CHAPTER 1:

GENERAL INTRODUCTION AND BACKGROUND STORY

1. GENERAL INTRODUCTION AND BACKGROUND STORY

1.1. Invasive plant species

An invasive alien species is considered, as those species whose introduction cause economic or environmental harm or harm to human health (Clinton 1999). Invasive plant species are notably greats threats to agricultural farms, croplands, orchards, aquatic bodies and wild forests around the globe. The accidental introduction of these species leaves a huge negative impact in the ecological and economical forefront, leading to loss of natural resources and their contamination. They appear to be more competitive than the native species (Vila and Weiner 2004). With high rate of propagation and utilisation of resources, agricultural crops and other natural resources are delimited, making these species a global threat. Immediate requirement and actions need to be implemented and prioritized to safeguard the global economy and environmental balance.

Waterhyacinth is world's one of the most notorious invasive aquatic plant species that have left a significant negative impact in the socio-economic and ecological aspects. The major infestation rate has impacted the waterbody, leading to subsequent ecosystem-level research programmes that can monitor and regulate the effects of this weed in multi-trophic levels, for proper understanding.

1.1.1. *Waterhyacinth*

Eichhornia crassipes (Mart.) Solms-Laub. (water hyacinth; Pontederiaceae) has attracted global attention for its rapid propagation rate, congested growth and the threat it imposes in the associated flora and fauna of the waterbodies, with disruption in the food-web structure, have invoked the need to study the plant and its characteristics.

1.1.1.1. **Taxonomic position and nomenclature**

Eichhornia crassipes, commonly known as waterhyacinth, belong to the family Pontederiaceae (including polyphyletic genus of heterostylous aquatic flowering plants). But off late because of the consistency of being recovered in three independent lineages, has been grouped under sub-genera Ponterderia, along with Monochoria. The three lineages are now grouped under the Pontederia are as follows:

- ***Pontederia*** subg. ***Cabanisia***, which includes *P. meyeri*, *P. paniculata* (the Brazilian waterhyacinth), and *P. paradoxa*
- ***Pontederia*** subg. ***Oshunae***, which includes the common waterhyacinth, *P. crassipes*
- ***Pontederia*** subg. ***Eichhornia***, which includes *P. azurea*, *P. diversifolia*, *P. heterosperma*, and *P. natans*

The taxonomic classification of waterhyacinth is as follows:

Kingdom	:	Plantae
Sub-kingdom	:	Tracheophytes
Super-division	:	Spermatophyta
Division	:	Angiosperms
Class	:	Monocot
Subclass	:	Commelinids
Order	:	Commelinales
Family	:	Pontederiaceae
Genus	:	*Pontederia* L.
Species	:	*Pontederia crassipes*

The plant, waterhyacinth, had earlier been described by Von Martius in 1824 under the name *Pontederia crassipes* from the pond of Minus Gerais region, adjoining Bahia state and river St. Francis, after extensive fossil survey in Brazil. Later it got changed to *Eichhornia speciosa* by Kunth, which was further corrected by Solm-Laubach as *Eichhornia crassipes* in 1883. The term 'Eichhornia' was coined by Kunth, a German Scientist in 1843, in honour of J.A.F. Eichhorn (1779-1856), the then Minister of Education, Culture and Medicine. The term 'waterhyacinth' was coined based on the resemblance of the flower of hyacinth (Family Liliaceae : genus *Hyacinthus*).

Waterhyacinth is the standardized spelling adopted by the Weed Science Society of America (WSSA 1984) to denote that it is not an aquatic relative of 'hyacinth' (*Hyacinthus* sp.). Due to high infestation caused in India, it is commonly known as "Blue Devil" or "Terror of Bengal".

1.1.1.2. **Morphological characters of the weed**

The morphological and biological characteristics of waterhyacinth has been reviewed in 1990 by Harley (Harley 1990). The aquatic weed is a perennial, herbaceous and free-floating in nature and it comprises of a shoot with a rosette of petiolate leaves, having terminal inflorescence and large number of roots hanging in water. The shoots are comprised of a sympodially branched, stoloniferous rhizome with several short intemodes. Each node bears a leaf and roots. The ·axillary buds are of two kinds according to their position and function. They grow out at an angle of about 60° from the rhizome and the elongation of the internode between the prophyll and the second leaf results in a stolon. They remain so or bend upwards on the side distal to the main axis in dense stands (or heavy infested space) but become horizontal in open stands (or less infested region). The axillary buds enclosed in prophyll, give rise to the primary leaf, consisting of a small petiole, a minute lamina and a large stipule. The foliage leaves are radical and are spirally arranged in a 3/8 phyllotaxy on the stem axis.

The leaf consists of a petiole, isthmus and a lamina. The petiole is sheathing at its base bearing a large membranous stipule, which forms a sheath around the next young leaf. The older leaves are slowly displaced downwards as they attain senescence and later submerged. If unshed and undamaged, they can remain attached to the stem until they decompose. The petioles are spongy and swollen in the middle and tapering slowly towards the lamina. The petiole's shape determines and influences the amount of air that can be sustained in them and consequently determine the floating capacity for the plant. The petiole may elongate from 30 to 125 cm and plays an important factor in the size and age of the plant. The leaf lamina is orbicular to ovoid in shape with a nearly cordate base. The laminae usually measure up to 15 x 13 cm but may grow up to 35 x 30 cm. On the basis of differences in leaf size, three biotypes of waterhyacinth can be distinguished and named 'small' (or normal), 'medium' (or stunted) and 'super' (large sized) hyacinths.

Roots of waterhyacinth are dark violet to bluish or pinkish violet in color. They are whitish when growing in total darkness but colored when exposed. They are adventitious and fibrous. The roots produce a large number of lateral roots of limited growth giving them a fine feathery appearance. Each lateral root has a prominent root cap. The root length may vary from 10 to 300 cm. Root system is generally extensive accounting for up to 50 % of the plant's biomass but may be too small particularly when plants grow in mud or shallow-deep aqueous bodies.

The flowers are borne on a terminal inflorescence, which is an attractive lavender blue spike with yellow center (**Figure 1.1**). The blue spike is subtended by two bracts and surmounted on an elongated peduncle. Of the two bracts the lower one generally has a distinct lamina, with each spike having 4 to 25 flowers. They are sessile, beautifully coloured (reason for its ornamentation) but short-lived. The perianth tube is 1.5 to 1.75 cm long with green base and pale top. Tepals are ovate to oblong to obovate, thin, liliac in colour and up to 4 cm long. Stamens have curved filament with glandular hairs and of the six stamens, three - close to the perianth tube are small, and the other three - borne near the mouth of perianth tube are long. Sometimes five or seven stamens are visible, size of which varies. The anthers are violet and measure up to 1.4 x 2.2 mm. Each anther contains up to 2000 pollen grains. Gynoecium is tricarpellary, syncarpous, superior, trilocular with axile placentation. Ovary is nearly conical, and produces about 500 ovules. The style is variable in length and bears nearly capitate sigma (three lobes closely appressed). The stigma may be longer than long stamens shorter than short stamens or of intermediate length. Thus, three hetrostylous forms of flower are found in different population.

Figure 1.1: The flower with lavender blue spike and yellow center of waterhyacinth

The fruit of the weed, observed rarely, is a thin-walled capsule enclosed in a relatively thick-walled hypanthodium developed from the perianth tube. Each inflorescence can produce more than 3,000 seeds and from a single rosette can several inflorescences can be produced every year (Barrett 1980). The mature number of seeds per capsule varies from just a few to 450. Seeds are 1 to 1.5 mm long and roughly egg-shaped with ridges from one end to another. The seeds of waterhyacinth are long-lived; remain viable for 5 to 20 years (Matthew et al. 1977). Seeds sink after the release from the seed capsule and may subsequently germinate with the fluctuating water level. Seeds germinate either on moist sediments or in warm shallow water (Center et al. 2002) and flowering usually occurs 10 to 15 weeks thereafter (Barrett 1980). Lack of germination sites, limits seedling development except during drought, on decaying mats after herbicide applications (Matthews 1967), or at the margins of water bodies.

Intraspecific variation

The varying growth forms of waterhyacinth ranges continuously from plants having bulbous petiole (float) to plants with petioles that are slender and non-bulbous. Plants with non-bulbous petiole are commonly found in dense infestations where space is limited mainly due to high intra-specific competition, and neighbouring plants support each other. Plants with bulbous petioles are seen to occur at the open water margins of dense infestations or in infestations of low plant density.

A variation in style form also occurs. Several reports (Barrett 1977, Gopal 1987) have studied heterostyly in the weed. Of the three forms (short-, mid- and long styled forms), the short-styled form is confined to its place of origin, the Amazonia. The style of the intermediate form predominates in all the introduced ranges while the long style form occurs less frequently and predominates only in Ceylon (Sri Lanka).

1.1.1.3. **Anatomical characters of the weed**

The anatomical features of the petiole show single-layered parenchymatous cell composition with the absence of a cuticle. The bands of parenchymatous cells are surrounded by hexagonal air spaces. The vascular bundle has sclrenchymatic cell-bearing bundle cup. Vascular bundles are immersed in aerenchyma and each bundle consists of tracheids, vessels, parenchyma cells and fibers. Phloem in turn is composed of sieve tubes and companion cells. Aerenchyma cells project in to the above-mentioned air spaces. Few raphides are also observed in parenchyma cells.

The epidermal peels (**Figure 1.2**) of waterhyacinth leaves, indicate no presence of trichomes. Stomata are paracytic type. The frequency of stomata is lower in the upper epidermis (about 2.83 mm^2) than the lower (3.32 mm^2), hence amphistomatic leaves (Qaisar et al. 2005). Transverse section of the lamina has a thin cuticle on the epidermal cells, rectangular in outline and forms a single layer. The mesophyll is differentiated into a palisade and spongy mesophyll. Palisade is present by both the upper and lower epidermis, but more in the upper than the lower side. The spongy mesophyll consists of a large number of air spaces surrounded by thin walls full of chloroplast. Sclereids are observed pointing at the air spaces (**Figure 1.3**). Smaller vascular bundles are present in both upper and lower epidermis sides; some of them are in contact with the epidermis. The vascular bundle is collateral with phloem towards upper epidermis and xylem towards the lower epidermal side. Large vascular bundles are present in the leaf center and extend from one end to the other of the leaf. Each vascular bundle is surrounded by a bundle sheath of parenchyma cells. Sclereids are observable in the palisade cells, and also in air spaces.

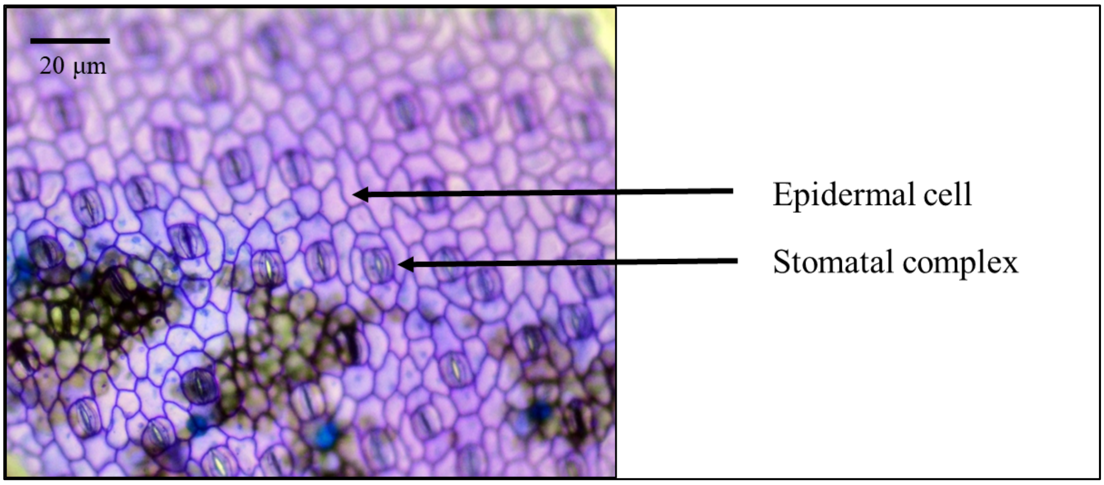

Figure 1.2: Upper epidermis of waterhyacinth leaf showing the stomatal openings

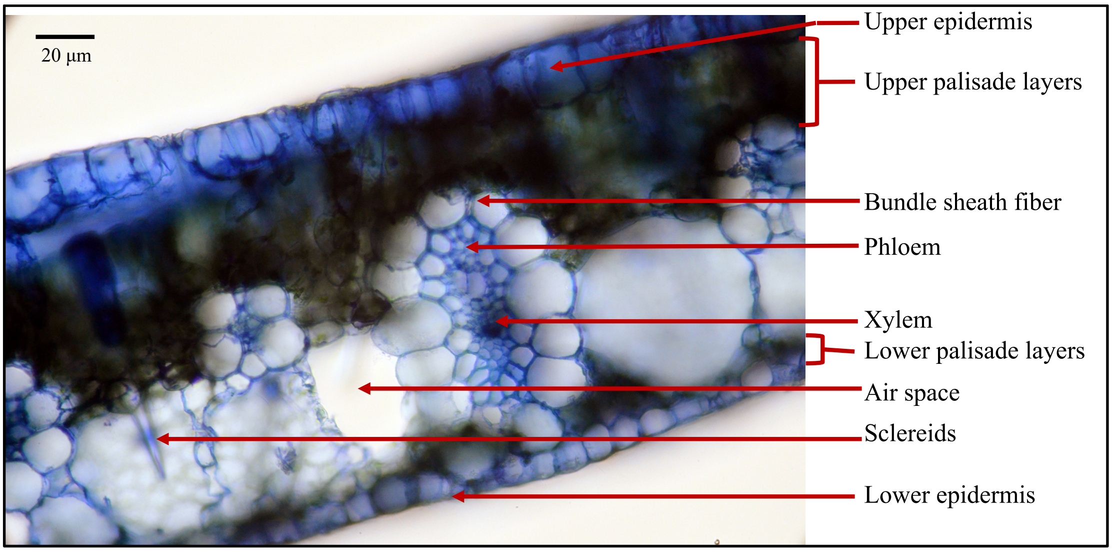

Figure 1.3: Cross sectional view of the waterhyacinth leaf

In case of the anatomy of root, epidermis is single-layered and compactly rectangular cells. Cuticle is absent. Hypodermis is 1 to 2 layered thick-walled cells, following which is the outer and inner cortex. The stele is surrounded on the outside by single-layered endodermis where Casparian strips are not prominent. Beneath the endodermis is a single-layered pericycle. The stele has alternating xylem and phloem. The center of the root is occupied by sclerified parenchyma cells.

In rhizome, similar rectangular cells are arranged to form a single layer of epidermis. The outer cortex, following it, has dispersed varying-sized vascular bundles, which are surrounded on the outside by a patch of sclerenchyma. Vascular bundles occupy the center of the rhizome. Xylem is V-shaped. Phloem is present in between the arms of the xylem. Air spaces are spherical.

1.1.1.4. **Other characteristics of the weed**

Reproduction: Waterhyacinth reproduces both vegetatively and sexually (Penfound and Earle 1948, Gopal, 1987). Vegetative reproduction is one of the most common and largely responsible method for a rapid spread and propagation of waterhyacinth into newer areas. The daughter plants are born from the horizontal stolons, from which roots develop and grow as a new plant, eventually separating from the mother plant following decay or breakage of the connecting stolon. Currents, winds, fishing nets and watercraft readily distribute these plants. Under favourable conditions a single plant can develop into a substantial infestation in a very short time. Earlier studies (Vietmeyer 1975) reported that in Louisiana two parent plants were surrounded by 300 offspring in 23 days and also that 10 plants under favourable conditions can multiply to 600,000 and over-spread an acre of water in just 8 months. Under such ideal conditions waterhyacinth plants can double their biomass in 10 days. Sexual reproduction in waterhyacinth was reported first by Muller in 1883 (Barrett 1980). Though little work has been done to understand the factors controlling flowering, seed formation and seeds germination and hence are still poorly understood. Many works (Baker 1965, Mulcahy 1975) have minimized the importance of sexual reproduction of waterhyacinth. Normally, leaf production occurs at a rate comparable to leaf mortality so that mature ramets retain six to eight leaves. Ramets produce about one leaf in about every five days and leaves survive about 2 to 2.5 months. So individual ramets are virtually immortal, but complete tissue replacement occurs every few months as the ramets decay at the bottom and grow from the top. Destruction of the apical meristem results in the death of the ramet through the loss of these regenerative capabilities.

Growing conditions: Waterhyacinth grows best in neutral pH, water rich in macronutrients, warm temperatures of around 28° to 30 °C, and high light intensities, making the tropics and sub-tropics most favourable as its breeding place. It tolerates pH levels from 4.0 to 10.0. It cannot survive in more than 20 to 25 % seawater. The plants survive frost if the rhizomes don't freeze, even then emergent portions may succumb. Prolonged cold kills the plants, but reinfestation from seed follows during later warmer periods. Growth is inhibited at water temperatures above 33 °C. Plants stranded on moist sediments can survive several months (Center et al. 2002).

1.1.2. *Invasiveness of the weed*

1.1.2.1. **Distribution of the weed**

The aquatic macrophyte, waterhyacinth, is free-floating in nature and considered as one of the ten worst invasive weeds of the world (Asmare et al. 2020, Holm et al. 1977). It is of South American origin, from the Amazon basin in Brazil. Julien (2001) though considered the upper reaches of Amazon and its tributaries as the source point. The floating habit of the plant, the water current of the river and the association of the surrounding fauna have led to the spread of the plant. However, all these helped in a local infestation. Invasion in other countries, worldwide, have been mostly human aided. By the late 20th century, the macrophyte was introduced worldwide into many countries as an ornamental plant, which slowly turned out to be a menace (**Figure 1.4**). With the rise in per degree temperature globally, the spread of the weed has further expanded. In US it was reported in 1884, while in Egypt, Australia and southern parts of Asia, by 1890s. Julien et al. (1999) highlighted the distribution in China and the Pacific region by the early 1900s. East Africa showed late introduction by 1970. In India, waterhyacinth was introduced in 1896, as an ornamental plant at the Royal Botanical Garden, Calcutta (now known as the Acharya Jagadish Chandra Bose Indian Botanic Garden) (**Figure 1.5**). A major river, like Ganga and her tributaries, along with water currents of other aquatic bodies have led to wide spreading of the weed in the country.

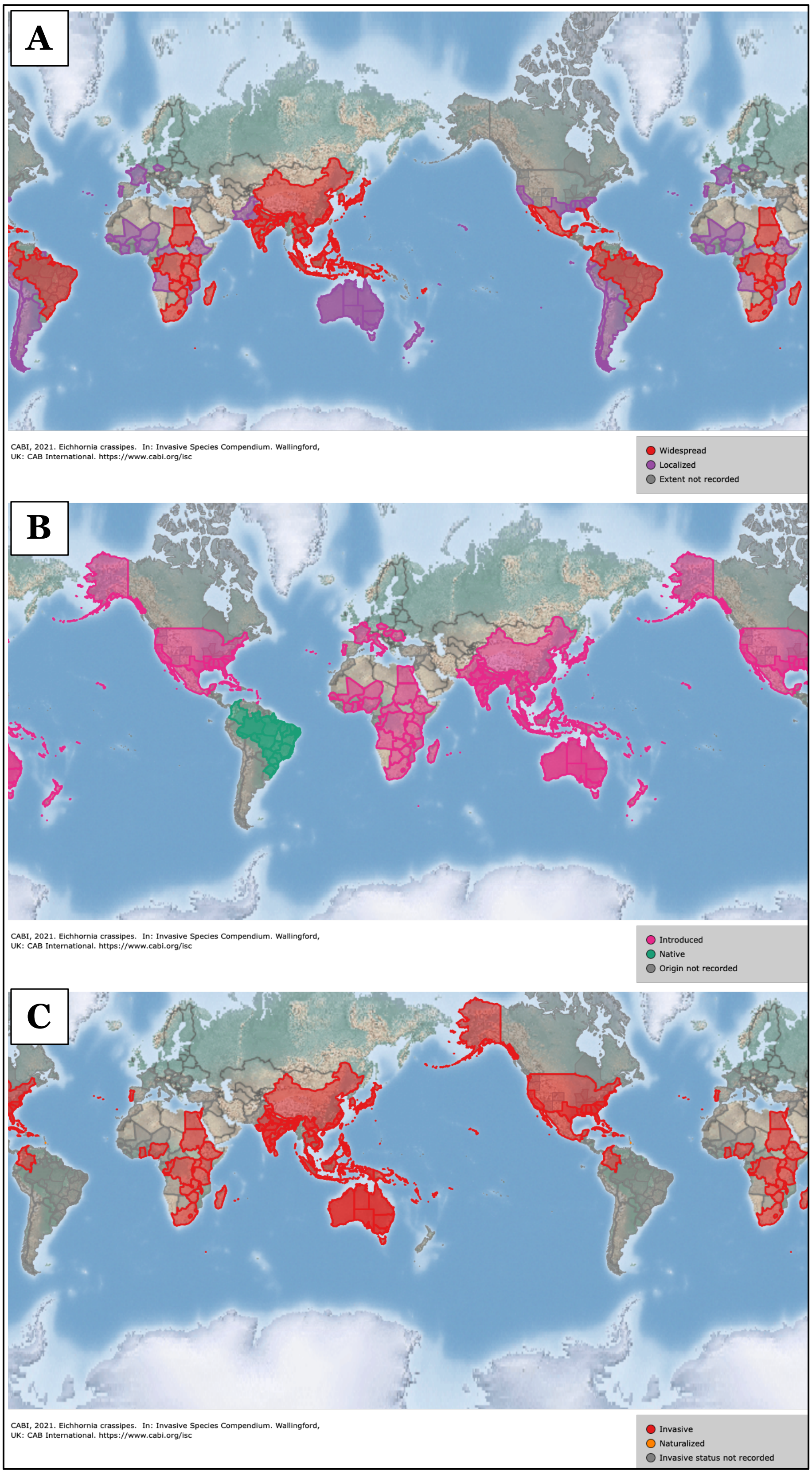

Figure 1.4: Global distribution of waterhyacinth based on **A**. extent of spread, **B**. origin of the weed and **C**. infestation rate of the weed (CABI, 2021).

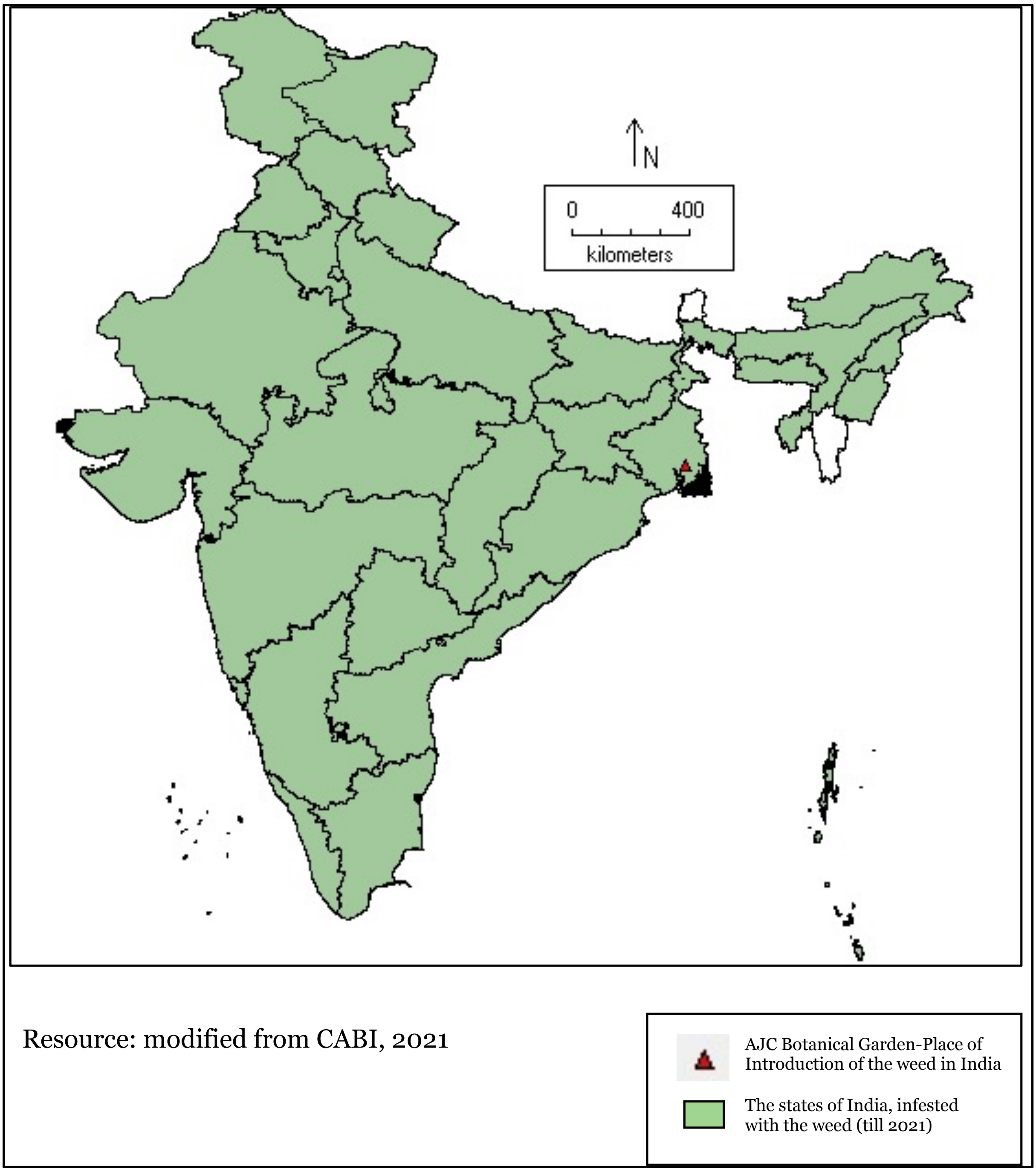

Figure 1.5: Distribution of waterhyacinth in various states of India (modified CABI, 2021) and the site where it was first introduced, in the country

1.1.2.2. Impact of the invasiveness

The favourable conditions of the tropics and subtropical countries have promoted high reproductive growth rate and heavy infestation of waterhyacinth (Gopal, 1987). The prolific mat-like infestation of the weed (**Figure 1.6**) soon covered a significant percentage of the water surface (around 200,000 ha) (Naseema et al. 2003, Visalakshy 2005).

Figure 1.6: Infestation of the weed at various regions in and around Kolkata

The weed exploits the freshwater habitat to its fullest utilising the oxygen from it and making it unfit and unhygienic for other aquatic fauna and flora. The temperature of the waterbody drops while the pH and alkalinity increase. The dead plant debris, increases the BOD and also leads to eutrophication. It is well known for its negative impact on the economy and ecosystem worldwide (Segbefia et al. 2019, Kriticos and Brunel 2016, Ray and Hill 2013). Loss of water by evapotranspiration, further depletes the water level, leading to water wastage.

The dense impenetrable mat of the weed alters the diversity of aquatic fauna and flora diversity. It suffices the breeding grounds for *Anopheles* sp., *Culex* sp., *Aedes* sp., the causative agent for diseases like malaria, encephalitis, filariasis etc. Some phytopathogens found infecting the weed, can cause diseases to some ecological economically important plants like tomato, brinjal, rice, jute, etc. (Dutta et al. 2015). Limited penetration of sunlight, impacted photosynthesis and oxygen production for life forms to sustain (Shabana 2005). A big reason for the depletion of the phytoplankton (the producers in the aquatic food chain), thus crippling the fauna dependent on them, especially a sink in the fishes' population.

Apart from the imbalance in the environment and in the biodiversity, the high infestation impedes the flow of water in waterways, like canals, channels, drainage etc. Blockages led to heavy flooding. Human activities connected to water for recreation or livelihood maintenance have further aggravated the infestation of waterhyacinth (Bateman 2001). The high invasiveness has hindered the water current and has impaired hydroelectric power generation, irrigation and transportation via water (Getsinger et al. 2014).

1.2. Control measures

The development and employment of management approaches to overcome and suppress this macrophytic weed, which includes mitigating their survival strategies, is imperative to safeguarding aquatic biodiversity. Several control mechanisms including manual, mechanical, chemical and biological methods have been implemented for controlling the unrestricted spread of the weed, with mixed results (Charudattan et al. 1996).

Manual control (**Figure 1.7**) is usually a very slow process with respect to the propagation rates of the weed, while usage of machines in mechanical control, might have shortcomings of spillage of petrol, adding on to the increase in pollution and cost. Chemical use in control, though provide quick solution, it leaves effects in intoxication and bleaching of several fauna and flora of the aquatic zone, along with alteration of the chemical balance of the water, making it unfit in several ways. The hazardous short-term effects of use of chemicals (Dagno et al. 2012) for weed control and laborious mechanical control have led to a myriad of ongoing research on trying to control it through various means including biocontrol (Sushilkumar 2011).

Figure 1.7: Demonstration of manual control of the weed, waterhyacinth

Apart from eradicating the weed population, utilisation of the weed in the form of control has also been implemented. The plant itself although comprises of more than 90 % water, has fibrous tissue - with high energy and protein content, which can be used in various applications. Reports of the weed been used for the production of fuel and biogas (Sen and Chatterjee 1931), paper-making industries, as fodder for cattle, as a source of potash or in making different accessories in small-scale industries or the substrates from the weed being used in mushroom cultivation (Puja 2008). The moisture content of the weed has been favourable for their use as mulch and compost or in the reclamation of the alkaline soils.

However, several environmental and financial challenges and losses are associated with the aforesaid mentioned methods (Bourguet and Guillemaud, 2016). Over the last six decades, a lot of emphasis has been on adapting eco-friendly measures, like biological control (the term was first used by H.S. Smith in 1919), where several biocontrol agents including arthropods and plant pathogens have been released around the world to control the weed (Ray and Hill 2013, Firehun et al. 2015). Biocontrol agents are often found to be slow and unpredictable (Ray and Hill 2016) but also provided an excellent means to control invasive species around the globe (Centre 1994, Jayanth 1988, Coetzee et al. 2011). Yet more efforts are required to control this disruptive macrophyte.

There have been instances, where the phytopathogens along with arthropod biocontrol agents are applied singly or in various combinations to understand the possible synergistic or antagonistic effect on the biocontrol of the weed (Dutta and Ray 2017). Such studies replicate the biotic interactions among different biocontrol agents in a small scale and provide alertness, prior to the release of those combinations in field conditions.

Apart from the use of biological control agents, metabolites (or chemical compounds) produced by phytopathogenic microorganisms and several plants and their phytochemicals are often effective in the controlling of the weed. Plants produce allelochemicals, and similar leachates produced by *Lantana camara* and dry powder of leaves and flower of *Parthenium hysterophorus* on waterhyacinth has been reported to prove fatal for the latter (Saxena et al. 2000, Zheng et al. 2006, Pandey et al. 2001). Some potential microbial toxins, A-AL toxin from *Alternaria* sps. and a few more, released have also shown biocontrol potential against the weed (Tegene et al. 2012). Waterhyacinth naturally shows the presence of kairomone, which are released when the plant is injured, drawing more biocontrol arthropods, like *Neochetina* sps., to the place of injury (Del Fosse and Perkins 1977).

1.3. Objectives and its significance

1) Survey for collection of diseased leaves of waterhyacinth
2) Isolation, purification and identification of fungal strains associated with waterhyacinth
3) Evaluation of the biocontrol potential of fungal pathogens against the weed
4) Studies on enzymes released from the selected fungal strains

5) Optimizing, characterizing and exploring possibilities of application of the isolated enzymes with emphasis on enhancing water hyacinth biocontrol

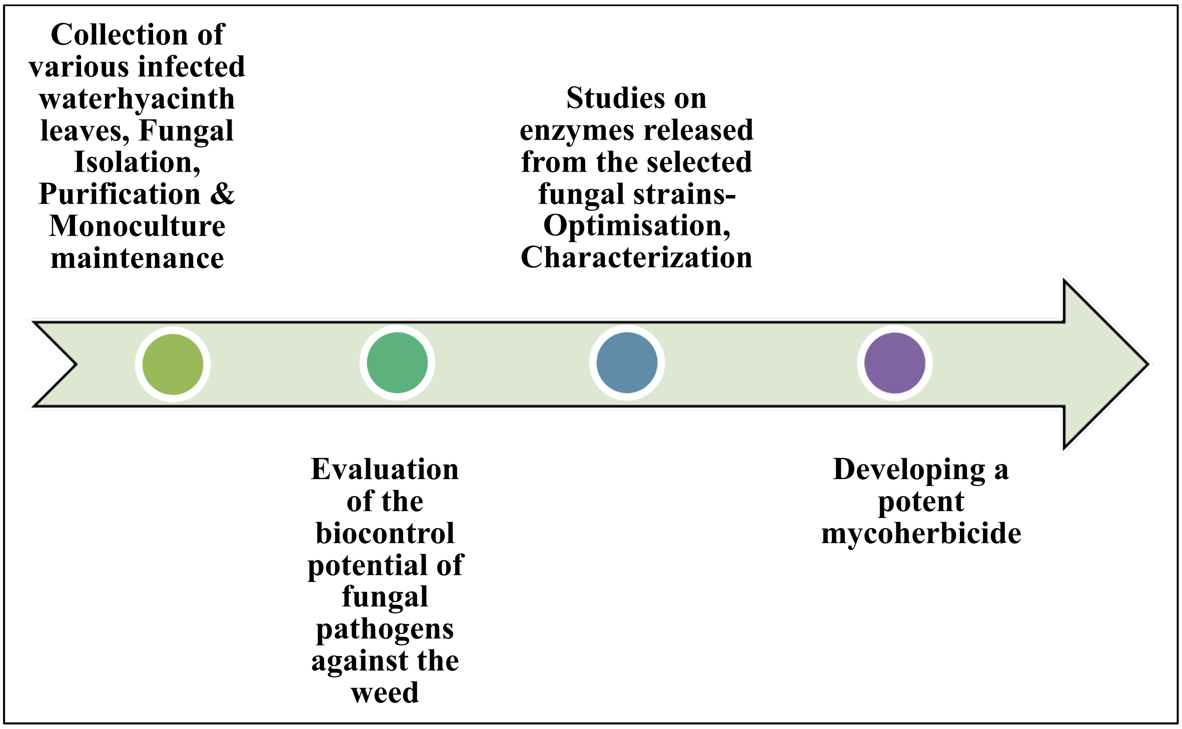

Figure 1.8: Schematic diagram of the plan of work, highlighting the objectives

<u>Chapter 2</u>: "*Survey for collection of diseased leaves of waterhyacinth and isolation, purification and identification of fungal strains associated with waterhyacinth*" is the amalgamation of both the first and second proposed objectives.

Negative impacts from some of the classical control methods and the rapid infestation rate of the weed, waterhyacinth, have opened more forefront into the biological control methods. With the opening of several options in mycobiota, undergoing evolutionary changes across the time lineage and providing much more fruitful, host specific measures, even for varying intraspecific species of the targeted weed, have prioritized the use of biological agents to control the infestation of the weed. With the increase in the diversity of the biological agents, identifying and understanding these species, becomes of utmost importance for mechanistic approach learning of their mode of action in the weed biocontrol. In fact, endemic phytopathogens are reported to be more host-specific (Freeman 1977). To use these agents, maintaining of these mycobiota for future use also becomes equally important.

<u>Chapter 3</u>: *"Evaluation of the biocontrol potential of fungal pathogens against the weed"* concentrates on the third objective of the proposed work plan.

With the isolation of array of mycobiota from the host weed, it becomes important to understand the efficacy of the biocontrol potential of these agents on the weed, before using them in field condition. Also, their impact on other species sharing the same ecological niche, or are of economic importance needs to be evaluated prior to their release. Further interaction with other biocontrol agents gives a glimpse in to their practical efficacy under field condition (Dutta and Ray 2017), because a particular niche is shared by an array of species. So cumulative trials are necessary for understanding the biocontrol efficacy of the agents, without hampering the balance of the ecosystem.

<u>Chapter 4</u>: *"Studies on enzymes released from the selected fungal strains-optimisation, characterisation"* concentrates on the fourth and partially the fifth objective of the proposed work plan.

Mostly secondary metabolites from various phytopathogens have previously been reported (Ray 2008) with biocontrol potential, against the weed, waterhyacinth, but limited knowledge is restricted for primary metabolites. However, several works about CWDEs (Cell Wall Degrading Enzymes) have drawn the attention towards their cell wall degradation properties (Ruiz et al. 1997, Cardinale and Matta 2001, Gómez-Gómez et al. 2002). Development of alternative weed control measures are also required to decrease the resilience of the usage of environmnetally damaging chemical herbicide (Singh and Pandey 2019). The enzymes help to overcome the plant cell wall barrier and help in breaking down the structural rigidity and complexity of the plant cell wall barrier, bringing about the initiation of the death of the weed, leading to a pause in the infestation caused by it. Enzymes help in degradation of the complex polysaccharides' moiety (**Figure 1.9**) into their simpler mono- or di-saccharides forms (**Figure 1.10**). Enzymes, are primary metabolites, produced by phytopathogens for the initial phase of their development in response to interspecific host-pathogen interactions, and it very essential to understand the physiochemical conditions, at which they are most active and produce the best results.

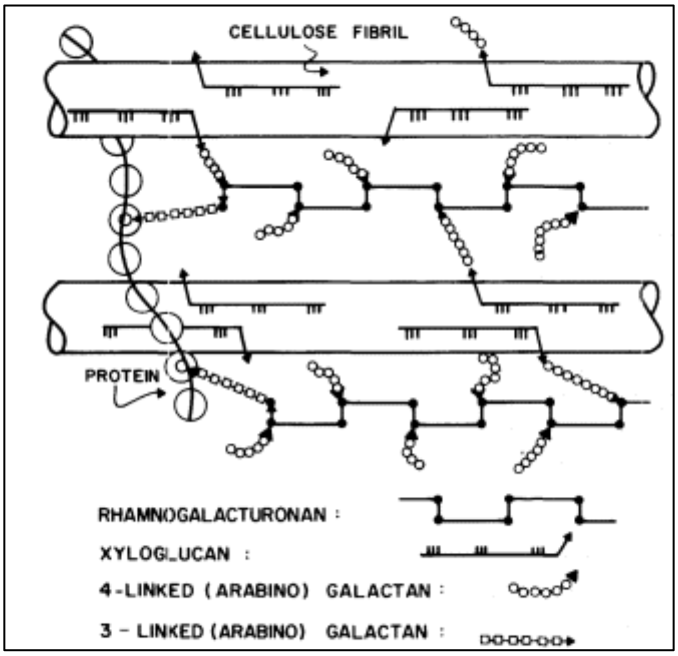

Figure 1.9: Interconnections between cell wall components. Schematic representation of the polymeric components of sycamore primary cell walls and their interconnections (Keegstra et al. 1973). Hemicellulosic xyloglucan polymers are cocrystalized with cellulosic glucan chains on the surface of the (two) microfibrils. The reducing ends of some—but not all—of the xyloglucan chains are glycosidically attached to some—but not all—of the β-1, 4-linked (arabino) galactan side chains of the rhamnogalacturonan polymers. The rhamnogalacturonan polymers and the structural protein are interconnected by 1, 3-linked (arabino) galactan bridges. Arrowheads indicate the reducing ends of polysaccharide chains.

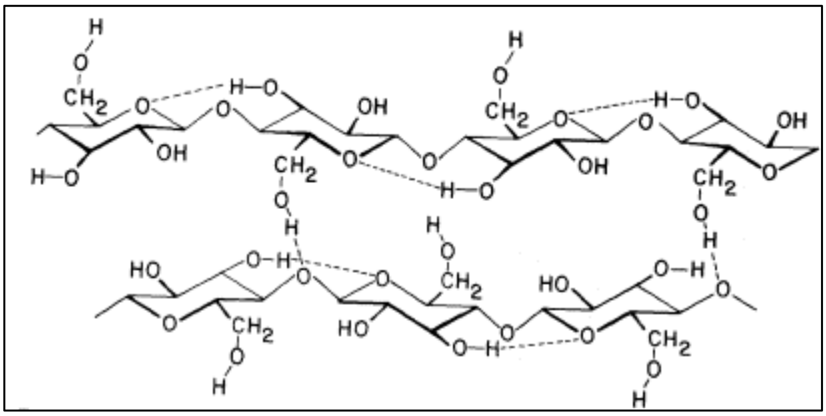

Figure 1.10: β-1, 4–linked glucan chains of cellulose (simpler forms of complex cell wall components).

Chapter 5: *"Exploring possibilities of application of the isolated enzymes with emphasis on enhancing waterhyacinth biocontrol"* explains the remaining part of the fifth objective of the proposed work plan.

Phytopathogens produce a plethora of primary metabolites of which some are more effective than others produced by a particular host-pathogen. Microbial metabolite production depends on the composition of the cell wall and how effectively the particular CWDEs can act. This chapter enhances on the distinctive feature of a particularly potent enzyme, out of the several, produced by one of the most effective biocontrol pathogens. It focuses on the analysis and understanding of the responsible part that plays a significant role in the biocontrol of the weed and better understanding of their mechanistic mode of action along with their evolutionary significance.