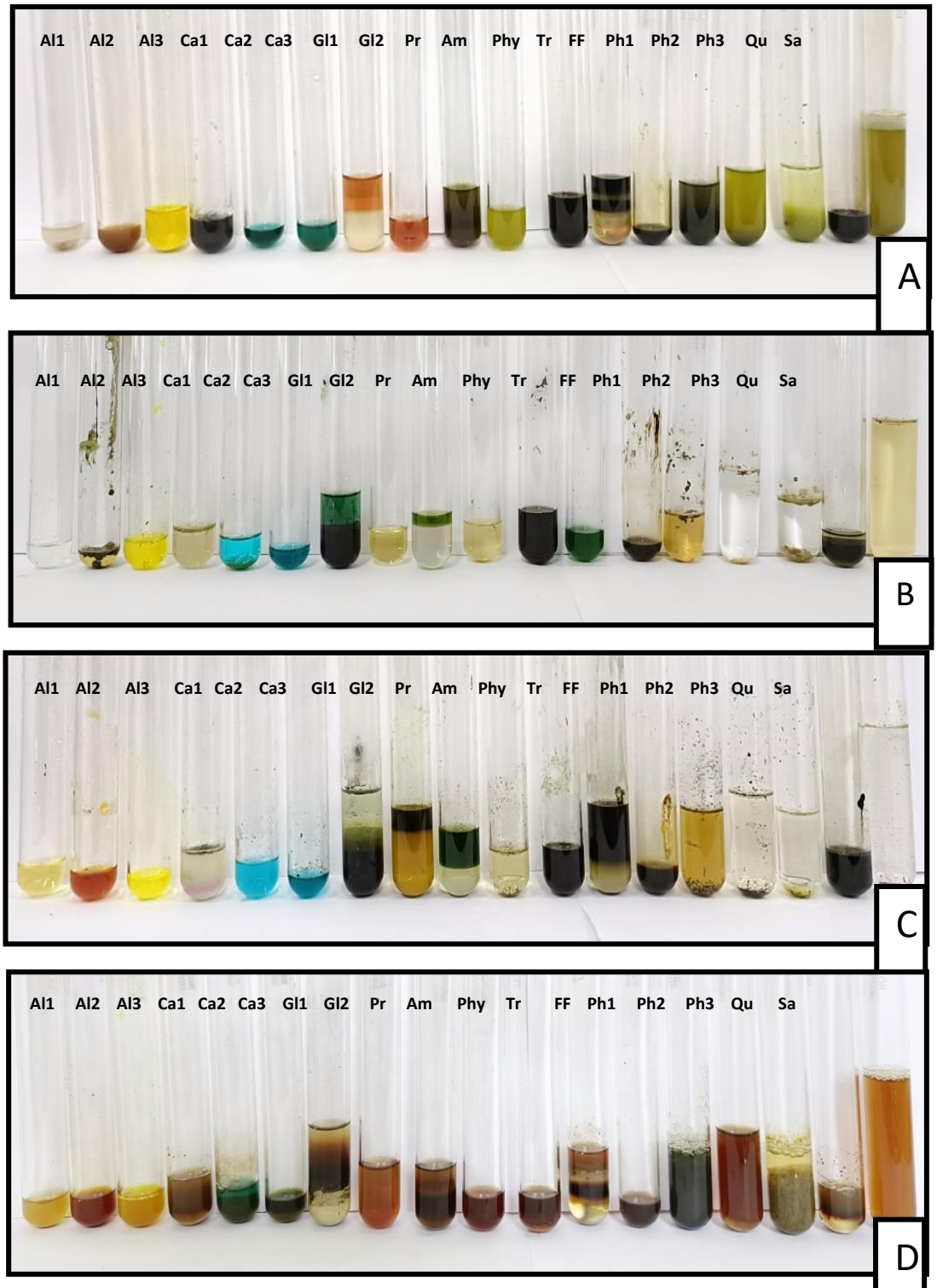


Plate 11: Preliminary qualitative phytochemical analysis of *S.trilobata* through Soxhlet extraction method using different solvents. (A) Methanol (B) Chloroform (C) Ethyl acetate (D) Distilled water.

(Al1–Al3) = Alkaloids, (Ca1-Ca3) = Carbohydrates, (Gl1-Gl2) = Glycosides, (Pr) = Proteins, (Am) = Amino acids, (Phy) = Phytosteroids, (Tr) = Terpanoids, (FF) = Fixed oils and Fats, (Ph1–Ph3) = Phenolic compounds, (Qu) = Quinones, (Sa) = Saponin

present in various solvent extracts. They are alkaloids, carbohydrates, glycosides, proteins, phytosteroids, terpenoids, fixed oils & fats, phenolic compounds, and quinones. The amino acids. For the distilled water for the Soxhlet extractor, it shows positive results for alkaloids

In all, more phytochemicals were found present in the extract prepared with methanol. For methanol in Soxhlet extraction, ten tests showed positive results, for chloroform just two showed the presence of phytochemicals, ethyl acetate showed the presence of seven phytochemicals and nine for water. For maceration despite using the same solvent, it showed a difference. As compared to the Soxhlet fewer phytochemicals showed the presence of the test. in maceration it showed positive results for nine tests, in chloroform, it showed 2, ethyl acetate 5 and in distilled water 7 positive results. The intensity was also noted.

Previously Akinmoladun (2007) conducted phytochemical investigations on *C.odorata* by using methanol and water as a solvent and they discovered steroids, alkaloids, terpenoids and glycosides but alkaloids were reported absent in water and saponins in methanol. Muricken and Joy (2015) conducted similar studies with the single solvent methanol and found the same result for saponins whereas phytosteroids and quinones. Whereas Panda *et al.* reported the presence of saponins, alkaloids, glycosides, and phenolic compounds, the solvent employed as ethyl acetate reported the presence of alkaloids, steroids phenolic compounds and saponins, glycosides, proteins were found missing. The current study showed similarities with the finding mentioned above with one or other tests but they did not report the presence of proteins but current study found the presence of proteins in the plant extract.

Rashid *et al*. (2022) conducted a study on the phytochemistry of *S. trilobata* which revealed the presence of terpenoids and saponins in water extract and amino acids, carbohydrates, alkaloids, steroids, proteins, fats in methanolic extract and only terpenoids

were reported present in chloroform. Similar results were given by Sutar and parveen in 2020, they used methanol as a solvent which reported the presence of saponins, alkaloids, phenolic compounds, proteins, and phytosteroids but they had shown the absence of glycosides. Prasanth's 2018, the study reported similar results as shown in the present documentation, aqueous and chloroform solvents were used the chloroform showed only the presence of steroids whereas the aqueous extract reported the presence of saponins, amino acids, carbohydrates, glycosides, steroids, phenolic compounds present study differs in the absence of amino acids when compared with other research findings.

Table 3. Preliminary phytochemical analysis of *Chromolaena odorata.*

Phytochemical test	Soxhlet Extraction				Maceration			
	M	**C**	**EA**	**DW**	**M**	**C**	**EA**	**DW**
1)Alkaloids: - a)Mayer's Test	+	-	+	-	-	-	-	-
b) Wagner's Test	+	-	-	+	-	-	-	-
c) Hager's Test	-	-	-	-	-	-	-	-
2)Carbohydrates: -a)Molish'sTest	+	-	+	+	+	-	+	+
b) Barfoed's Test	-	-	-	-	-	-	-	-
c) Benedict's Test	-	-	-	-	-	-	-	-
3)Glycosides:-a) Borntrager's Test	-	-	-	-	-	-	-	-
b) Legal's Test	+	-	+	+	+	-	-	-
4)Protein:-a) Biuret Test	+	-	-	-	+	-	-	-
5) Amino acid:- a) Ninhydrin Test	-	-	-	-	-	-	-	-
6)Phytosteroids:- a) Liebermann-Burchard's Test	++	++	++	+	+	+	+	+
7)Test for Terpenoids	++	-	+	+	+	-	-	+
8)Fixed oil &Fats: - a)Saponification Test	++	+	+	+	+	+	+	+
9)Phenolic Compounds: - a)Ferric Chloride Test	++	-	-	++	+	-	-	++
b) Gelatin Test	++	-	-	-	-	-	-	-
c)Lead acetate Test	++	-	-	++	+	-	-	++
10)Test for Quinones	++	-	+	+	+	-	-	-
11)Saponins - Foam Test	+	-	-	+	+	-	-	+

(++): Very intense ; (+): Intense ; (-) :Absent

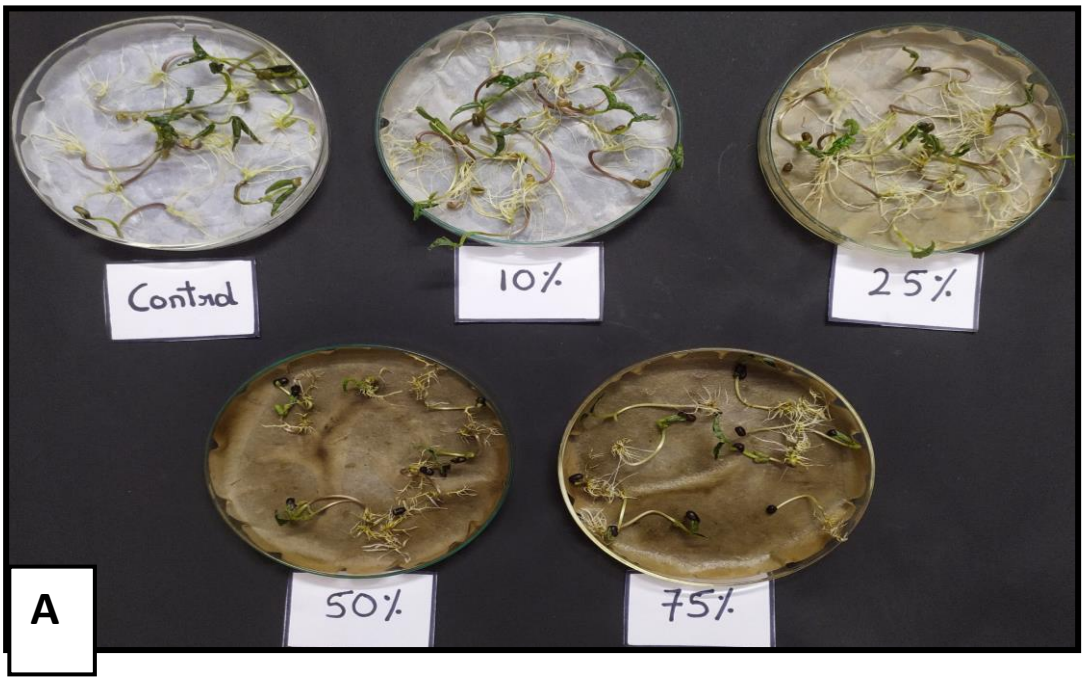
Table 4. Preliminary phytochemical analysis of *Sphagneticola trilobata*

Phytochemical test	Soxhlet Extraction				Maceration			
	M	**C**	**EA**	**DW**	**M**	**C**	**EA**	**DW**
1)Alkaloids: - a)Mayer's Test	++	-	+	-	-	-	-	-
b) Wagner's Test	+	-	-	+	-	-	-	-
c) Hager's Test	+	-	-	-	-	-	-	-
2)Carbohydrates: -a) Molish'sTest	+	-	+	+	+	-	+	+
b) Barfoed's Test	-	-	-	-	-	-	-	-
c) Benedict's Test	-	-	-	-	-	-	-	-
3)Glycosides a) Borntrager's Test	-	-	-	-	-	-	-	-
b) Legal's Test	+	-	+	+	-	-	-	-
4)Protein a) Biuret Test	+	-	-	-	+	-	-	-
5) Amino acid a) Ninhydrin Test	-	-	-	-	-	-	-	-
6)Phyto-steroids a) Liebermann-Burchard's Test	++	++	++	+	+	+	+	+
7)Test for Terpenoids	++	-	+	+	+	-	-	-
8)Fixed oil &Fats: - a)Saponification Test	+	+	+	+	+	+	+	+
9)Phenolic Compounds: - a) Ferric Chloride Test	++	-	-	++	++	-	+	++
b) Gelatin Test	+	-	-	-	-	-	-	-
c)Lead acetate Test	++	-	-	++	++	-	+	++
10)Test for Quinones	++	-	+	+	+	-	+	+
11)Saponins - Foam Test	+	-	-	+	-	-	-	+

5.5. Allelopathic effect

The allelopathic effect shoot elongation of three receptor agricultural seeds to distilled water (T_0) and different concentration of *C. odorata* leaf extracts (T_1 - T_4) was measured and the results are given in Table no.5 for *C. odorata* and Table no.7 for *S. trilobata*. The results clearly showed that the leaf extract of *C. odorata* showed an inhibitory effect on all three *V. radiata, V. mungo* and *B. nigra*. It was observed that as the concentration increases the inhibitory effect also increases that is they suppress the growth of another crop plant. But when compared to the shoot elongation of *V. radiata*, *V. mungo* and *B. nigra* By using the leaf extract of *S. trilobata* as the donor plant different results were depicted than the *C. odorata.* When aqueous extract of the *S. trilobata* plant was used in some cases stimulation of the shoot was observed. The highest stimulation effect (+3.096) was observed in *B. nigra* at 25% concentration, then it was also noted that 25% for *V. radiata* did not show the stimulation whereas in the same concentration *V. mungo* and *B. nigra* showed the stimulation. In 10% concentration, all three seeds showed an increase in growth length and more in *B. nigra*. The other two concentrations decreased the length of the shoot. for *V. mungo* 10% showed more increase in length than 25% similar results were observed for *V. radiata*. Among the survivors, the highest inhibitory effect in *C. odorata* (-9.626) was found on *V. mungo* at 75% concentration and in *S. trilobata* (-6.174) for the same plant and same concentration, while the lowest inhibitory effect of *C. odorata* (-0.504) was found on *B. nigra* at 10% concentration and in *S. trilobata* (-0.19) was found in *V. radiata* at 20% concentration.

The allelopathic effect root elongation of three receptor agricultural seeds to distilled water (T_0) and different concentration of *C. odorata* leaf extracts (T_1 - T_4) was measured and the results are given in Table no.6 for *C. odorata* and Table no.8 for *S. trilobata*. For *C. odorata* all the concentrations showed the inhibitory effect. Among the survivors plant the



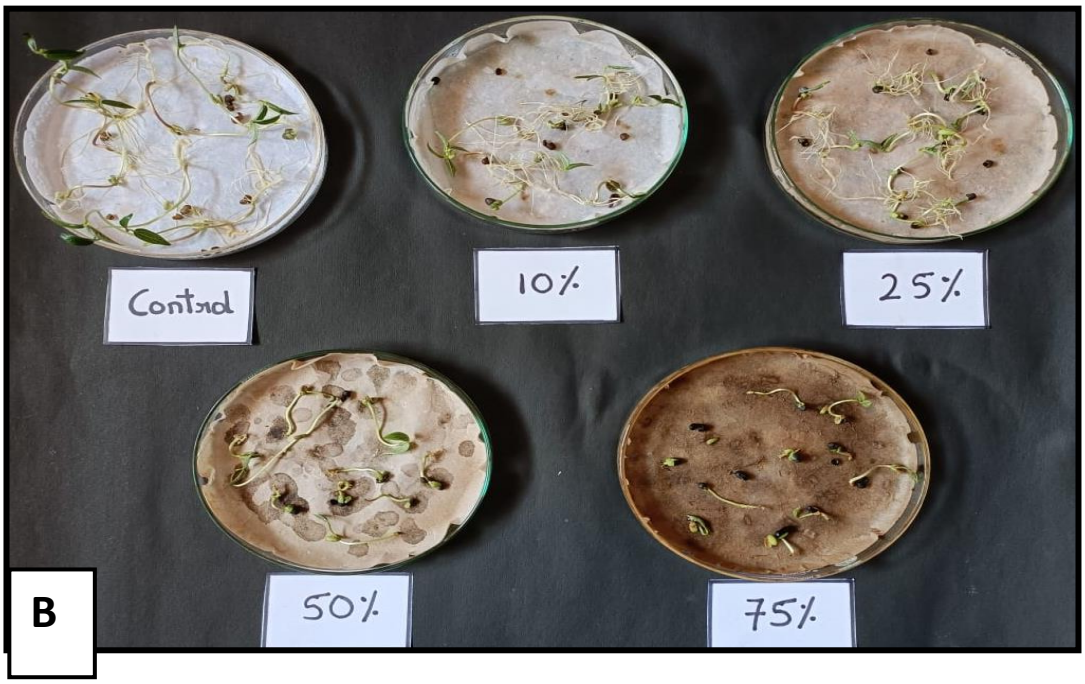


Plate 13: Seeds treated with leaf extract of *C. odorata*: (A) *V. radiata* seeds, (B) *V. mungo* seeds. The % given is the concentration of the extract

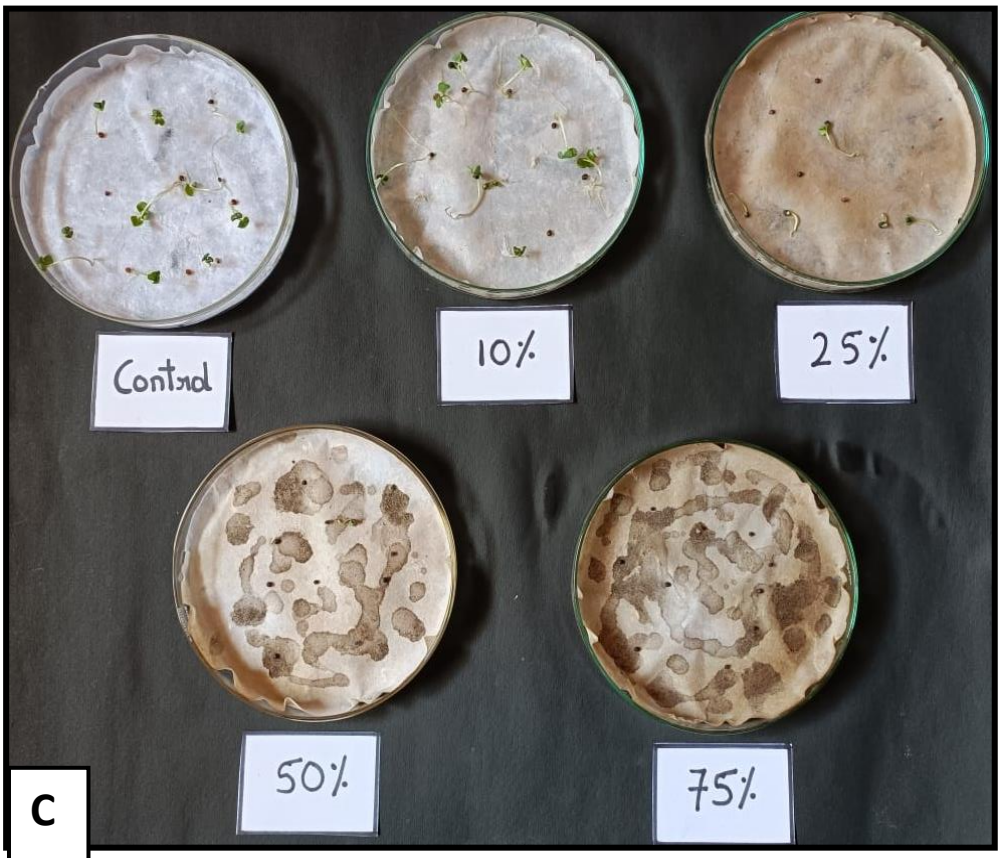


Plate 14: Seeds treated with leaf extract of *C. odorata* (C) *B. nigra* seeds. The % given is the concentration of the extract

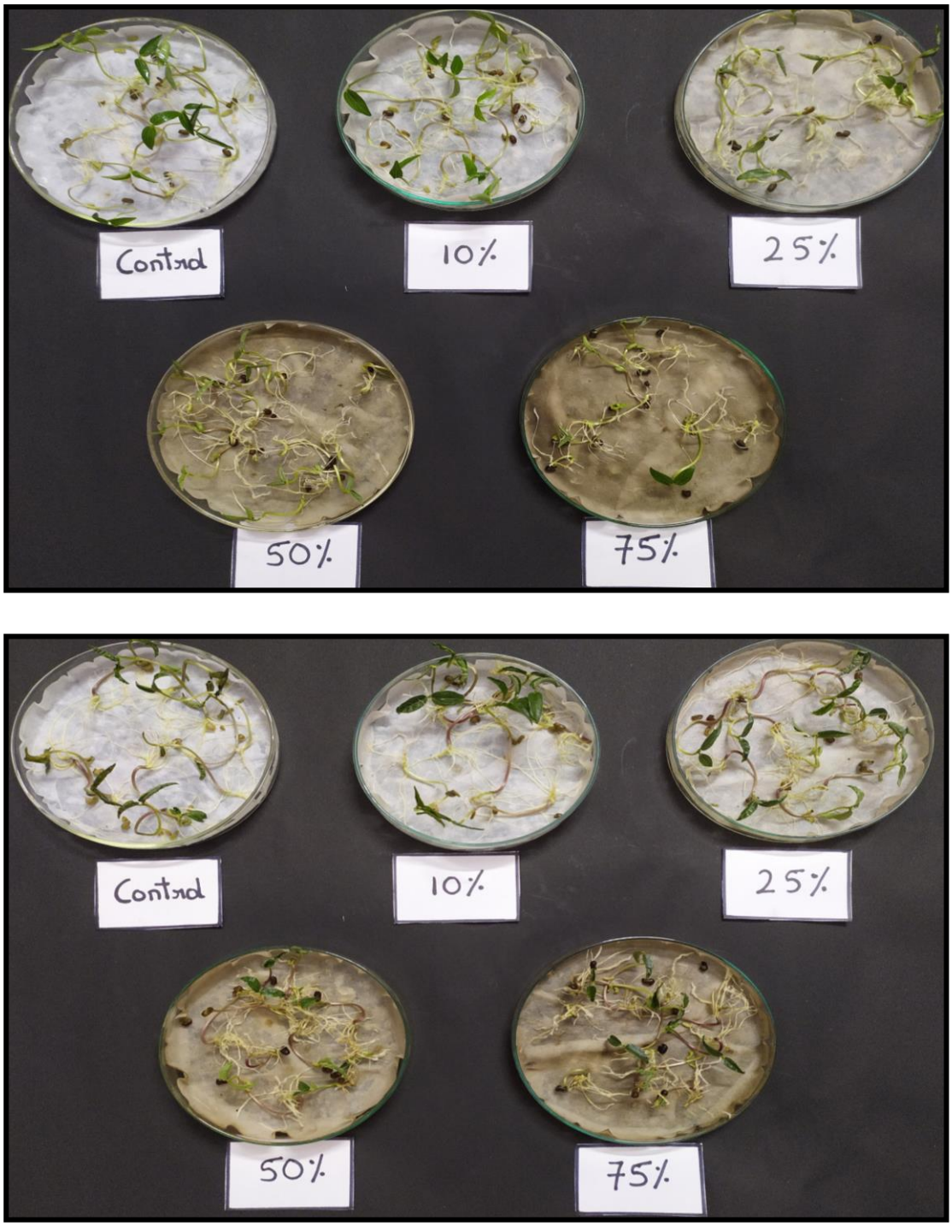


Plate 15: Seeds treated with leaf extract of *S. trilobata* (A) *V. radiata* seeds (B) *V. mungo* seeds. The % given is the concentration of the extract.

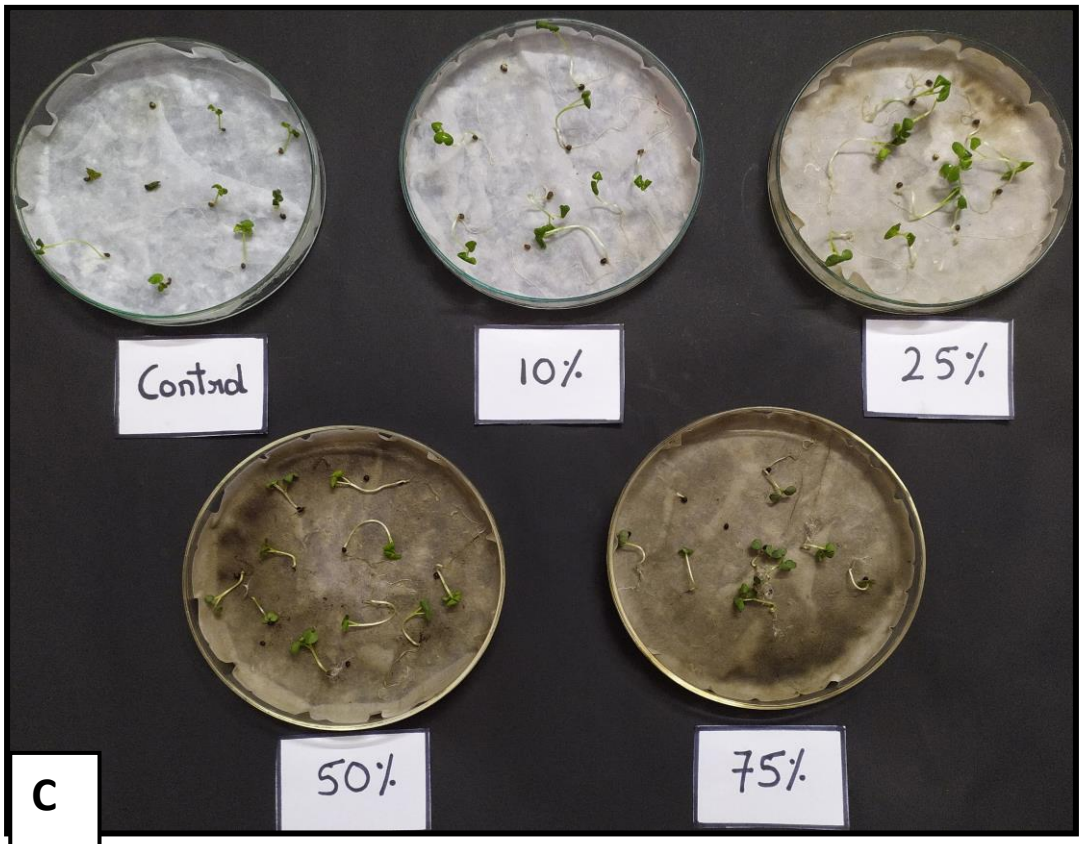


Plate 16: Seeds Treated with leaf extract of *S. trilobata* (C) *B. nigra* seeds

The % given is the concentration of the extract.

highest inhibition effect (-6.37) was found in *V. mungo* at 75% followed by (-5.562) found in the same crop plant at 50% concentration. 10% concentration showed the lowest inhibition it increases as the concentration increases. For *S. trilobata*. Stimulatory growth was observed for all three crops two of them showed stimulation in two concentrations namely for 10% and 25% of *V. mungo* and *B. nigra* and for only *V. radiata* 10% showed stimulatory growth. The highest stimulatory effect (+1.64) was found to be present *in V. mungo* and the lowest (+0.17) was found in *B. nigra*.

These findings are correlated with the findings of Hoque (2003); Ahemad (2008); Sharma *et al*. (1967); Eyini *et al*. (1989) reported the inhibitory effect of leaf extract of some agroforestry tree species on certain agricultural crops. A similar observation was found by Rizvi and Rizvi (1987); Hernadez *et al*. (2016). Ghayal and Nirgundikar (2020); Hoque (2003); Kumar *et al*. (2007); Madane and Bhimrao (2017); Zhang (2013); Shahena (2021); Who found the inhibitory effect of leaf extracts of *C. odorata* and *S. trilobata* on the different varieties of agricultural crops and they found the similar results that at certain concentration for certain crop plant root or shoot growth increases and as the concentration increases the root and shoot length decreases.

Table 5. The shoot elongation (cm) receptor agricultural seed to distilled water (T_0) and different concentration of *C. odorata* leaf extracts (T_1 - T_4).

S. No	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	**T_0**	8.786 ± 0.205993527	9.926 ± 0.060277138	3.41 ± 0.4327817
2	**T_1**	7.536 ± 0.36828431 (-1.25)	7.7 ± 0.199749844 (-2.226)	2.906± 0.205507502 (-0.504)
3	**T_2**	4.033 ± 0.221885857 (-4.753)	6.546 ± 0.361985267 (-3.38)	0.96 ± 0.045825757 (-2.45)
4	**T_3**	2.46 ± 0.398873414 (-6.326)	4.546 ± 0.28501462 (-5.38)	0.17 ± 0.052915026 (-3.24)
5	**T_4**	0.893 ± 0.077674535 (0.893)	0.3 ± 0.055677644 (-9.626)	0

(-) Shows the inhibitory effect when compared to the control

Table 6. The root elongation (cm) receptor agricultural seed to distilled water (T_0) and different concentration of *C. odorata* leaf extracts (T_1 - T_4).

S. No	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	**T_0**	6.08 ± 0.413279566	6.576 ±0.355011737	4.65 ± 0.563205114
2	**T_1**	5.51 ± 0.300499584 (-0.57)	5.816 ±0.170098011 (-0.76)	3.473 ± 0.398036849 (-1.177)
3	**T_2**	3.833 ± 0.661991944 (-4.03)	4.826 ±0.378197479 (-5.563)	0.36 ± 0.11 (-4.29)
4	**T_3**	2.05 ±0.226053091 (-4.03)	1.013 ±0.205264058 (-5.563)	0.07 ± 0.02 (-4.58)
5	**T_4**	0.876 ± 0.070237692 (-5.204)	0.206 ±0.066583281 (-6.37)	0

(-) Shows the inhibitory effect when compared to the control

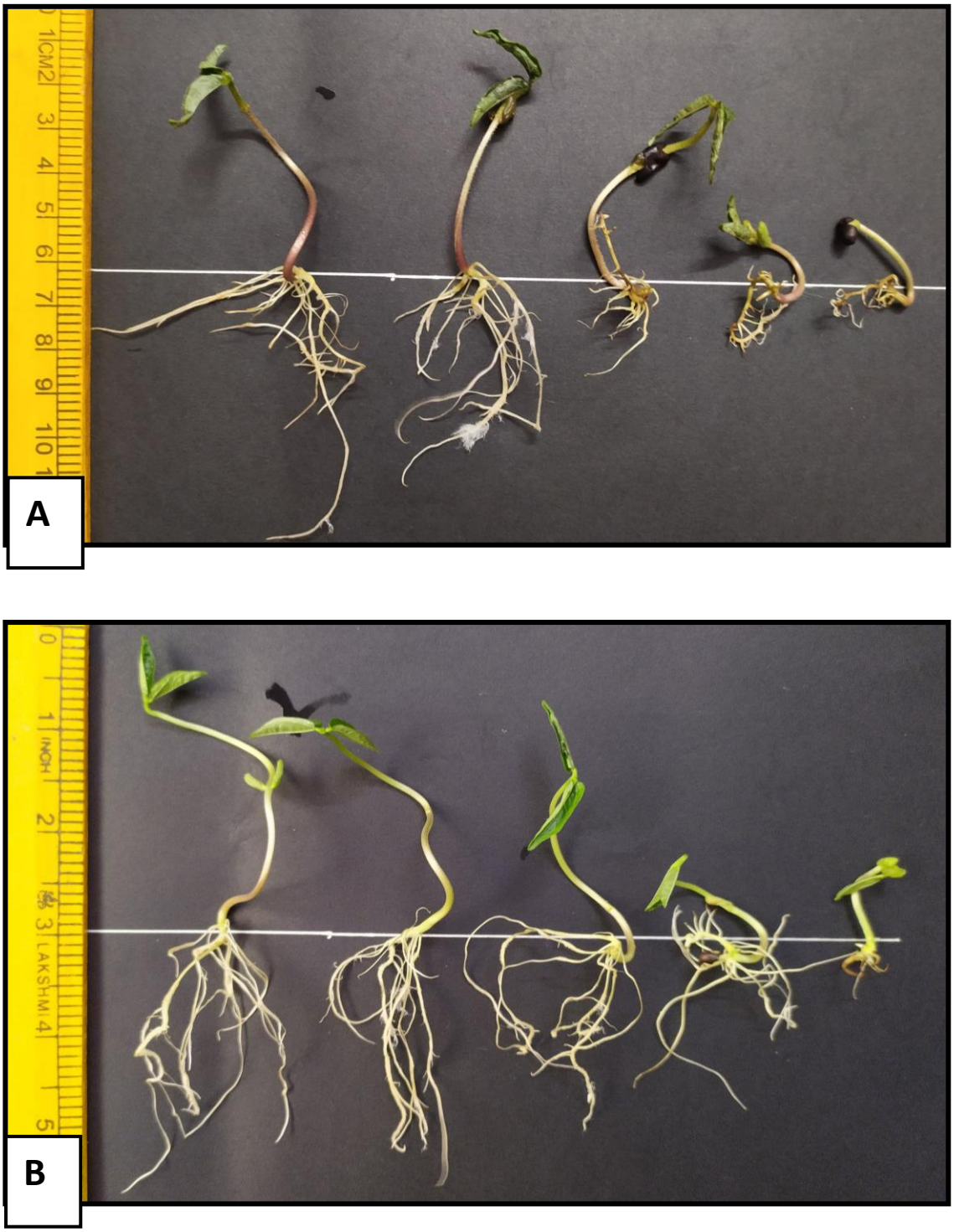


Plate 17: Seedling Growth after 7 days treated with *C. odorata* leaf extract: (A) *V. radiata* seedlings, (B) *V. mungo* seedlings.

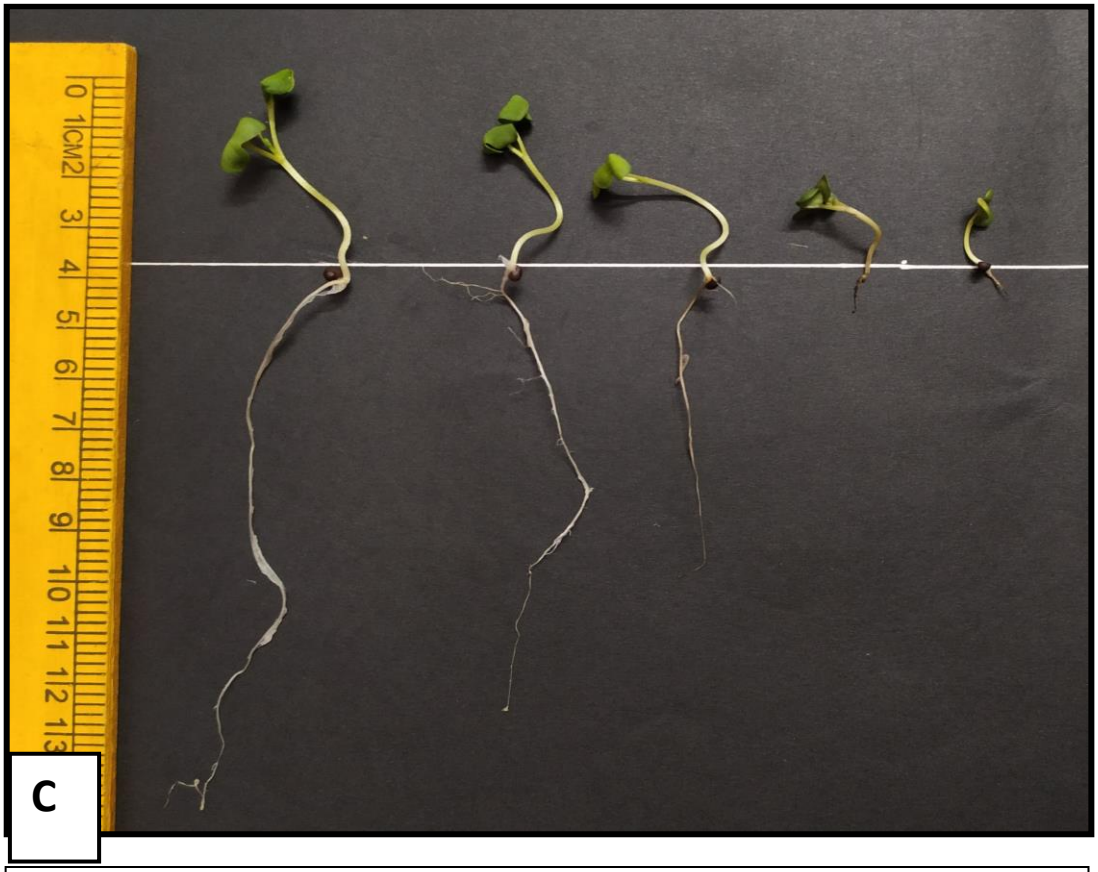


Plate 18: Seedling Growth after 7 days treated with *C. odorata* leaf extract

(C) *B. nigra* seedlings

Table 7:- The shoot elongation (cm) receptor agricultural seed to distilled water (T_0) and different concentration of *S. trilobata* leaf extracts (T_1 - T_4).

S. No	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	T_0	9.193 ± 0.0321455	10.72 ± 0.121243557	2.2 ± 0.117898
2	T_1	10.486 ± 0.07023769 (+1.293)	11.653 ± 0.155026879 (+0.933)	5.103 ± 0.066583 (+2.903)
3	T_2	9.003 ± 0.07023769 (-0.19)	10.946 ± 0.085049005 (+0.226)	5.296 ± 0.050332 (+3.096)
4	T_3	7.99 ± 0.45033321 (-1.203)	8.863 ± 0.185831465 (-1.857)	1.87 ± 0.052915 (-0.33)
5	T_4	7.146 ± 0.19857828 (-2.047)	4.546 ± 0.473532822 (-6.174)	0.93 ± 0.065574 (-1.27)

(-) Shows the inhibitory effect and (+) shows the stimulating effect when compared to the control

Table 8:- The root elongation (cm) receptor agricultural seed to distilled water (T_0) and different concentration of *S. trilobata* leaf extracts (T_1 - T_4).

Sr.no	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	T_0	8.13 ± 0.03	5.873 ± 0.125033329	9.666 ± 0.083267
2	T_1	8.533 ± 0.08962886 (+0.403)	7.513 ± 0.126622799 (+1.64)	9.836 ± 0.090185 (+0.17)
3	T_2	7.4 ± 0.45639895 (-0.266)	6.8 ± 0.181934054 (+0.927)	10.346 ± 0.315647 (+0.68)
4	T_3	5.47 ± 0.43714986 (-2.66)	5.25 ± 0.122882057 (-0.623)	3.14 ± 0.216333 (-6.256)
5	T_4	4.726 ± 0.09073772 (-3.404)	4.506 ± 0.372334975 (-1.367)	1.336 ± 0.336502 (-8.33)

(-) Shows the inhibitory effect and (+) shows the stimulating effect when compared to the control

Plate 19: Seedling Growth after 7 days treated with *S. trilobata* leaf extract

(A) *V. radiata* seedlings, (B) *V. mungo* seedlings.

Plate 20: Seedling Growth after 7 days treated with *S. trilobata* leaf extract (C) *B. nigra* seedlings.

Table 9. Germination % of seeds treated with *C. odorata*

Sr.No	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	**T_0**	100%	100%	96.66%
2	**T_1**	100%	100%	83.33%
3	**T_2**	100%	96.66%	56.33%
4	**T_3**	86.6%	86.66%	33.33%
5	**T_4**	80%	56.66%	0

Table 10. Germination % of seeds treated with *S. trilobata*

Sr.No	Treatment	*V. radiata*	*V. mungo*	*B. nigra*
1	**T_0**	100%	100%	100%
2	**T_1**	100%	100%	100%
3	**T_2**	100%	100%	100%
4	**T_3**	100%	100%	96.66%
5	**T_4**	100%	100%	86.66%

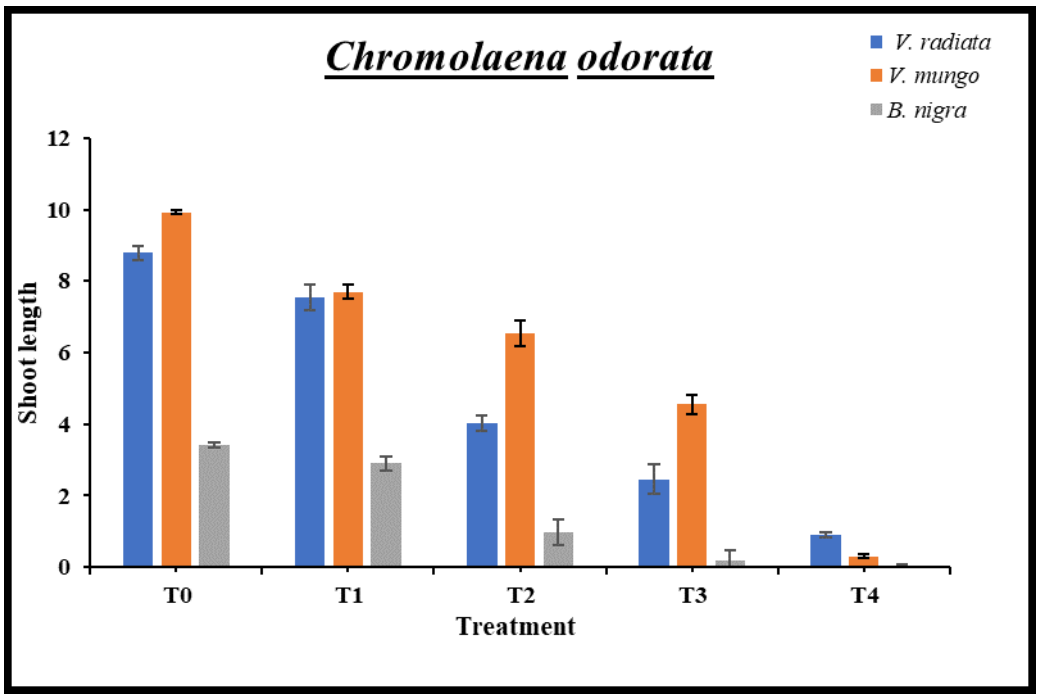


Fig 4: Shoot elongation in the bioassay of species grown in Petri plate at different concentrations of *C. odorata extract*

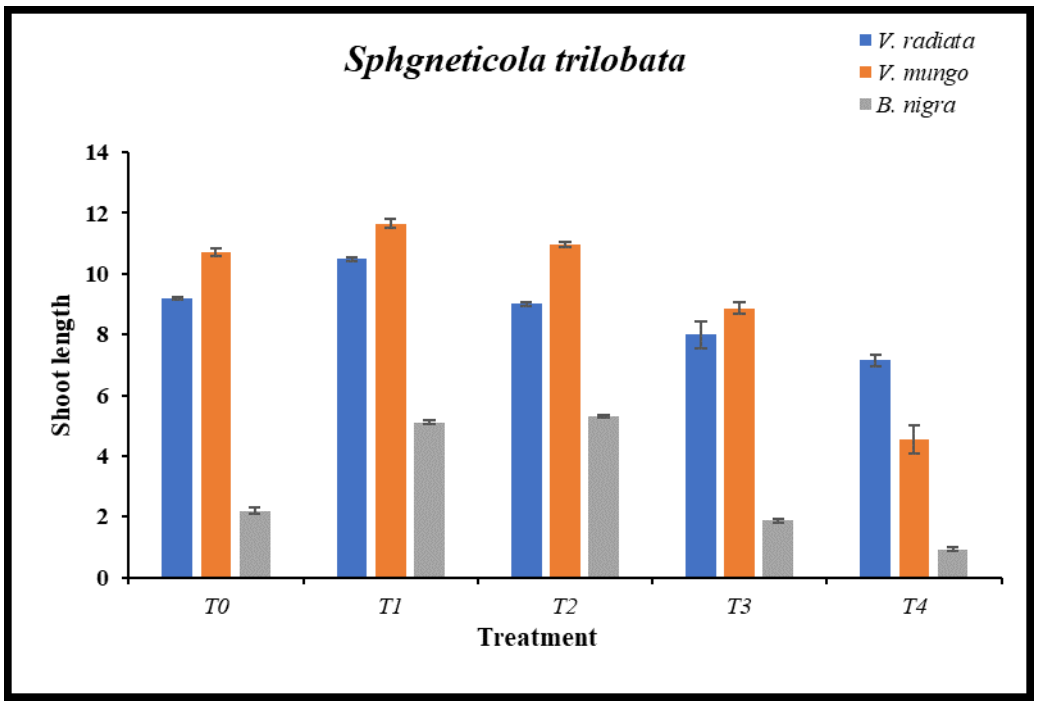


Fig 5: Shoot elongation in the bioassay of species grown in Petri plate at different concentrations of *S. trilobata extract*

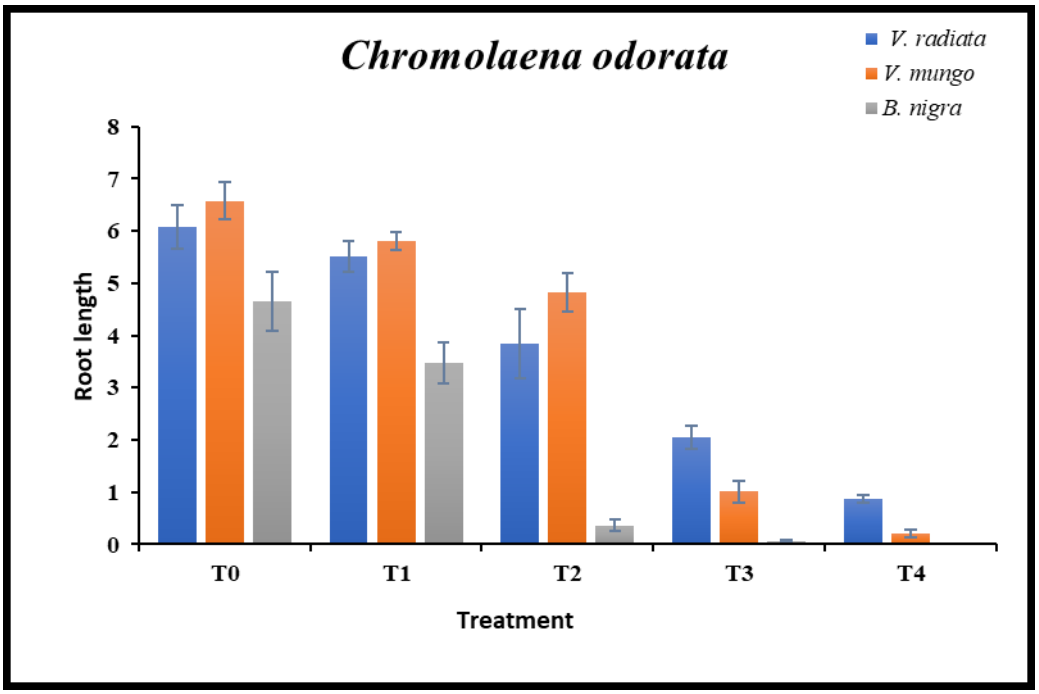


Fig 6: Root elongation in the bioassay of species grown in Petri plate at different concentrations of *C. odorata extract*

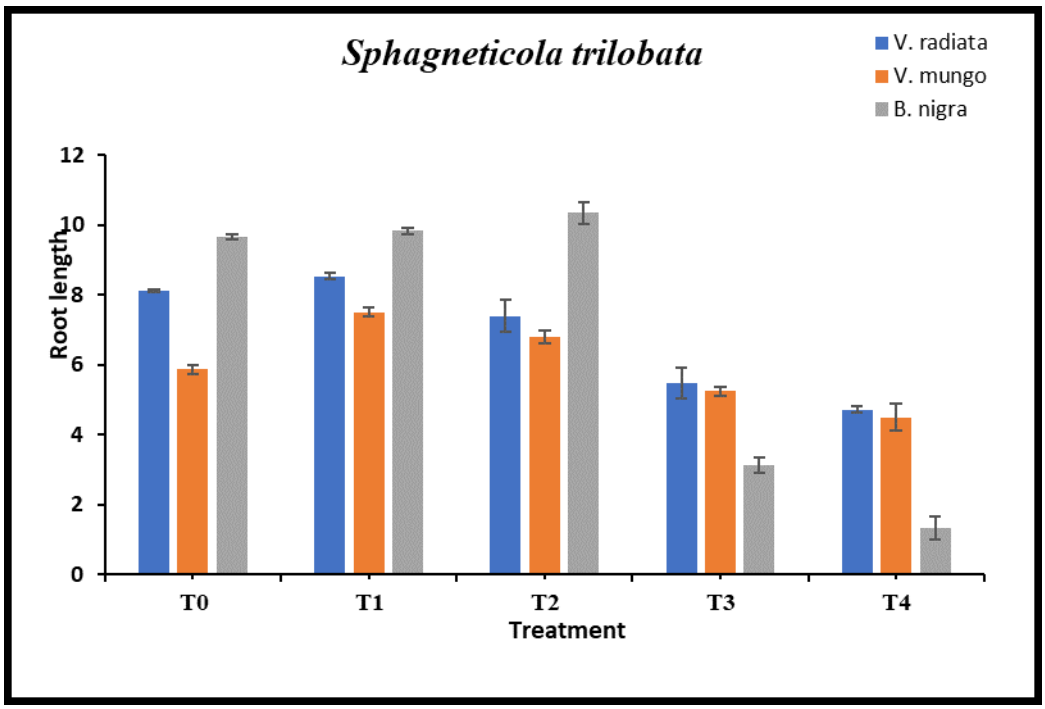


Fig 7: Root elongation in the bioassay of species grown in Petri plate at different concentrations of *S. trilobata extract*

CHAPTER 6: CONCLUSION

CONCLUSION

The present study on the anatomical characterization of two invasive plants viz. *Chromolaena odorata* and *Sphagneticola trilobata* revealed the characteristics which are present in the invasive plants and are responsible for the invasiveness of the plants. The notable among these characteristics is the presence of sclerenchymatous tissues in the section of the stem. The sclerenchyma tissue forms a cap around the vascular bundles. Similar results were shown in the study conducted before. Sclerenchymatous tissue is adapted to withstand compressive stresses in plants. These tissues give support to the conductive tissues for the transport of nutrients and water and strengthen vessel walls. The abundance of sclerenchymatous tissues is an indication of xerophytism. There is the presence of a lot of parenchymatous cells and closely packed. The previous documentation revealed that more closely arranged parenchyma cells play an important role in repairing the damage. The presence of wider xylem pores and short vessels both function as support and strength to the stem during the stress period. The medullary rays were prominent in both plants. The long-coiled trichomes present in *C.odorata* is a xeromorphic feature present in invasive species as reported earlier. These plants having a higher amount of trichomes resist herbivory. Trichome has secondary metabolites stored which can be harmful to them. there are other characteristics which are commonly found in plants like secretory cavities. The data recorded in this study shows the anatomical invasiveness of the plant.

A similar trend was also seen in the histochemical tests which were carried out for alkaloids and phenolics. The histochemical studies were carried out on the localization of the secondary metabolites present in both plants. Through the literature review, it was clear that some of the secondary metabolites whose derivatives behave as allelochemicals can either stimulate or suppress the growth of surrounding plants. So phytochemical analyses were performed for detecting the primary and secondary metabolites. There were two methods

used for the preparation of leaf extract; Soxhlet and maceration. Likewise, four solvents were used for extraction viz; methanol, chloroform, ethyl acetate and distilled water. Eleven phytochemical tests were carried out and out of eleven, ten being alkaloids, carbohydrates, glycosides, proteins, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins were found to be present in the methanolic extract of Soxhlet extraction method and only amino acid was found to be absent. It was concluded that methanol is the best solvent for extraction and it depends on the polarity of the solvent. Chloroform showed the least detected phytochemicals. Likewise, the Soxhlet extraction was the suitable extraction method which has been widely used when the desired components have limited solubility in a particular solvent.

As it was concluded from the preliminary test and the secondary metabolites do have allelochemicals so, the allelopathic effect of the plant was checked by carrying out a Petri dish bioassay. The leaves were kept in water for 24h and that was used as a plant extract. The serial dilutions were made and were experimented on three agricultural crops viz; *Brassica nigra*, *Vigna mungo* and *Vigna radiata*. The parameter noted were root and shoot length. The germination % was calculated. *C.odorata* showed the inhibitory effect for all concentrations whereas *S.trilobata* showed an increase in the root and shoot growth at a certain concentration that is 10% and 20% concentration. Similar results have been documented before for different plants and different agricultural crops. Some studies have revealed that *C.odorata* stimulates the growth of certain plants.

CHAPTER 7: SUMMARY

SUMMARY

The two plants from the family Asteraceae were selected namely *Chromolaena odorata* and *Sphagneticola trilobata* both being listed by IUCN red list as invasive plants in Goa. *Chromolaena odorata* is known for its invasive nature and *Sphagneticola trilobata* has invasive nature but because of a lack of knowledge, it is cultivated as an ornamental plant. It has been seen spreading in agricultural land and even on the roadside and not allowing other plants to grow similar in nature with *C. odorata*. The plants were collected from the Goa University campus and further studies were carried out.

The common characteristics found from the anatomical studies were sclerenchymatous tissue, different types of trichomes, the presence of compactly arranged parenchymatous cells, and more common characteristics but this is mainly present in invasive plants. The histochemical test showed the presence of alkaloids and phenolics in higher concentration in the section of the leaf, petiole and stem. The preliminary phytochemical study revealed the presence of many primary and secondary metabolites viz; alkaloids, carbohydrates, glycosides, proteins, phytosteriods, terpenoids, fixed oils and fats, phenolic compounds, quinone and saponins. Methanol is confirmed to be the best solvent and the Soxhlet extraction method showed the best result compared to the maceration method and the yield was the highest in water extract with the Soxhlet extraction method. To confirm the invasive nature, the allelopathic test was carried out, which indicated that when the concentration increases the inhibitory effect also increases and at a certain concentration it shows the stimulation in growth. The germination % was calculated and it shows negative in the *C.odorata* plant. *Sphagneticola trilobata* stimulated the growth of seedlings in the concentration of 10% and 20%. So the allelopathy shows that it can have either a good effect

or a bad effect depending on the concentration and the other plant which is affected. Allelochemicals can be used for better purposes in agriculture with suitable concentrations.

CHAPTER 8: REFERENCES

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National Conference on Recent Trends in Plant Sciences & Biotechnology

Certificate

This is to certify that Dr./Mr/Ms ✓ Sanketa Gawande has attended/ ~~Delivered Lead Lecture~~/ Presented a paper (~~Oral~~/✓Poster) entitled Preliminary phytochemical analysis of "Sphagneticola trilobata (L.) Pruski leaves using maceration and Soxhlet extraction in the National Conference on Recent Trends in Plant Sciences & Biotechnology, held during 3rd & 4th November 2022, at the School of Biological Sciences and Biotechnology (Botany), Goa University, Goa

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