STUDY ON THE IMPACT OF ALLELOCHEMICALS OF *SENNA SPECTABILIS* (DC.) IRWIN & BARNEBY INVASION IN WAYANAD, KERALA

Thesis submitted to the University of Calicut in partial Fulfillment of the requirements for the degree of Doctor of Philosophy in Botany

by

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January 2025

CERTIFICATE

This is to certify that the thesis entitled “Study on the Impact of Allelochemicals of Senna spectabilis (Dc.) Irwin & Barneby Invasion in Wayanad, Kerala” submitted to the University of Calicut for the award of degree of Doctor of Philosophy in Botany by Ms. Suby is the result of bonafide research work carried out by her under my guidance in the Department of Forest Genetics and Tree Breeding, KSCSTE – Kerala Forest Research Institute, Peechi. Further, I certify that this or part thereof has not been the basis for the award of any other diploma or degree either in any institution or university

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DECLARATION

I, Suby hereby declare that the thesis entitled “Study on the Impact of Allelochemicals of Senna spectabilis (Dc.) Irwin & Barneby Invasion in Wayanad, Kerala” embodies the results of bonafide research work done by me under the guidance of Dr. TK Hrideek TK, Senior Scientist, Department of Forest Genetics and Tree Breeding, KSCSTE – Kerala Forest Research Institute, Peechi. I further declare that this or part thereof has not been the basis for the award of any other diploma or degree either in any institution or university.

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Abbreviations

SOD	Superoxide dismutase	WWS	Wayanad Wildlife Sanctuary
APX	Ascorbate peroxidase	CCA	Canonical correspondence analysis
CAT	Catalase	GC-MS	Gas chromatography – Mass spectrometry
PPO	Polyphenol oxidase	ROS	Reactive oxygen species
MDA	Malondialdehyde	IAPS	Invasive alien plant species
AT	*Ailanthus tryphysa*	PPH	Propagule Pressure Hypothesis
PP	*Pongamia pinnata*	PGPF	Plant growth-promoting fungi
TG	*Tectona grandis*	PGPR	Plant growth-promoting rhizobacteria
HP	*Hopea parviflora*	IAA	Indole-3 acetic acid
DS	*Dendrocalamus strictus*	NWH	Novel weapon hypothesis
PSF	Plant-Soil Feedback	ERH	Enemy Release Hypothesis
EICA	Evolution of Increased Competitive Ability	NBR	Nilgiri Biosphere Reserve
DMRT	Duncan's Multiple Range Test	TTC	Triphenyl tetrazolium chloride
SE	Standard error	GBH	Girth at breast height
TCA	Trichloroacetic acid	LC	Leaf Chlorophyll content
SPAD	Soil-Plant Analysis Development	NIST	National Institute of Standard and Technology
SMC	Soil moisture content	SOC	Soil organic carbon
ρB	Soil Bulk Density	θv	Moisture Content
CDA	Casein Digest Agar	PSM	Phosphate Solubilizing Microbes
EC	Electrical conductivity	DHAR	Dehydroascorbate reductase
GR	Glutathione reductase	AsA	Ascorbic acid
GSH	Glutathione		

CHAPTER 1

INTRODUCTION

Chapter 1
Introduction

Invasive species affect biodiversity through different mechanisms; one such mechanism is allelochemicals. It disrupts microhabitats that dominate forest ecology. The phenomenon known as plant invasion encompasses the range expansion of plant species facilitated by human or natural means, or a combination thereof, resulting in modifying plant community structure to the near-complete dominance by successful alien plant species. Despite its global occurrence across diverse ecosystems, the processes and consequences of plant invasion are not always immediately apparent and necessitate systematic exploration. Such invasions often involve shifts in soil quality and intensified belowground competition, enabling invasive species to outcompete and displace native species.

Allelopathy is a biological occurrence where organisms generate biochemical substances capable of either augmenting or hindering other organisms' physiological and morphological attributes (Cheng & Cheng, 2015). Allelochemicals are released through various mechanisms in various plant tissues, including exudation from roots, leaching from the upper parts of the plant and plant decomposition (Latif, Chiapusio & Weston, 2017). Allelochemicals also elicit the production of reactive oxygen species (ROS) (Weir et al. 2004), important signalling molecules that stimulate developmental responses in plants. Additionally, they can act as stress indicators in plants under stress conditions; however, their overproduction may interact with normal cellular processes and can destroy cellular compartments or denature DNA (Rahal et al., 2014). Invasive alien species can alter the prevailing structure and species composition, often suppressing or displacing native species. Biological invasion entails the total or near-total domination of local plant or animal communities by a single or combination of invasive species, often alien to the location. Most invasive species possess chemical advantages over their competitors, contributing to their

extraordinary success and domination in biotic communities. Invasive alien species proliferate rapidly, monopolize ecosystem resources, and displace native plants, resulting in large monocultures of invasive species. They alter community structure and appropriate energy flows and material cycles to their advantage, facilitated by ecological, socio-political factors, and successful physiological and life history strategies.

Before delving into the impacts of plant invasion on soil microbial diversity, it is imperative to elucidate the role of the soil microbiome in ecosystem processes. Soil constitutes a multifaceted and ever-changing environment wherein microorganisms determine the biological processes.

Kerala has undergone several waves of alien invasion of both plants and animals. *Chromoleana odorata*, *Lantana camara*, *Mikania micrantha*, *Mimosa diplotricha*, *Sphagenticola trilobata*, *Senna spectabilis*, *Acacia mearnsi*, giant African snail, papaya mealy bug *etc.* are the species which arrived from other countries and have caused significant impact on the ecology and economy of Kerala. Tree invasions are also one of the major biological invasions posing important threat to Kerala's biodiversity, especially in Wayanad Wildlife Sanctuary. Introducing alien invasive tree species has imposed alarming levels of biodiversity loss and shifts in vegetation composition. Here, tree invasions mount an overarching influence on ecosystems. Invasion of *Senna spectabilis* and *Maesopsis eminii* in Wayanad Wildlife Sanctuary and *Acacia mearnsii* in various National Parks of the high ranges of Kerala are notable instances of tree invasions.

Senna spectabilis belongs to the Fabaceae family; it is native to South America and an invasive species in many parts of the world. It is a deciduous tree that grows quickly, reaching 15-20 meters in height and producing many seeds after flowering. The tree tends to fork near the ground and spreads widely with drooping, leafy branches. The rapid growth of *Senna spectabilis* in Wayanad Wildlife Sanctuary (WWS), Kerala, India, is riskier than other exotic

species (Prajitha and Sudhabai, 2022). The WWS, spanning an area of 344.44 square kilometers, is an integral component of the Nilgiri Biosphere Reserve and is a significant habitat for Asiatic elephants in India. Currently, the sanctuary is under severe threat from the rapid proliferation of invasive plants, causing a food shortage for herbivores. *S. spectabilis* was introduced as an avenue tree and started establishing itself extensively in new areas, and its management has become a challenging task (Vinayan et al., 2020). A survey conducted by the Wildlife Trust of India and the Kerala Forest department has identified the major occurrence of *S. spectabilis* in the Muthanga, Sulthan Bathery, and Tholpetty forest ranges within the sanctuary (APFISN, KFRI, 2014).

S. spectabilis was initially introduced to the Wayanad Wildlife Sanctuary in the early 1980s and has since proliferated to encompass roughly 23% of the sanctuary's entire area over four decades (Anoop et al., 2022). There are also reports of such accidental introduction in the Caribbean. It has also escaped from Trinidad and Tobago and invaded the northern parts of Orinoco in Venezuela (Irwin and Barneby, 1982). In addition, the regeneration and growth of native tree species are also suppressed by *S. spectabilis* (Wakibara, 1998; Wakibara and Mnaya, 2002). Prawoto et al. (2006) revealed that planting *S. spectabilis* as shade trees in coffee plantations can inhibit the growth of Arabica coffee, attributed to the chemicals it releases with allelopathic potential. By investigating alterations in the native plant's physiology, we seek to uncover the direct impact of *S. spectabilis* on native plant species.

This research addresses critical objectives focused on understanding the multifaceted impacts of *Senna spectabilis* invasion on both the abiotic and biotic components of the environment. The primary aim of this study was to explore the allelopathic impacts of *S. spectabilis* extract on the growth of seedlings from chosen native species. Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbic peroxidase (APX) are

known natural control strategies of plants to limit the damage caused by ROS (Huseynova et al., 2014 & Sekmen et al. 2012) and were determined in this experiment to study allelochemical stress. To reduce the impact of induced oxidative stress, plants activate a complex system of enzymatic antioxidants (SOD, CAT, POD, and APX), which are highly accumulated under stress conditions (Farhoudi and Lee, 2013). The other test assay was to determine the accumulation of proline in plants, which is a useful assay to monitor and evaluate the physiological tolerance of plants due to stress (Ábrahám et al., 2010). Cellular respiration is a vital phenomenon during germination, providing a supply of ATP to the embryo and allowing it to resume its metabolic activities. Reduced seed respiration is subjected to plant extracts action during germination. Furthermore, in second objective we documented the major allelochemicals present in *S. spectabilis* leaf extracts using gas chromatography–mass spectrometry (GC–MS). In summary, this research helps to find the affect allelochemicals impart on native species and their response. The third objective is an effort to analyse the whole genome and extent of Bacteria and fungi, its plant growth promoting and antagonistic activity of fungi in the rhizosphere soil of *Senna spectabilis* invaded forest and natural forest in Wayanad Wildlife Sanctuary.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2
Review of Literature

Invasive alien plant species

Invasive alien plant species (IAPS) is a significant driver of biodiversity loss, leading to far-reaching impacts on habitat and socioeconomic conditions through various mechanisms (Rai and Singh, 2020). Invasive species can cause ecosystem degradation and fragmentation, with consequences recognized on multiple levels, including alterations in the structure and composition of flora, flora complexes, and plant communities. This broader-scale impact can ultimately result in a homogenization of both fauna and flora worldwide, leading to biodiversity loss (Shuvar et al., 2021). The potential of invasive alien plant species to disrupt habitats is a concern, leading to the displacement of native species and the introduction of new species that may not be well adapted to the prevailing ecosystem (Lazzaro et al., 2020). Research suggests that habitat filtering can support the naturalization and invasion of alien plants when native and invaded ranges share similar ecological conditions (Hejda et al., 2015; Buckley and Catford, 2016; Divíšek et al., 2018). Economic uses of introduced plants in the horticulture sector are a major driver of their naturalization (Hulme et al., 2017; van Kleunen et al., 2018; Guo et al., 2019; van Kleunen et al., 2020; Arianoutsou et al., 2021; Sîrbu et al., 2022).

Invasive plants can trigger significant environmental disturbances, resulting in long-lasting impacts on global economic stability, ecological balance, and biodiversity preservation (Elton, 1933; Coblentz, 1990; Aronson and Handel, 2011; Crystal-Ornelas et al., 2021) and are often associated with the risk of extinction for many plants listed in the IUCN Red List of Threatened Species (Downey and Richardson, 2016; Dueñas et al., 2021). Managing invasive species costs billions of US dollars (Pejchar and Mooney, 2009; Haubrock et al., 2021). The impact of

invasive alien plant species on the distribution of native plant species is multifaceted as they directly compete with native species for essential resources such as water, nutrients and light, leading to a decline in the abundance and diversity of native species (Zheng et al., 2015; Čuda et al., 2015; Gioria and Osborne, 2014). They can also induce habitat alterations, resulting in the displacement of native species and the introduction of new species that may not be well-suited to the existing ecosystem (Langmaier and Lapin, 2020). Invasive species have negative ecological and economic impacts on recipient environments, resulting in loss of biodiversity and commercially important species (Charles and Dukes, 2007; Pejchar and Mooney, 2009; Barney and Tekiela, 2020). Biological invasion is now a global concern, the second major threat to global biodiversity after habitat destruction (Hulme et al., 2009; Hulme and Weser, 2011). Although the invasive species negatively affect native plant communities and soil processes, their actual impact on plant and soil communities is inadequately documented and poorly understood (Vujanović, 2022). Invasive plants reduce the diversity of native plants by altering habitats or disturbance regimes, but it's unclear whether they do so via competitive exclusion or some other process (Valone and Weyers, 2019). The two major aspects of IAPS on the ecosystems are the invasive potential of the alien plant and the susceptibility of the native plant species.

The invasive potential of exotic species can be characterized by a combination of morphological, ecological and phenological traits, as demonstrated by several studies (Siemann and Rogers, 2001; Chaneton et al., 2004). These plant functional traits interact with surrounding biotic and abiotic factors, responding to novel environmental conditions (Pyšek et al., 1995; Thompson et al., 2001; Pyšek et al., 2009; Drenovsky et al., 2012). Some exotic plants exhibit high adaptability to extreme conditions, supporting the concept of "survival of the fittest" (Cronk and Fuller, 2014). Understanding the invasive attributes of successful invaders and analyzing the traits of newly introduced species contribute to our understanding of invasion

biology. Trait based research has surged, revealing the interrelationship between functional traits and invasion ecology. Reproductive traits play a crucial role in the invasive potential of the plant species. Factors such as the duration of the juvenile period, length of the flowering period, propagule pressure, seed characteristics (especially seed mass and viability) and seed dispersal ability have been emphasized by several ecologists (Rejmánek and Richardson, 1996; Hamilton et al., 2005; Schmidt and Drake, 2011; Castro-Díez et al., 2014; Godoy and Levine, 2014; Moravcová et al., 2015; Oliveira et al., 2017). Additionally, ecological, physiological, and genomic attributes accompany reproductive traits in contributing to the invasive potential of plants (Küster et al., 2008; van Kleunen et al., 2010; Kubešová et al., 2010; Hamelin and Roe, 2020). The invasive potential and success of plants in an ecosystem can be explained through various hypotheses, such as:

Disturbance Hypothesis: According to the disturbance hypothesis, disturbance events such as natural disasters, land-use changes, or anthropogenic activities create open spaces and resource availability that invasive plants can exploit (Mack, D'Antonio, & Laura, 2002). Furthermore, disturbed environments frequently have less competition from native plants, giving invasive species a competitive advantage leading to abundance (Rejmanek and Richardson 1996). Hobbs and Huenneke (1992) reported that invasive plant species were more common in areas that had experienced fire, logging, or grazing than in areas that had not been disturbed. In Europe, Prach and Pyšek (2001) observed that invasive plant species were more likely to occur in habitats with higher levels of human disturbance, and in Hawaii, Vitousek et al. (1996) discovered that plant species were more abundant in areas with higher levels of soil disturbance.

The relationship between disturbance and invasion, however, is not always clear. The severity and frequency of disturbance events can significantly impact the success of invasive plant species, with some species better adapted to moderate or infrequent disturbances than to frequent or severe disturbances (Mack, 1996). The disturbance hypothesis has important

implications for invasive plant species management. Restoration of disturbed habitatscan aid in the prevention of invasive plant establishment and spread. Furthermore, land-use planning that considers the potential for disturbance can aid in preventing the introduction and spread of invasive species.

Propagule Pressure Hypothesis: The propagule pressure hypothesis explains the success of IAPS by estimating the number of individuals or propagules introduced into a new range and assuming that at least some will survive and establish a viable population (Simberloff, 2009). Higher genetic diversity and a greater chance of encountering favourable environmental conditions increase the likelihood of establishment. According to this hypothesis, more propagules increases the likelihood of successful establishment and growth, resulting in greater environmental impacts. Several studies have found evidence to support the propagule pressure hypothesis. According to Pyšek et al. (2012), higher numbers of invasive plant species introduced into Europe were associated with higher invasion success.

According to a study conducted by Richardson et al. (2000), the likelihood of an invasive plant species becoming naturalized was positively correlated with the number of introductions. Similarly, Catford et al. (2016) observed that propagule pressure was a key factor in predicting the success of invasive plant species in a meta-analysis. The PPH, however, does not fully explain the success of invasive plant species. Other considerations include environmental suitability, biotic interactions, and management efforts. Furthermore, not all invasive species have high propagule pressure, and some species can establish and spread with a small number of individuals. Effective invasive plant species management strategies should consider the PPH and other factors contributing to invasion success. Reducing the number of propagules can effectively control invasive plant spread, but efforts should also be directed toward preventing new introductions and minimizing the impact on existing populations.

Niche Preemption Hypothesis: The niche preemption hypothesis proposes that invasive species occupy and dominate new environments and can monopolize resources by pre-empting available resources and preventing native species from using them, leading to their success. According to this hypothesis, invasive plants may have certain traits or adaptations that allow them to effectively compete for resources and establish themselves in a new environment (Shea and Chesson, 2002). *Alliaria petiolata* was able to outcompete native plant species for light and nutrients in forest understory (Blossey and Nötzold, 1995). *Falcataria moluccana* monopolised soil nutrients, reducing the availability of resources for native plant species (Funk and Vitousek, 2007).

The NPH suggests that invasive plants have a competitive advantage over native species in the invaded ecosystem because they can occupy a specific niche not occupied by native species. This niche is the specific set of environmental conditions and resources a species needs to survive and reproduce. Invasive plants are considered more successful than native species in occupying this niche due to their unique traits or adaptations, such as faster growth rates, higher reproductive output, or broader tolerance for environmental conditions (Catford et al., 2016). Hulme (2006) found that invasive plant species had greater niche overlap with native species than non-invasive species, suggesting that they were better able to occupy and compete for resources in the invaded ecosystem. Similarly, a meta-analysis by Vilà et al. (2011) found that invasive plant species had higher competitive ability than native species. Additionally, not all invasive species occupy a unique niche, and some species may be successful due to their ability to adapt to a wide range of environmental conditions. Preventing the introduction and spread of invasive species, as well as promoting the establishment and growth of native species, can help reduce the competitive advantage of invasive plants and improve the resilience of native ecosystems.

Novel Weapons Hypothesis: The novel weapons hypothesis states that invasive plants have an evolutionary advantage over native species because they produce allelochemicals that are toxic to native plants, giving the invaders a competitive advantage in the ecosystem (Callaway and Aschehoug, 2000). These novel allelochemicals are not found in the native range of invasive species and thus are not recognized by native plants, allowing the invasive species to outcompete them. According to Callaway et al. (2004), the invasive plant species *Centaurea maculosa* produces allelochemicals that inhibit the growth of native plant species. Another study by Blumenthal (2009) reported that invasive plant species like cheat grass (*Bromus tectorum)* released allelochemicals that reduced the growth of native plant species in the Great Basin region of the United States. Allelochemicals can inhibit native plant growth and development by interfering with various physiological processes, such as seed germination, root growth, and photosynthesis (Hierro et al., 2006). Numerous plant species have been successful in invading landscapes due to their ability to generate allelochemicals and suppress native vegetation. *Centaurea stoebe*, an invasive species, was successful in North America due to its allelopathic effects (Callaway et al., 2004). Another study showed that the allelopathic effects of the invasive species *Eucalyptus globulus* were responsible for its success in Portugal (Pereira, 2007). *Centaurea diffusa* was more toxic to native plants than native plant allelochemicals (Callaway et al., 2004). Another study found that the invasive plant *Lonicera japonica* produced novel allelochemicals, which were more effective in inhibiting the growth of native plants (Cappuccino and Arnason, 2006).

The NWH also suggests that invasive plant species may use allelochemicals to attract mutualistic organisms, such as mycorrhizal fungi, which can provide plant nutrients and other benefits (Callaway and Aschehoug, 2000). The invasive plant *Alliaria petiolata* proved capable of recruiting mycorrhizal fungi, which increased its growth and reproduction (Wolfe and Klironomos, 2005). Although the NWH has received widespread support in the literature, it

does have some limitations. One limitation is that the allelopathic effect of invasive plants may not be the only factor contributing to their success. Other factors, such as seed production, dispersal, and environmental conditions, may also be important (van Kleunen et al., 2010). Another limitation is that the allelopathic effect of invasive plants is not always negative. In some cases, invasive plants may produce allelochemicals that benefit native plants by suppressing competitor growth (Kosola et al., 2001).

Invasive plants pose a significant challenge to ecosystems worldwide, characterized by their rapid growth in alien environments. The escalation of global human movement has fueled the introduction of non-native plant species, intentionally or inadvertently, leading to their proliferation (Bradley et al., 2012). These invasions, facilitated by introducing seeds, spores, and other vegetative materials, have grave consequences for biodiversity and ecosystem integrity (Koshila et al., 2019). Invasive plants not only outcompete native species but also induce biodiversity loss, alter ecosystem dynamics, and disrupt native animal and microbial communities (Peter et al., 2021). Furthermore, they contribute to the homogenization of geographical biota and drastically impact soil and environmental conditions (Bradley et al., 2012; Richardson et al., 2000).

The detrimental effects of invasive plants extend beyond ecological disruption. They exert direct competitive pressure on native flora, inhibiting their germination and survival. Additionally, invasive plants alter soil biotic and abiotic properties, further impeding the growth of native species. The intensity of competition is inversely related to resource availability, exacerbating the challenge posed by invasive species (Davis et al., 1998).

Invasive species invasion, a global phenomenon, carries multifaceted consequences for ecological, social, and economic systems (Vitousek et al., 1996). These invaders can significantly alter community and ecosystem properties, affecting hydrology, fire regimes, light

penetration, nitrogen cycling, and mycorrhizal associations (Walker and Smith, 1997; Sala et al., 1996; Brooks et al., 2004; Reinhart et al., 2005; Vitousek and Walker, 1989; Stinson et al., 2007; Gomez-Aparicio and Canham, 2008). The success of invasive plants in new environments is contingent upon propagule pressure, species traits, and environmental responsiveness (Lonsdale, 1999). Moreover, their invasion alters soil microbial communities, influencing subsequent plant establishment and growth (Kirk, 2002; Callaway et al., 2004; Bever, 2002).

Invasive plants also disrupt native symbiotic networks, alter grazing patterns, and release allelopathic compounds, further inhibiting native species' growth (Putten, 2010; Lankau, 2012; Cipollini, 2012). The resulting changes in soil detritus-based food webs enhance microbial biomass and decomposition rates. Consequently, native plant communities face partial or complete destruction, with cascading effects on wildlife habitats and ecosystem services (Pyšek et al., 2011; Foxcroft et al., 2017; Simberloff et al., 2013).

Despite the critical ecological significance of invasive species, there are inadequate attention and management efforts, particularly in regions like India, where the Western Ghats harbor unique biodiversity. The Western Ghats, a globally recognized ecologically sensitive zone, boasts a rich diversity of endemic plants and species (Volga, 2013). However, invasive species such as *Acacia mearnsii*, *Lantana camara*, and others threaten this biodiversity, causing widespread destruction and displacement of native flora (Sankaran, 2014). Urgent measures are required to address the escalating threat posed by invasive species to the fragile ecosystems of the Western Ghats.

Allelopathy and invasive alien species

One of the major factors determining plants' invasive potential is allelopathy. Plants can produce chemicals that affect the growth and survival of other plants and microorganisms in their environment, which is a pervasive trait of invasive plant species, occurring in 51.4% of the

invasive species in a database of 524 known invasive plant species (Kalisz et al., 2021). Invasive species diminish the richness and abundance of native biota, leading to a decline in local species diversity and the distinctiveness of biological communities across different spatial scales (Pyšek et al., 2012). Invasive species can also serve as ecological indicators of environmental pollution (Rai and Singh, 2020).

There are also several examples where certain invasive species have modified habitats and altered ecosystems. In the New England region, *Fallopia japonica* (Houtt.) invaded roadsides and riparian ecosystems, leading to alterations in species diversity, aboveground biomass, and nitrogen concentrations, resulting in the development of significantly transformed monocultures (Aguilera et al., 2010). *Microstegium vimineum* (Trin.), another invasive plant, has been recoreded to decrease the diversity of native plant species, particularly during the peak growth season of *M. vimineum*, causing a reduction in species richness and suppression of native plant species (Adams et al., 2009). *Alliaria petiolata* (M. Bieb.) Cavara and Grande, known for its shade tolerance and prolific growth, typically thrive in disturbed environments and dense canopied ecosystems. This species was found to affect the functional capacity of arbuscular mycorrhizal fungi negatively, leading to a reduced growth rate of indigenous hardwood tree species (Stinson et al., 2006). Some allelochemicals also impact the regeneration of neighbouring tree seedlings (Schnitzler, 1994; Rahmonov, 2009; Rusterholz et al., 2018). These impacts include changes in biochemical soil properties, resulting in variations in above and belowground native species richness (Rusterholz et al., 2018). Another species, *Impatiens glandulifera* was found to alter soil bacterial and fungal communities by releasing naphthoquinones (Gaggini et al., 2018).

Another aspect of allelochemical impact is the chemical composition of plant litter from alien plants, such as *Robinia pseudoacacia*, leading to higher nitrogen levels in the upper soil horizons, affecting regeneration (Rahmonov, 2009). Mahall and Callaway (1991, 1992) found that in

Larrea tridentata, the allelopathy effects of roots inhibit the elongation of nearby *L. tridentata* or *Ambrosia dumosa* roots, and the presence of activated carbon significantly reduces this inhibition.

Allelopathic interactions may significantly contribute to the success of invasive plant species by modulating physiological processes, thereby exerting influence on community structure (Rice, 1992; Bais et al., 2002, 2003; Inderjit and Duke, 2003). Allelochemicals exhibit a diverse array of effects on plants, including plant growth and development inhibition on and eliciting stress responses. Allelochemicals released by the invasive species spotted knapweed (*Centaurea maculosa*) inhibited root growth of native bunch grass (*Festuca idahoensis*) (Ridenour and Callaway, 2001). The invasion of *Centaurea* is frequently marked by the total displacement of native plants due to intense competition, leading to the formation of thick, single-species patches. Allelochemicals, in addition to their effects on plants, can interact with soil microbes such as bacteria, fungi, and other microorganisms. Depending on the allelochemical and microbe involved, these interactions can be positive and negative. Nilsson (1994) found that both allelopathic leaf leachates of *Empetrum hermaphroditum* and competition for resources suppressed the seedling growth of *Pinus sylvestris*. Understanding these interactions is critical for developing invasive species management in natural ecosystems. Allelopathy research is likely to yield new insights into these complex interactions and new avenues for utilizing the potential of allelochemicals in agriculture and natural resource management.

The allelopathic characteristics of plants and microbes play a crucial role in coping with various biotic and abiotic stresses (Muzell et al., 2016). Abiotic stress factors include extreme temperature changes, drought, floods, and radiation imbalances, including infrared and ionization, as well as external pressures like wounds, wind, and magnetic fields, which are significant contributors (Muzell et al., 2016). Biotic stresses, mediated by interactions with microbes, animals, and other plants, initiate signal perception and transduction processes, which

subsequently trigger metabolic responses and gene expression changes (Maqbool et al., 2013). The exposure of plants to biotic stress enhances allelopathic activity and may increase the concentration of allelochemicals produced. The production of these allelochemicals is dynamic and influenced by environmental conditions, causing allelopathic potential to appear and disappear in specific geographic regions (Scavo et al., 2018). Studies on *Quercus rubra* L. and *Acer rubrum* L. revealed an overall increase in allelopathic potential due to high temperature and drought, resulting in heightened tannin production (Top et al., 2017). The synthesis of allelochemicals varies based on the type and magnitude of stress, as reported by Pedro et al. (2016). Allelopathy can exert positive or negative effects through biochemical constituents produced by plants or microbes, affecting plant-plant, plant-microbes, and microbes-microbes interactions. Most allelochemicals originate from acetate or amino acids and play a role in the shikimic acid pathway (Li et al., 2010). The effects of allelochemicals, introduced into the environment with numerous secondary metabolites, include disruptions in growth, reproduction, germination, and distribution (Muzell et al., 2016). Ethylene exudation from the roots of *Striga lutea* Lour. serves as a natural biocontrol agent, inhibiting weed germination (Li et al., 2010; Gfeller et al, 2018). Certain bacterial species, like rhizobacteria, act as plant growth-promoting microbes through symbiotic behaviour, while some bacteria producing allelochemicals compete with plants for macro- and micronutrients (Basu et al., 2021).

The heightened degree of competitiveness observed in certain plant species may stem from the production of allelochemicals, compounds impede the germination and growth of neighbouring flora (Baker, 1974). This inhibition mechanism can occur by directly suppressing plant growth or inhibiting symbiotic microorganisms associated with the plants (Stinson et al., 2006). Notably, the novel-weapons hypothesis posits that certain invasive plants succeed in their proliferation due to the production of allelopathic substances that native plants lack adaptation (Callaway and Ridenour, 2004). Substantial empirical evidence supports this hypothesis (Ridenour and

Callaway, 2001; Prati and Bossdorf, 2004; Abhilasha et al., 2008; Gómez-Aparicio and Canham, 2008; Thorpe et al., 2009; Li and Jin, 2010; Inderjit et al., 2011; Becerra et al., 2018; Irimia et al., 2019), underscoring the significance of allelopathy in the invasive potential of certain plant species to suppress native vegetation. These allelopathic compounds are typically disseminated through various means, including leaf leachates, root exudates, volatile emissions, and decomposition of both above- and below-ground organic matter (Callaway and Ridenour, 2004; Callaway et al., 2005; Inderjit et al., 2011).

Problem description: *Senna spectabilis* as an invasive plant

Among tree invasives, one of the most prominent is *Senna spectabilis,* belonging to the Fabaceae family. This species is native to South America and is recorded as an invasive species in many parts of the world. It is a deciduous tree that grows quickly, reaching 15-20 meters in height and producing many seeds after flowering. The tree tends to fork near the ground and spreads widely with drooping, leafy branches. According to Irwin and Barneby (1982), it is one of the "most handsome ornamental sennas recommended for rapid growth". *S. spectabilis* is a nitrogen-fixing tree widely planted for ornamental purposes or as a shade or boundary tree. It can withstand a wide range of environmental conditions. *S. spectabilis* competes aggressively in disturbed forests, forest gaps, open vacant spaces, parks, riverbanks, and plantations but not in closed canopies (Irwin and Barneby, 1982), which is typical of most invasive plant species (Kornas, 1990; Duggin and Gentle, 1998). The invasion of *S. spectabilis* has significantly depleted the native vegetation in Wayanad Wildlife Sanctuary. The invaded region lacked ground cover, with a noticeable proliferation of *S. spectabilis* seeds (Prajitha and Sudhabai, 2022). It grows and spreads rampantly and prospers on acidic and even infertile soils. It flowers and sets seed precociously, and the viability of the seed remains for up to three years. It also has a great coppicing ability (Kerala Biodiversity Board, 2012). It resprouts quickly, profusely, and persistently when cut. The species is non-nodulating but accumulates nitrogen efficiently,

sometimes exhausting soil nitrogen reserves and is regarded as a nitrogen-fixing tree (Kerala Biodiversity Board, 2012). The rapid growth of *S. spectabilis* in Wayanad Wildlife Sanctuary (WWS), Kerala, India, is noted to be prominent compared to other exotic species (Prajitha and Sudhabai, 2022). The WWS, spanning an area of 344.44 square kilometers, is an integral component of the Nilgiri Biosphere Reserve and is a significant habitat for Asiatic elephants in India. Currently, the sanctuary is under severe threat from the rapid proliferation of invasive plants, causing a food shortage for herbivores. *S. spectabilis* was introduced as an avenue tree in the 1980's and started establishing itself widely in new areas, and its management has become a challenging task (Vinayan et al., 2020). A survey conducted by the Wildlife Trust of India and the Kerala Forest department has identified the major occurrence of *S. spectabilis* in the Muthanga, Sulthan Bathery, and Tholpetty forest ranges within the sanctuary (APFISN, KFRI, 2014). Initially, it was introduced as an ornamental in botanical gardens in India, followed by further accidental introduction from cultivated areas to forest areas in Sikkim and Mysore (Singh, 2001). Satyanarayana and Gnanasekaran (2013) reported this species in the forest areas of Sathyamangalam, suburban areas of Coimbatore and Wayanad Wildlife Sanctuary and confirmed that it has a high potential for flourishing rapidly and producing numerous viable seeds. It was first introduced to the Wayanad Wildlife Sanctuary in the early 1980s and has since expanded to approximately 23% of the sanctuary's total area in 40 years (Anoop et al., 2022). There are also reports of such accidental introduction in the Caribbean. It has also escaped from Trinidad and Tobago and invaded the northern parts of Orinoco in Venezuela (Irwin and Barneby, 1982). In addition, the regeneration and growth of native tree species are also suppressed by *S. spectabilis* (Wakibara, 1998; Wakibara and Mnaya, 2002). However, it is interesting to note that *S. spectabilis* was not recorded in the Global Invasive Species Database (2021) till that year.

Some adverse reports on this species have emerged since the last few decades. *S. spectabilis*

invaded forests in Uganda. *S. spectabilis* is typically avoided by timber loggers and is largely unattractive to forest herbivores, such as chimpanzees (NARO, 2004). While the detrimental effects of numerous invasive exotic species have been extensively studied, limited information is available regarding the invasiveness of *Senna spectabilis*. Nevertheless, its robust growth patterns, even in nutrient-poor soils, have been acknowledged (Balasubramanian and Sekanaynge, (1991), McClean *et al.,* (1992)). Ladha *et al.,* (1993) determined that *S. spectabilis* cannot fix atmospheric nitrogen, relying instead on its extensive root system to extract nitrogen from deep soil layers. The species has a proclivity for naturalizing in arborescent forests, particularly in disturbed environments. *S. spectabilis* reduced the overall seed germination percentage, indicating the presence of potential allelopathic inhibitory substances in such extracts (Pélagie et al, 2016). The Asian elephant (*Elephas maximus*) plays a crucial role as the primary disperser of Senna seeds, with its dung serving as a vital factor in facilitating the germination of the species (Anoop et al.,2022). However, studies under controlled agronomic conditions have not shown allelopathic effects on maize and rice (McClean *et al*., 1992). Despite Mahale being home to at least nine primate species, over 2.5 km^2 of its forest is currently infested by *S. spectabilis*, and the invasion shows signs of escalating (Wakibara, 1998).

Native species selected for understanding allelochemical stress were *Tectona grandis. Ailanthus tryphysa, Dendrocalamus strictus, Pongamia pinnata* and *Hopea parviflora.* These species were selected due to their ease of availability and their being members of the native plant community of Wayanad Wildlife Sanctuary. These plant species were selected to showcase the response of native plants and to understand the depth of impact imparted by the allelochemicals of *S. spectabilis*.

2.1. To analyze plant stress due to the invasive tree species *Senna spectabilis* on selected native plant species

Allelopathic compounds produce reactive oxygen species (ROS), leading to oxidative stress in plants (Sunmonu et al., 2014). During allelopathic interactions, ROS function as signalling molecules in plants (Bais et al., 2003; Harun et al., 2014). The generation of free radicals induced by allelochemicals affects membrane constituents and influences physiological processes such as membrane potential and lipid peroxidation (Rice, 1984; Lin, 2010). The root is the primary organ that encounters allelochemicals in the soil and rhizosphere, affecting water and ion uptake (Yu and Matsui, 1997). Plants employ various mechanisms to combat oxidative damage, with antioxidant enzyme activity a crucial component. Resistant plant genotypes often maintain elevated levels of antioxidants, leading to reduced oxidative damage. While antioxidants play a vital role in regulating intracellular ROS levels, an imbalance in their concentrations can prompt compensatory mechanisms (Apel and Hirt, 2004). In plant species, barley aqueous extract has been found to enhance lipid peroxidation (Farhoudi et al., 2012; Farhoudi and Lee, 2013). Cinnamic acid and benzoic acid in cucumber plants induce electrolyte leakage, thereby altering membrane permeability (Yu and Matsui, 1997).

To counteract the damaging effects of ROS, plants have developed cellular adaptive responses, which involve up-regulating oxidative stress protection mechanisms and accumulating protective solutes (Jiang and Zhang 2002; Hu and Kitts 2005). ROS primarily include the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO), perhydroxyl radical (HO_2), and singlet oxygen ($^1O_2$). In the absence of protective mechanisms, ROS can severely disrupt normal plant metabolism by oxidizing membrane lipids, proteins, and nucleic acids, as noted by researchers (Mittler 2002). Key components of this defence include antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX),

catalase (CAT), glutathione-S-transferase (GST), guaiacol peroxidase (POD), and glutathione reductase (GR).

Enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), catalase (CAT), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) act as protective agents during stress conditions. These enzymes help safeguard plants from oxidative stress. Non-enzymatic antioxidants, including glutathione (GSH), ascorbic acid (AsA), carotenoids, and tocopherols, also contribute to ROS scavenging in plants (Vranova et al., 2002). These enzymes play pivotal roles in balancing the production and elimination of ROS within plants. It's important to note that no single antioxidant enzyme can effectively combat all forms of ROS on its own, which could explain why they work synergistically (Miller et al. 2008). Superoxide dismutases (SODs) are a class of metallo-enzymes that include Fe-SOD, Mn-SOD, and Cu/Zn-SOD, each characterized by the specific metal ions they bind to (Pawlak et al. 2009). They are classified into three known classes and play a critical role in protecting plants from oxidative stress. These various SODs are localized in different subcellular compartments, such as chloroplasts, mitochondria, and the cytoplasm of plant cells. SODs function by catalyzing the dismutation of O_2 - to generate stable hydrogen peroxide (H_2O_2) and oxygen (O_2). Thus SOD is the initial line of defence against ROS-induced damage (Wang et al. 2009). Catalase (CAT) and peroxidase (POD) are essential in scavenging H_2O_2 within plant cells. While CAT can directly convert H_2O_2 into water, it exhibits a lower affinity for H_2O_2 than GPX (Miller et al. 2008). These antioxidant systems, including SODs, CAT, and POD, have been demonstrated to play crucial roles in enhancing plant tolerance to extreme environmental conditions (McKersie et al. 1999; Wang et al. 2009; Gill and Tuteja 2010). APX uses ascorbate as an electron donor to catalyze the conversion of H_2O_2 into H_2O. Guaiacol peroxidase (POD) scavenges H2O2 to regulate abiotic stress responses, participates in lignin biosynthesis, and modulates cell wall properties during plant growth (Lee and Lin, 1995).

Increased POD activity, however, can limit cell expansion due to lignin deposition, impacting overall growth (Dichio et al., 2002).

Previous studies have identified allelopathic phenolic compounds, including gallic acid, chlorogenic acid, and ferulic acid, in *Momordica charantia*, all of which possess strong antioxidant potential (Singh and Sunaina, 2014). In the context of root development, caffeic acid alters the activities of enzymes such as proteases, peroxidases, and polyphenol oxidases (PPOs) in mung beans (Batish et al., 2008). Similarly, cinnamic acid-treated cucumber roots exhibit increased activity of SOD, APX, CAT, and POD (Ye et al., 2006).

2.2. To identify, isolate, and elucidate the structure of allelochemicals in *Senna spectabilis*

Allelochemicals are excreted from various plant parts, such as roots, flowers, seeds, stems, leaves, and pollen grains. These low molecular weight allelochemicals possess medicinal and agricultural value, functioning as growth regulators, herbicides, insecticides, and antimicrobial crop-protective agents. Commercially, allelochemicals are extracted from plants using leaching, volatilization, exudation, and decomposition of plant residues (Collier, 2016). Traditionally, metabolite extraction involves solid-liquid extraction, followed by distillation and refining. Phenolic acids, quinones, flavones, flavonoids, tannins, and coumarins, obtained through this process, are known for their allelopathic activities. Solubilization of bioactive compounds employs various liquid or gaseous solvents depending on the compound's nature (Collier, 2016). With advancements in metabolic engineering, certain plant/microbe-derived compounds are explored for the industrial production of medicines, flavourings, antioxidants, plant growth promoters, and pesticides. The bioprocess should be designed to obtain low-volume, high-value-added products, emphasizing suitable downstream processing strategies for extraction and purification (Preece, 2017).

Plant biomass is initially crushed into a powder form to obtain a concoction of chemicals, including potential allelopathic compounds. Subsequently, extraction is carried out using solvents such as ethanol, methanol, acetone, ether, and chloroform (Neiva et al., 2019). The primary objective of employing extraction techniques is to optimize the recovery of metabolites from the plant matrix, ensuring the preservation of target molecule integrity and simultaneously reducing the presence of undesired compounds (Godoy et al., 2014; Kapoor et al., 2019).

Soxhlet extraction, a classical automatic continuous system that integrates the percolation and reflux principles, is employed to constantly extract herbs using fresh solvents. This method demonstrates high extraction efficiency with reduced solvent consumption and time compared to maceration and percolation. The process involves a batch extraction procedure featuring intermittent solvent infusion, akin to static extraction, once the soxhlet thimble is filled with liquids. Throughout the extraction, solvent recirculation occurs. Soxhlet extraction remains a frequently used technique for extracting natural antioxidants from plant materials, offering high extraction yields (Camel, 2006). Solvent extraction (SE) is the most extensively used extraction technique, wherein the efficacy of extraction is influenced by various factors such as the properties of the solvent, solvent/solid ratio, particle size of the raw materials, extraction time, and extraction temperature (Zhang et al., 2018). Screening the solvent is a crucial step in this method, and considerations such as solvent solubility, selectivity, safety, and expenditure should be considered. The effectiveness of extraction can be enhanced by reducing particle size and facilitating better diffusion of solvents and solutes.

Phytochemicals of *S. spectabilis* have been extensively studied and documented. Selegato et al. (2017) have examined the chemical aspects of *S. spectabilis*. Forty secondary metabolites were isolated from *S. spectabilis*, with a predominant presence of pentacyclic triterpenes and piperidine alkaloids exhibiting various health-promoting properties and known for its antimicrobial properties, attributed to a range of bioactive compounds such as steroids,

flavonoids, anthraquinones, anthrones, and various other substances. These bioactive components are distributed throughout the plant, including the leaves, stems, roots, flowers, bark, seeds, and fruits (Alshehri, 2022). Among the compounds isolated from the leaves by Silva *et al.* (2010) are caffeine, lupeol, α-amyrin, β-amyrin, cycloeucalenol, friedelin, ursolic acid, oleanolic acid, betulinic acid, sitosterol, and stigmasterol, along with their respective glucosides. Lim et al. (2018) also contributed to the understanding of *S. spectabilis* by isolating (+)-spectaline and iso-6-spectaline from the leaves. The methanol leaf extract and DCM flower extract of *S. spectabilis* significantly inhibited the germination and growth of *C. barbata* and *B. chinensis* at 10,000 ppm (Mongkol and Chavasiri 2018).

Many allelochemicals have been reported in the last 40 years. The first reported and verified allelochemical is Juglone, a phytotoxic compound found in black walnuts in a non-toxic naphthol O-glycoside form. This glycoside is biosynthesized in living tissues and can be released from leaves, bark, and roots into the environment. Through hydrolysis or interactions with soil microbes, the naphthol O-glycoside rapidly transforms into aglycone, a less phytotoxic naphthol. Subsequently, the aglycone is oxidized to form the phytotoxic juglone (Rietveld, 1983).

Cunninghamia lanceolata, commonly known as Chinese fir, holds significant importance as a conifer tree species in industrial wood production. The failure of regeneration and declining productivity in Chinese fir is a critical concern, primarily attributed to the persistent practice of monocultures (Bi et al., 2007; Xia et al., 2015). A pivotal factor contributing to this problem is the emergence of a novel allelochemical cyclic dipeptide (6-hydroxy-1,3-dimethyl-8-nonadecyl-[1,4]-diazocane-2,5-diketone), autonomously released by Chinese fir trees themselves. The cyclic dipeptide, released from the roots of Chinese fir, accumulates in the soil during successive monoculture cycles. Its increasing concentration leads to self-inhibition through intraspecific allelopathy (Kong et al., 2008; Chen et al., 2014). This allelochemical has strong phytotoxic effects, limiting the growth of Chinese fir offspring in plantations. Interestingly, when Chinese

fir is co-cultivated with other tree species like Michelia macclurei, the release of the allelochemical is reduced, and its degradation in the soil is improved. Consequently, this dynamic interplay transitions from self-inhibition to facilitation within the Chinese fir ecosystem (Xia et al., 2016). 1α-Angeloyloxycarotol, a carotene-type sesquiterpene, has been isolated and identified from giant ragweed (*Ambrosia trifida)* infested wheat fields and inhibited their growth (Kong et al., 2007).

Fine fescue grasses (*Festuca* spp.) play a crucial role in enhancing ecological function in stressed environments of rangelands. These grasses could outcompete neighbouring plants by releasing phytotoxic root exudates into the soil. A significant allelochemical called m-tyrosine, a non-protein amino acid, has been identified within these exudates. The allelopathic action of fine fescue grasses is attributed to m-tyrosine, which interferes with the root development of other plants. Consequently, this interference leads to the displacement of neighbouring plants by the fescue grasses (Bertin et al., 2007).

2.3. To analyze the microbial diversity in Senna spectabilis-invaded areas

Plant invasions have a notable impact on soil composition and nutrient cycles, and the extent of this impact is influenced to some degree by the similarity in morphological and phenological traits between the invading species and the native ones it displaces (Ehrenfeld 2003, Vanderhoeven et al. 2005, Ashton et al. 2005). Depending on the characteristics of the native soil, invasive plants often lead to increased biomass and net primary production, resulting in significant alterations to soil composition and nutrient cycling (Ehrenfeld, 2003). These alterations manifest as changes in nitrogen availability, soil pH, phosphorous levels, aluminum concentration, and nutrient dynamics due to the presence of invasive plants, which, in turn, can impact forest biota, reduce microarthropod diversity, and disrupt the natural progression of forest succession (McLendon and Redente, 1992, McGrath and Binkley 2009). When released

into the novel environment, IAPS produce allelochemicals that can disrupt regular decomposing processes in soil communities (Qu et al., 2021). IAP's alter the soil microbial community structure and carbon biomass, which can promote their growth and survival (Elsheikh et al., 2021). In turn, soil microbes affect the growth and survival of invasive plants by influencing nutrient availability and uptake (Morgan et al., 2016). IAPS alters the composition of rhizosphere microbial communities, affecting their growth and survival (Rout and Callaway, 2012). IAPS harbours endophytes that produce secondary metabolites promoting their own growth and survival (Elsheikh et al., 2021). IAPS disrupts the mutualistic relationship between native plants and mycorrhizal fungi, negatively impacting native plant growth and survival (Grove et al., 2017).

The invasion of plant species can also bring about shifts in microbial communities, with the most pronounced changes occurring in cases of multiyear successions. These changes can potentially disturb ecosystems' fitness and functioning (Batten et al., 2006, Rodrigues et al., 2015). *Lespedeza cuneata* was found to significantly alter soil bacterial and fungal communities, with the degree of change increasing with the invasion duration (Yannarell et al., 2011). In areas where long-lasting plant invasions have occurred, the impacts on soil biota and chemistry can persist for many years, even after removing invasive species, hindering the ability of native plants to progress through their natural seral stages. (Corbin and D'Antonio, 2004; Flory and Clay, 2010; Corbin and D'Antonio, 2012). *Imperata cylindrica* (L.), a non-native invasive plant spreading throughout the southeastern United States, did not significantly change soil nitrate availability between invaded and uninvaded control plots. However, eradicating this invasive species led to short-lived but significant alterations in soil nutrient cycling (Hagan et al., 2013a). *Imperata cylindrica* produces allelopathic exudates that disrupt mycorrhizal symbiosis and inhibit the development of fine roots (Hagan et al., 2013b).

Invasive plants can significantly alter various aspects of soil, including its pH level, carbon (C)

and nitrogen (N) content, rates of nitrification and mineralization, and concentrations of essential elements like calcium (Ca), magnesium (Mg), and potassium (K) (Simba et al., 2013). Wang et al. (2015) conducted research showing that plant invasion can lead to a notable increase in soil acidity. They explained that this increase in acidity is related to the release of hydrogen ions (H+) during the nitrification process in the soil, which, in turn, further contributes to soil acidity. Furthermore, the acquisition of nitrogen by invasive plants can also influence the pH levels of the soil. Invasive plants change their surroundings in a way that makes more nitrogen available and facilitates invasion (Wang et al., 2015). Additionally, Stefanowicz et al. (2018) found that when invasive plants take over an area, they can change the chemical composition of the topsoil by extracting nutrients from deeper soil layers. Invasive species alter the soil physico-chemical parameters and create a microenvironment that favours their growth and establishment and suppresses the growth of native species (Varughese and Joseph, 2023).

The biological diversity observed in plant communities is a reflection of the ongoing process of co-evolution, influenced by a multitude of factors across various levels of interaction, including climatic, edaphic, and both inter- and intraspecific interactions among microbes and host plants, as well as human agency (Berendsen et al., 2012; Bulgarelli et al., 2012; Ehrenfeld, 2003; Hardoim et al., 2015; Sanon et al., 2009; Stone et al., 2018; Torsvik and Øvreås, 2002; Williams and de Vries, 2020). Perceptions of the utility of biodiversity elements in socio-cultural and economic contexts drive the actions of human agents. Above-ground plant communities play a significant role in shaping the structure of soil microbial communities, with microbial abundance and activity exhibiting horizontal and vertical patterns along soil profiles (Constancias et al., 2015). Spatial heterogeneity in microbial distribution spans from millimetre to meter scales, influenced by soil properties such as texture, aggregation, moisture, pH, organic matter content, oxygen concentration, nitrogen availability, precipitation levels, and vegetation dynamics (Constancias et al., 2015). While some properties exert significant effects at

microscopic scales, others impact microbial distribution over larger distances, with less disturbed systems often exhibiting high spatial heterogeneity (Constancias et al., 2015).

Soil microbes are vital components of ecosystems, significantly impacting various ecological processes such as nutrient acquisition, nitrogen and carbon cycling, and soil formation. They constitute a substantial portion of Earth's genetic diversity, yet often remain unseen (Smith and Read, 1997; Sprent, 2001; Tiedje, 1988; Kowalchuk and Stephen, 2001; Hogberg et al., 2001; Rillig and Mummey, 2006; Whitman et al., 1998). The interaction between soil microbes and native and non-native plant species, particularly through mycorrhizal and bacterial symbiosis, plays a crucial role in plant invasion ecology, influencing soil nutrient cycling and enzyme activity (Koshila, 2019). Plants introduced to foreign environments can overcome ecological barriers and proliferate, driven by various mechanisms contingent upon plant and soil community dynamics (Bever, 2003). Despite being a primary challenge to ecosystems, the factors facilitating plant invasion remain elusive, often resulting in global environmental changes, biodiversity loss, and displacement of native species (Mooney and Cleland, 2001).

Rhizosphere, the soil zone surrounding plant roots, strongly influences plant invasion processes by mediating microbial interactions. Plants can modify soil microbial composition, particularly mycorrhizal fungi and bacteria, altering ecosystem functions (Ehrenfeld, 2003). Soil microbes are pivotal in facilitating or inhibiting invasive species, thereby shaping ecological changes and successional patterns (Bever, 2012). Plant invasions can modify soil physiochemical properties, affecting pH, moisture, and temperature, thereby influencing microbial community dynamics (Yelenik, 2019). Introducing and preventing invasive plants hinge upon the symbiotic relationships between soil microorganisms, impacting plant growth and establishment (Sánchez, 2014). Notably, invasive plants such as *Bidens pilosa* can accumulate soil microorganisms, alter soil enzyme activities and nutrient availability, and impact native plant species (Jing et al., 2016). The microbial biomass composition in invaded soils differs from non-invaded soils, with

implications for ecosystem functioning (Xiao et al., 2014). Furthermore, invasive plants may disrupt mutualistic relationships between native plants and mycorrhizal fungi, promoting their growth at the expense of native species (Pysek and Richardson, 2010). The success of plant invasions is often attributed to the release of allelopathic compounds, which influence soil microbial flora and inhibit the growth of native species (Wardle et al., 2012).

Plant invasions alter soil microbial communities and functions, impacting nutrient cycles and ecosystem stability. Understanding these complex interactions is crucial for effective management and conservation efforts in invaded ecosystems.

Soil structure dynamics and microbial response

Soil biota inevitably aids plant invasions (van der Putten et al., 2005; Wolfe and Klironomos, 2005; Inderjit and van der Putten, 2010). Introducing and spreading invasive species can result in the displacement of native plant species and changes in soil chemistry and microbial communities. Microbial communities may change composition and function in response to plant species invasion, resulting in changes in ecosystem dynamics. Enhancement of antagonistic activity is one of the responses of microbial communities to invasive plant species. Invasive plants typically enhance both the diversity and population levels of soil organisms. The characteristics of invasive plants and the taxonomic group they belong to significantly impact the microbial community's diversity. Some invasive plant species significantly impact bacterial diversity (Torres et al., 2021). Invasive plants can also alter the structure of the microbial community by altering soil pH, which can affect the growth of specific microbial populations.

Microbial communities can respond to the invasion of plant species by enhancing their antagonistic activity. Antagonistic activity is the ability of microorganisms to inhibit the growth of other microorganisms. Several studies have reported enhancing the antagonistic activity of microbes in response to invasive plant species. *Solidago canadensis* enhanced the antagonistic

activity of soil bacteria against the plant pathogen *Rhizoctonia solani* (Callaway et al., 2004). The enhancement of antagonistic activity was attributed to the increase in the production of antifungal compounds by soil bacteria. The enhancement of the antagonistic activity of microbes in response to invasive plant species can also be mediated by changes in the microbial community structure.

The enhancement of the antagonistic activity of microbes in response to invasive plant species can be mediated by various mechanisms and can provide a natural and sustainable method of controlling invasive species. One of the mechanisms is the production of antifungal compounds by soil bacteria. Antifungal compounds can inhibit the growth of plant pathogens, and the presence of invasive plant species can induce the production of such compounds (Callaway et al., 2004). Another mechanism is the alteration of the microbial community structure, which can increase the abundance of microorganisms with antagonistic activity (Li et al., 2015).

The use of biocontrol agents, such as beneficial microbes, is a promising alternative to synthetic pesticides, which can negatively affect the environment and human health. Several studies have investigated the potential use of microbes in the biocontrol of invasive plant species. The bacterium *Pseudomonas fluorescens* inhibits the growth of the invasive plant species *Centaurea maculosa* by producing antifungal compounds (Bais et al., 2003). *Bacillus* as a biocontrol agent has also been investigated in managing the invasive plant species *Spartina alterniflora* (Li et al., 2015). Microbes can be more effective as biocontrol agents when their ability to fight invasive plant species is boosted.

Plant-bacteria-soil interactions play a crucial role in both plant growth and soil fertility. Understanding the specific associations between soil microbes and plants is imperative for harnessing the benefits of beneficial microorganisms to enhance plant growth (Antoun, 2012). Coined by Kloepper in 1978, Plant Growth Promoting Rhizobacteria (PGPR) refers to soil

bacteria that inhabit the root zone and contribute directly or indirectly to plant growth by producing various chemical compounds. Rhizospheric microorganisms facilitate phosphate and potassium solubilization, nitrogen fixation, and soil organic matter decomposition (Flaishman et al., 1996). Additionally, rhizobacteria can secrete plant growth regulators such as indole acetic acid, ethylene, cytokinin, and gibberellic acid (Yahya and Al-Azawi, 1989).

Microbial community diversity mirrors that of plant communities, manifesting across various levels of variability and complexity, encompassing genotypic diversity within species populations, species richness, relative abundance, and variability in functional group composition (Torsvik and Øvreås, 2002). However, gaps remain in understanding the repercussions of introducing plants into new environments with varied soil microbiomes, particularly in terms of plant-microbe interactions and ecosystem dynamics (Berendsen et al., 2012; Bulgarelli et al., 2012; Sanon et al., 2009).

Plant-associated microbes inhabit various plant tissues and surfaces, significantly influencing ecosystem-scale changes in soil biochemistry (Berendsen et al., 2012; Bulgarelli et al., 2012; Sanon et al., 2009). Changes in soil microbial community composition following invasion by exotic plant species can profoundly impact ecosystem functioning and native plant community composition (Ehrenfeld, 2003). The interaction between plants and their associated microbiota is governed by a myriad of biotic and abiotic factors, with soil properties such as pH, salinity, structure, moisture, and organic matter content exerting significant influence (Fierer, 2017; Hardoim et al., 2015). Host- and compartment-specific assembly indicates a strong functional relationship between plants and their above-ground microbiota, necessitating further research for deeper comprehension (Hardoim et al., 2015).

Endophytes and above-ground microbiota are recognized for their potential to promote plant growth, enhance disease resistance, and alleviate stress (Stone et al., 2018; Hardoim et al.,

2015). The invasive ability of plants is often bolstered by complex feedback loops of plant-soil microbe interactions, with allelopathic effects mediated by microbial and non-microbial transformations of soil chemicals (Richardson et al., 2000; Weidenhamer and Romeo, 2004). Microbial traits in the rhizosphere are shaped by the evolutionary history of host-microbe associations, influencing plant invasion dynamics (Lugtenberg and Kamilova, 2009).

Soil microbes respond to plant root exudates, forming a nutrient-rich rhizosphere that fosters competitive interactions among microbial strains, ultimately selecting for symbiotic traits beneficial to host plants (Lugtenberg and Kamilova, 2009). Invasive plants may disrupt soil microbial communities through the production of allelochemicals, altering nutrient cycling patterns to favour their growth (Kourtev et al., 2002; Blank and Young, 2004; Caldwell, 2006; Lankau, 2012; Morris et al., 2016). Root exudate variation among plant species contributes to the 'rhizosphere effect,' influencing microbial composition around roots (Mendes et al., 2013; Hartmann et al., 2008). Plant-microbe relationships play a crucial role in plant growth and survivorship, with implications for plant community dynamics (Bulgarelli et al., 2013; Mikiciński et al., 2016).

Phytohormones selectively impact bacterial phyla in microbial communities, while soil microbes influence plant signalling and metabolism through the production of secondary metabolites (Pieterse et al., 2012; Carvalhais et al., 2014; Rout and Southworth, 2013). Plant growth-promoting bacteria directly facilitate plant growth by providing necessary compounds or modulating plant hormone levels while indirectly mitigating pathogenicity (Glick, 2012).

The rhizosphere harbours diverse microorganisms that play pivotal roles in synthesising bioactive compounds, including antibiotics, enzymes, and plant growth-promoting factors (PGPF). Notably, certain bacterial and fungal inhabitants of the rhizosphere function as facilitators of plant growth by augmenting the provision of PGPF, thereby earning designations

such as plant growth-promoting fungi (PGPF) and plant growth-promoting rhizobacteria (PGPR) (Murali et al., 2012). These entities also mitigate soil-borne pathogens, fostering plant health and vigour.

In plant nutrition, essential macronutrients such as nitrogen, phosphorus, and potassium are fundamental for plant growth and development. Studies emphasize the significance of microbial mediation in transforming insoluble nutrient forms into accessible forms for plants. Certain fungal species exhibit prowess in combatting phytopathogens, solubilizing nutrients like phosphorus and potassium, and synthesizing Indole-3-acetic acid (IAA), a crucial plant growth regulator (Ek et al., 1983). The synthesis of auxins by plants and fungi is paramount as hormones govern plant growth and development (Gabriel et al., 2009). Interactions between plants and microbes, particularly those involving IAA, are pivotal in shaping plant health and resilience. Plant growth-promoting fungi demonstrate diverse mechanisms for pathogen suppression, including antibiosis, antagonism, mycoparasitism, and predation (Shivanna et al., 1996). Furthermore, certain fungi capable of IAA reduction exhibit defensive capabilities against plant pathogens, thereby eliciting plant immune responses (Kumla et al., 2014). The symbiotic association of plant growth-promoting microorganisms with host plants can significantly bolster plant invasiveness. Consequently, understanding the dynamics of plant-bacteria-soil interactions is imperative for optimizing soil fertility and enhancing plant growth (Antoun, 2012).

The term "Plant Growth-Promoting Rhizobacteria" (PGPR), coined by Kloepper in 1978, pertains to soil bacteria that colonize the rhizosphere and exert direct or indirect influences on plant growth via the production of various chemical compounds (Kloepper, 1978). Rhizospheric microorganisms enhance soil fertility by promoting phosphate and potassium solubilization, nitrogen fixation, and the decomposition of organic matter (Flaishman et al., 1996). Furthermore, these bacteria can secreter plant growth regulators such as indole acetic acid,

ethylene, cytokinin, and gibberellic acid (Yahya and Al-Azawi, 1989).

Phosphorus, an indispensable nutrient for plant growth, can be rendered accessible through phosphate-solubilising microorganisms, encompassing various bacterial, fungal, actinomycetal, and algal species (Antoun, 2012). Among these, bacteria exhibit higher phosphate solubilizing capacities than fungi (Alam et al., 2002). Phosphate-solubilizing bacteria can enzymatically convert insoluble organic phosphate into a utilizable form, facilitating plant nutrient uptake (Satyaprakash et al., 2017).

Similarly, potassium-solubilizing bacteria play a pivotal role in plant growth by converting insoluble potassium reservoirs into absorbable forms. Bacterial genera such as Pseudomonas, Bacillus, Rhizobium, and Enterobacter exhibit potassium solubilizing capacities (Whitelaw, 1999). The principal active form of auxin, Indole-3-acetic acid (IAA), is synthesized by soil microbes such as bacteria and fungi, promoting plant growth (Harikrishnan et al., 2014). Notably, bacteria such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Micrococcus*, *Cellulomonas*, and *Rhodococcus* have been identified as producers of IAA (Tsavkelova et al., 2005; Shahab et al., 2009; Patel and Patel, 2014).

Furthermore, ammonia production by rhizosphere bacteria supports plant growth by facilitating nitrogen fixation via ammonification. Bacteria such as *Serratia*, *Bacillus*, and *Pseudomonas* are known for their high ammonia production rates, contributing to soil nitrogen availability and plant nutrient uptake (Agbodjato et al., 2015). Soil harbours a diverse array of microbes that exhibit antagonistic properties against both gram-positive and gram-negative bacteria. Notably, bacteria such as Pseudomonas and actinomyces play a pivotal role in inhibiting the proliferation of gram-negative bacteria within the soil ecosystem (Waksman and Woodruff, 1940). Among the identified antagonists, *Bacillus subtilis, B. megaterium, Streptomyces spp., B. cereus, B. polymyxa, Pseudomonas putida,* and *P. fluorescence* were prominently isolated (Broadbent et

al., 1971).

The significance of soil microbial community diversity and composition concerning plant health is well recognized in ecological literature (Allison and Vitousek, 2004). Microbial diversity positively influences plant survivorship by enhancing tolerance to abiotic and biotic stresses (Callaway et al., 2004). Invasive plant species have been shown to alter fundamental ecosystem processes, such as nutrient cycling and energy flow, to their advantage upon invasion (Aon and Colaneri, 2001; Flory and Clay, 2010). Litter decomposition, a critical aspect of nutrient cycling, is heavily influenced by soil enzymes regulated by soil microbial communities (Allison et al., 2007). The dominance of invasive plants in a habitat disrupts soil networks and alters soil enzyme activity, affecting soil fertility and maintenance (Aon and Colaneri, 2001; Flory and Clay, 2010). These alterations extend belowground, leading to shifts in soil microbial communities, creating positive feedback loops for invasive species and negative feedback for native species (Kulmatiski et al., 2008).

Plant-Soil Feedback (PSF) studies investigate interactions between invasive plants and soil microbes, revealing potential competitive advantages for invasive species over natives (Dawson and Schrama, 2016). Instances from PSF studies demonstrate that exotic plants may experience fewer negative effects from soil biota compared to native plants, potentially contributing to their invasion success (Reinhart et al., 2003; van der Putten et al., 2007; Kulmatiski et al., 2008). For example, the absence of specialist soil pathogens in exotic environments, posited by the Enemy Release Hypothesis (ERH) (Keane and Crawley, 2002; Liu and Stiling, 2006), may confer a competitive advantage to invasive species. The Evolution of Increased Competitive Ability (EICA) hypothesis, an extension of ERH (Blossey and Notzold, 1995), suggests that selection favours exotic genotypes that invest less in defence and more in growth, enhancing competitiveness against native species.

Studies supporting EICA have demonstrated shifts in biomass allocation favouring stem growth in exotic environments (TeBeest et al., 2009). However, conflicting findings exist, with some studies reporting lower biomass in exotic genotypes than in natives (Zheng et al., 2015). Assessing the role of "pathogen release" in invasions requires biogeographic comparisons between exotic and native ranges (Hierro et al., 2005). Despite various theoretical frameworks, empirical evidence linking aboveground plant communities to belowground microbial populations is often inconclusive (Wardle et al., 1999). Nonetheless, it is suggested that upper trophic levels may be more influenced by plant diversity variations than lower trophic levels, such as microbes (Mikola et al., 2001), emphasizing the complexity of forecasting the effects of aboveground plant communities on higher trophic levels (Mikola and Setala, 1998).

Implications for controlling invasive plant species:

The links between microbial chemotaxis and invasive plant species have important implications for developing novel strategies for controlling invasive plants. For example, it may be possible to develop targeted approaches for controlling invasive plant species by understanding how invasive plants impact microbial communities and alter microbial chemotaxis. One potential approach is using microbial inoculants to restore or enhance the diversity of soil microbial communities, which may limit the growth and spread of invasive plant species (Hartmann et al., 2008). Another potential approach is using allelopathic compounds to disrupt the ability of invasive plant species to compete with native plant species. For instance, some allelopathic compounds may selectively inhibit invasive plants' growth while having little impact on native plant species (Callaway and Ridenour, 2004).

The potential applications of research on microbial chemotaxis and invasive plant species extend beyond developing control strategies. For example, understanding how microbial communities respond to environmental changes may provide insights into the mechanisms by

which ecosystems adapt to novel conditions. Additionally, research on the interactions between invasive plants and microbial communities may have important implications for understanding the impacts of invasive species on ecosystem functioning and predicting future invasions' outcomes. By understanding how invasive plants impact microbial communities and alter microbial chemotaxis, it may be possible to develop novel strategies for controlling invasive plant species. Furthermore, research on the links between microbial chemotaxis and invasive plant species may have broader implications for understanding the impacts of invasive species on ecosystem functioning and predicting future invasions' outcomes.

Lankau et al. (2011) showed that invasive plants can cause a reduction in soil microbial diversity and alter the structure of microbial communities. Similarly, Kourtev et al. (2002) found that invasive plants can alter the composition and function of soil microbial communities, leading to changes in nutrient cycling and other ecosystem processes. Invasive plants may also impact microbial chemotaxis through their production of allelopathic compounds. Allelopathy is the ability of plants to produce chemicals that influence the growth, development, or behaviour of other plants or microorganisms (Bais et al., 2006). These allelopathic compounds may also impact microbial chemotaxis by influencing the behaviour of microbial communities in the soil. Some invasive plant species produce toxic allelopathic compounds to certain soil microbes, leading to changes in the structure and function of soil microbial communities (Frey-Klett et al., 2007). Similarly, allelopathic compounds produced by invasive plants may alter the production of chemotactic compounds by soil microbes, leading to changes in microbial behaviour and potentially impacting the ability of native plants to compete with invasive species (Hartmann et al., 2009). The impacts of invasive plant species on microbial chemotaxis have important implications for developing control strategies for these species. For example, using microbial inoculants that enhance the growth and activity of native soil microbes may provide a natural means of suppressing the growth of invasive plants (Allison and Martiny, 2008). Additionally,

the identification of allelopathic compounds produced by invasive plants may provide targets for developing selective herbicides that target these species without impacting native plant species (Callaway and Ridenour, 2004).

The intricate relationship between plants, allelochemicals, invasion, and soil microbes has captivated researchers for decades. Allelochemicals are secondary metabolites produced by plants that influence the growth and development of neighbouring organisms, including both competing and beneficial species. Understanding the interplay between allelochemicals, invasion dynamics, and soil microbial communities is crucial for comprehending the ecological processes that shape plant communities. However, despite significant progress in this field, several research gaps remain.

Although numerous studies have explored the effects of allelochemicals on soil microbes, there remains a need to elucidate the underlying mechanisms of these interactions. Allelochemicals can positively and negatively affect microbial growth, activity, and community composition. Investigating the molecular and biochemical mechanisms underlying these interactions will provide valuable insights into how invasion influences soil microbial communities and subsequent invasion dynamics. Soil microbes play a pivotal role in the fate and activity of allelochemicals in the rhizosphere. They can facilitate the degradation, transformation, or detoxification of allelochemicals, influencing their bioavailability and impact on plant communities. However, the specific microbial taxa involved in these processes and their functional contributions are poorly characterized. Investigating the microbial mechanisms underlying allelochemical metabolism and their potential implications for plant invasion will deepen our understanding of the complex tripartite interactions between allelochemicals, invasion, and soil microbes. This review section aims to provide a thorough understanding of invasion hypotheses, the mode of action of allelochemicals produced by invasive alien plants, their physiological pathways, and current research on the enhancement of antagonistic activity

of microbes in response to invasive alien plants.

Biological invasions have negatively influenced human civilization. Invasive tree species pose a significant threat to native ecosystems, disrupting the delicate ecological balance that sustains biodiversity. Invasive alien species are the second leading cause of global biodiversity loss (Drake et al. 1989). The current research addresses the impact of *Senna spectabilis*, a particularly invasive tree species that has been unintentionally introduced to the forest ecosystems in India, particularly at the Wayanad Wildlife Sanctuary study area. As invasive species are known to outcompete native flora and establish monospecific stands, altering the structure and function of ecosystems, the current research aims to address the multifaceted impacts of *S. spectabilis* invasion on the environment's abiotic and biotic components.

The first objective aims to elucidate the physiological and morphological changes in selected native plant species when exposed to known stressor *S. spectabilis*. By investigating alterations in the native plants' biology, we seek to uncover the direct impact of *S. spectabilis* on their overall health and adaptability.

The second objective focuses on identifying, isolating, and elucidating the structure of allelochemicals in *S. spectabilis*. Understanding the chemical compounds produced by this invasive species is crucial for comprehending its ecological interactions and potential allelopathic effects on coexisting vegetation.

The third objective investigates the influence of *S. spectabilis* on the composition and abundance of microbial communities in the rhizosphere and surrounding soil. This exploration aims to unravel the intricate relationships between the invasive tree species and the microbial realm. Another aspect of the third objective delves into the role of microbial communities in mediating the interaction between *S. spectabilis* and native plant species. Understanding this interplay is crucial for assessing the overall impact on ecosystem health. The final component in the third

objective investigates how the invasive behaviour of *S. spectabilis* influences soil nutrient availability and water dynamics. This, in turn, has implications for the stress levels of coexisting native plant species, providing a comprehensive perspective on the ecological consequences of *Senna spectabilis* invasion.

This research aims to contribute valuable insights into the complex web of interactions involving *S. spectabilis*, native plant species, and microbial communities. By addressing these objectives, we hope to advance our understanding of the ecological consequences of invasive species and contribute to developing effective management strategies and mitigating allelochemical effects in soil with a restoration programme for preserving biodiversity.

CHAPTER 3

METHODOLOGY

Chapter 3
Methodology

3.1. Site description

The present study was conducted in the Wayanad Wildlife Sanctuary (11°44 N–11°97 N and 75°77 E–76°43 E), located on the crest of the Western Ghats in northeast Kerala, India. The altitude of the study area varies from 700 to 2100 metres above mean sea level (m.asl), covering an area of 344.44 km^2. The annual average temperature is 19 to 22°C in this area. January or February are the coldest months, with monthly average temperatures of 14–17 °C; August is the hottest month, with average temperatures of 23–32 °C. The annual precipitation is 2322 mm. The precipitation is the highest from June to September, with more than 50% of the expected annual precipitation (800–2000 mm) during this period (CABI, 2021) and receives full sunlight (PIER, 2014). The average relative humidity ranges between 60.4% in January and 87.6% in June. The hydrothermal conditions and geographical location of Wayanad Wildlife Sanctuary provide natural environmental conditions for the invasion and reproduction of *Senna spectabilis*. The district is contiguous to the Nilgiri Biosphere Reserve (NBR). Wayanad district is Kerala's only district that shares a border with the Indian states of Karnataka (north and northeast) and Tamil Nadu (southeast).

Our study selected three sites: the pristine forest (control – Uninvaded), *Senna spectabilis* invaded, and *Senna* managed forest. Forest protected zones cover over 40% of the district's land area (Sand, 2016). Southern moist deciduous and dry deciduous forests are the principal vegetation, along with monoculture plantations of teak and eucalyptus (Anoop and Ganesh, 2020). The moist deciduous forest consists of *Terminalia elliptica*, *Terminalia crenulata*, *Shorea roxburghii*, *Dalbergia latifolia*, *Pterocarpus marsupium, Grewia tiliifolia, Strychnos nux vomica, bamboos* and more, while the semi-evergreen patches comprise of *Veteria indica, Lagerstroemia lanceolata, Terminalia paniculata.* A diverse range of flora and wildlife and rich biodiversity is

harboured in the district, a UNESCO World Heritage site and a global biodiversity hotspot (Johna et al., 2020). The sample plots' broad soil type is Ferroiite, and sub type is Ustic Altisol. The key attributes of the sites under examination are outlined in Figure 1.

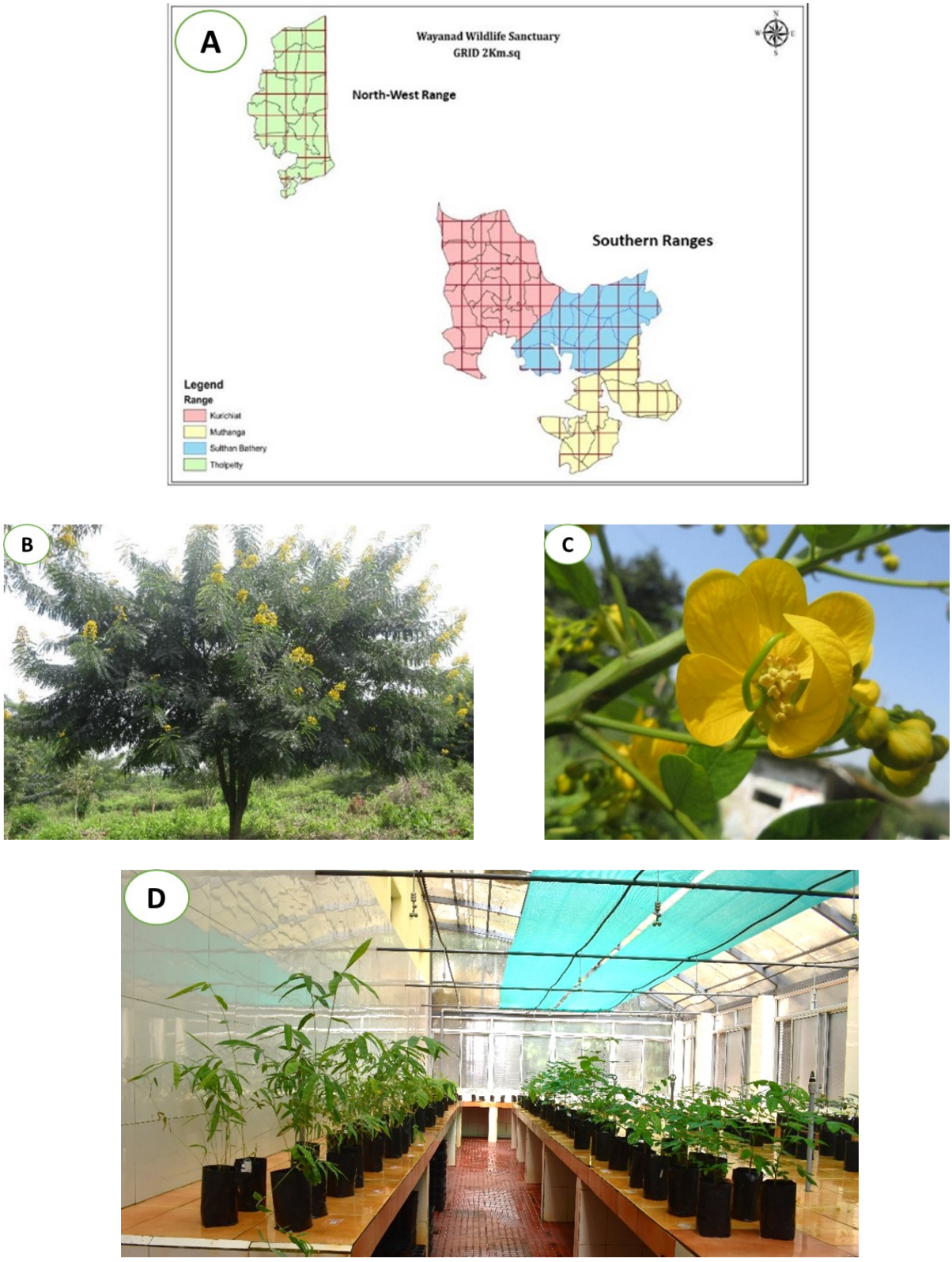


Figure 1. A. Study area: Map of Wayand Wildlife Sanctuary (WWS), B. *Senna spectabilis* tree

habit C. Flower of *Senna spectabilis*, D. Green house for maintaining various tree seedling for allelopathic experiments

3.1.1. Laboratory preparations for conducting the experiments

The first line of the experiment was to maintain the required aseptic conditions, including cleaning glassware for the physiological studies. The clear solution of chromic acid was prepared for cleaning the apparatus, and glasswares required in the experiments by dissolving 80 g of $K_2Cr_2O_7$ in 300 mL of distilled water and to this 400 mL of concentration, analytical grade H_2SO_4 was added gradually. The glasswares were dipped in chromic acid solution, washed with tap water, cleaned with detergent and rinsed with distilled water. Only borosil glassware, double-glass distilled water and AR-grade chemicals were used for the experiments.

3.1.2. Experimental design and collection of plant material

All the experimental plots were arranged in a completely randomized block design. Only the fallen senescent leaves of *S. spectabilis* were collected for the analysis. After removing dust and contaminants, all samples underwent air drying and grinding. Ground dried powder (5 kg) of the above plant parts of *S. spectabilis* was extracted using distilled water periodically.

3.1.3. Aqueous extract preparation

Fallen senescent leaves of *S. spectabilis* were collected for the analysis. Plant leaves of *Senna spectabilis* were separated from the shoots and were shade-dried until the removal of moisture. The sample was powdered (~5 kg) in a blender and stored in airtight glass jars until further use. *S. spectabilis* was extracted using distilled water, and sample solutions of varied concentrations with increasing order (0 (control), 10, 25, 50, 100 and 200 mg ml^{-1}) were prepared by soaking overnight for 24 hours. The crude extracts were filtrated through Whatman No. 2 filter paper and used to treat the seedlings of native plants (Ghimire et al., 2020).

3.2. Analysis of the stress due to invasive tree species *Senna spectabilis* on selected native plant species

Under this broad objective, three experiments were conducted: (i) Stress induced by allelochemicals of *S. spectabilis* on native plants was studied by enzymatic and non-enzymatic activity of antioxidants (ii) Metabolic activity and estimation of the rate of lipid peroxidation and (iii) The effect of allelochemicals on morphological changes of native plant species.

3.2.1. Analysis of the stress on selected native species due to allelochemicals of *S. spectabilis*

3.2.1.1.Seed Germination and growth records

The germination test was carried out in sterile Petri dishes of 9cm in size, placing a Whatman® No.1 filter paper on Petri dishes. The seeds were surface-sterilized with sodium hypochlorite and used for further bioassay studies. Each concentration's extract was added daily to the respective petri dish of treatment in an amount sufficient to moisten the seeds. Distilled water alone was used for the control group. Six seeds of each receptor plant species were placed in the Petri dish, replicating three times. The Petri dishes were set at room temperature of 28−30°C. The experiment was extended for seven days to allow the last seed germination and the shoot and root length measurement. When the radicle emerged, A seed was considered germinated (Hou et al 1998). The germination was recorded daily, and the results were determined by counting the number of germinated seeds, lateral roots and measuring the length of primary root and main shoot on the seventh day of the experiment. Data obtained through the study were analyzed in variance and Duncan's Multiple Range Test (DMRT). The germination and elongation ratios were calculated as follows (Rho and Kil, 1986).

Relative germination ratio =

$$\frac{Germination\ percentage\ of\ test\ seed}{Germination\ percentage\ of\ control\ seed}\ X\ 100$$

Relative elongation ratio of plumule = $\frac{\text{Mean length of plumule of test plant}}{\text{Mean length of plumule of control}}$ X 100

Relative elongation ratio of radicle $= \frac{\text{Mean length of radicle of test plant}}{\text{Mean length of radicle of control}} X\ 100$

3.2.1.2. Antioxidant Enzymatic Assay

Six months healthy seedlings of five native plants (*Ailanthus tryphysa* (AT)*, Pongamia pinnata* (PP)*, Tectona grandis* (TG)*, Hopea parviflora* (HP)*,* and *Dendrocalamus strictus* (DS)) were root fed with 5ml of plant extract every alternate day for over 3 years (2019-2022). These native plant species were maintained in a greenhouse with six replicates for different concentrations. Below mentioned assays were performed with the fresh mature leaves of treated and untreated (control) seedlings of native plant species by aqueous extracts.

Fresh leaves of treated and untreated (control) native plant seedlings (1 g each) were ground into a fine powder using liquid nitrogen in a mortar and pestle. This powder was mixed with 5 mL of extraction buffer (20 mM Tris-HCl in 1% polyvinylpyrrolidone, pH 7.4). After filtration to remove debris, the homogenate was centrifuged at 10,000×g for 30 minutes at 4°C. The resulting supernatant, separated from the solution, was utilized for antioxidant assays.

For the assessment of superoxide dismutase (SOD) activity, fresh seedling leaves weighing 1 gram each from both treated and untreated (control) native seedlings were crushed in a mortar and pestle using liquid nitrogen, within 5 mL of extraction buffer (consisting of 20 mM Tris-HCl with 1% polyvinylpyrrolidone, pH 7.4), following the protocol outlined by Beauchamp and Fridovich (1971). Subsequently, the extracted solution underwent filtration to eliminate debris, and the homogenate was then centrifuged at 10,000×g for 30 minutes at 40°C. The resulting supernatant was isolated and utilized for antioxidant assays. An initial reaction mixture was prepared by blending 50 mM phosphate buffer (pH 7.8), 75 µM nitroblue tetrazolium, 13 mM methionine, 100 nM EDTA, 2 µM riboflavin, and distilled water. Following a two-minute incubation, 50 µL of the enzyme extract was introduced. The absorbance of the reaction mixture was subsequently measured at 560 nm. One unit of SOD activity (U) was defined as the quantity

of enzyme required for 50% inhibition of the initial reaction rate.

Ascorbate peroxidase (APX) activity was determined following the method outlined by Nakano and Asada (1981). A mixture containing 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbic acid ($C_6H_8O_6$), 2% hydrogen peroxide (H_2O_2), 0.2 mM Ethylenediaminetetraacetic acid (EDTA), and 0.1 mL enzyme extract was prepared to a final volume of 3 mL. The absorbance of the solution was measured at 290 nm using a Shimadju UV-VS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The quantity of ascorbate oxidized was calculated using the extinction coefficient (2.8mM−1cm−1). APX was defined as 1 mmol mL^{-1} per min at 25◦C. One unit of ascorbate was oxidized as 1 mmol mL^{-1} ascorbate oxidized per min at 25◦C.

Catalase (CAT) assay indicates the variation in the quantity of H_2O_2 due to the presence of CAT in the test samples, indicating the potency of the activator/inhibitors under assessment. The catalase (CAT) assay was conducted following the methods described by Aebi (1984). Various extracts with different concentrations were initially added to the 4 uL of plant extracts in 67 mM sodium potassium phosphate at pH 7.4. The mixture was further incubated for 10 min at 37^0C. Then, 1.2 mL of H_2O_2 (0.6%) was added to the mixture, and the change in absorbance was assessed at 240 nm for 2 min. Sodium azide was used as a positive control.

Polyphenol oxidase activity (PPO) was determined following the method described by Saeidian (2013). The reaction mixture consisted of 100 mM phosphate buffer (pH 7.0), 1 mL (0.1 M) catechol, and 0.5 mL of enzyme extracts mixed to a final volume of 3 mL. The mixture was incubated at room temperature for five minutes. The PPO activity assay used a Shimadju UV-VS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) measuring absorbance at 420 nm.

For proline estimation, 0.5g fresh leaves were taken and properly homogenized using 3% sulfosalicylic acid. The homogenates were filtered through filter paper. 2 ml each of the filtrate, ninhydrin and glacial acetic acid were mixed altogether and incubated at 100^0C for 1 hour (Bates

et al., 1973). The reaction was later stopped by keeping test tubes in an ice bucket. Toluene (4 ml) was added, and the mixture was vigorously shaken for a few seconds. The separated aqueous toluene layer was warmed at room temperature, and the coloured sample was measured at 520nm wavelength.

3.2.2. Estimation of metabolic activity and the rate of lipid peroxidation

3.2.2.1. Analysis of the metabolic activity

For the analysis of the metabolic activity (Sampietro et al, 2006), the fresh plant material (100 mg) was washed and dried quickly between blotting paper, then incubated in 5 mL of TTC (0.2%, pH = 7) at 37^0C for four hours in the dark. The reaction was stopped by adding 0.5 mL of sulfuric acid (1 M). To examine metabolic activity (Sampietro et al, 2006), 100 mg of fresh plant material was swiftly washed and dried between blotting paper. Subsequently, it was immersed in 5 mL of TTC solution (0.2%, pH = 7) and kept at 37°C for 4 hours in darkness. The reaction was halted by adding 0.5 mL of 1 M sulfuric acid. After that, the plant material was removed, washed with distilled water, and dried quickly between filter paper and ground in a mortar placed in ice containing 3.5 mL of ethyl acetate. The homogenate was filtered through Whattman No. 1 paper, and the volume was adjusted to 7 mL with ethyl acetate. The absorbance at 485 nm was determined, and the quantity of formazan was calculated as follows (Sampietro et al., 2006).

Formazan content (%) = DO485 treatment / DO485 control

3.2.2.2. Analysis of lipid peroxidation

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) content using the Heath and Packer (1968) methodology. Fresh leaves (100 mg) were ground in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000×g for 10 min. One mL supernatant was mixed with 4 mL of 0.5% thiobarbituric acid prepared in 20% (w/v) TCA. After cooling, the mixture was then incubated at 950C for 30 min and again centrifuged at 10,000×g for 10 min. The absorbance of the supernatant was measured at 532 nm and adjusted by subtracting the non-

specific absorbance at 600 nm. The concentration of MDA was determined utilizing the extinction coefficient of 155 mM^{-1} cm^{-1}.

3.2.3. Study of the effect of allelochemicals on morphological changes of native plant species

For finding out the effect of allelochemicals on plants in the study area, growth retardation in terms of visually observable morphological changes induced by the allelochemicals in selected native plants was assessed by Tolerance Index percentage of the plants calculated according to the method of Turner (1994).

3.2.3.1. Morphological parameters

The native plants' seedlings were measured every year for three years. The parameters measured were the plant above ground height, girth at breast height (GBH) and leaf area.

3.2.3.2. Chlorophyll content

Leaf Chlorophyll content (LC) was measured using an instant SPAD (Soil-Plant Analysis Development) 502 plus chlorophyll meter. Before measuring the LC, the instrument was calibrated. Measurements were taken from the leaf lamina where leaf veins were not prominent. Six measurements were taken from a single leaf, and the average value was calculated using the LC.

3.3. Identification, isolation and elucidation of the structure of allelochemicals of *Senna spectabilis*

The biochemical profiling of the invasive species was carried out using GC– MS, and the potential allelochemicals were identified and quantified. Allelochemicals from root, bark and leaves were assessed. The allelopathic impact study was conducted under lab conditions wherein the plant part extracts were used to evaluate the effects on the germination and growth of native species.

3.3.1. Soxhlet Extraction

The Soxhlet extractor was placed onto a flask containing the extraction solvent, and the plant material was placed inside a thimble made from cotton, which was loaded into the main chamber of the Soxhlet extractor to cover the siphon (1:10, w/v). The Soxhlet was then equipped with a condenser. The temperature was set in the heating mantle according to the solvent's boiling point. The apparatus was run until the solvent turned colourless in the siphon (Falaki, 2019).

3.3.2. GC - MS Analysis

GC - MS analysis of extracts was performed using a Shimadzu GC-MS QP2010S equipped with Rxi-5Sil MS, fused silica capillary column (30 m length x 0.25 mm ID x 0.25 µm thickness). An electron ionization system employing an ionizing energy of 70 eV was utilized for GC-MS detection. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2µ1 was employed (split ratio of 10:1); injector temperature 2600 C; ion-source temperature 2000 C.

Interpretation on mass spectrum GC - MS was conducted using the National Institute of Standard and Technology (NIST 11) database with more than 62,000 patterns and Wiley 8. The unidentified component's spectrum was compared with that of the known components. Subsequently, the name, molecular weight, and structure of the test materials' components were determined.

3.4. Analysis of the microbial diversity in *S. spectabilis* invaded areas.

3.4.1. Experimental design and soil sample collection

In May 2020, we selected a pristine forest and *S. spectabilis* invaded forest of Wayanad Wildlife Sanctuary for sampling. The invasion time of *S. spectabilis* is approximately 30 years, and the coverage of *S. spectabilis* invasion is 25%. In the forest invaded by *S. spectabilis*, soil was

sampled from the rhizosphere zone of *S. spectabilis*. Three standard sampling plots measuring 20 m × 20 m were established within each stand, with 5 m × 5 m sampling areas arranged randomly within each plot. Each small sample area was surveyed using the "S" mixed sampling method. Following litter removal, five topsoil cores (5 cm in diameter and 10 cm deep) were gathered from random locations within each plot, then pooled and homogenized. Each of the three sampling plots was positioned more than 100 m apart at a given location. Six samples (2 treatments × 3 replications) were placed in sealed plastic bags and then immediately transported to the laboratory. The debris was removed, and each fresh soil sample was ground to pass through a 2-mm mesh sieve and then divided into two subsamples. One subsample of soil was prepared to determine bulk density, moisture content, soil organic carbon, pH, electrical conductivity, GC-MS analysis and DNA extraction.

3.4.2. Measurements of soil properties

The Soil moisture content (SMC) was determined by oven drying for 48 h at 105 °C and calculated using the wet and dry weights. Soil pH was measured using a pH meter in a 1:2.5 (w/v) soil–water suspension. The soil organic carbon (SOC) contents were determined using the high-temperature external heat potassium dichromate oxidation volumetric method (Walkley & Black, 1934).

3.4.2.1. Soil Bulk Density (ρB) and Moisture Content (θv) by Core Method

Soil core sampling is a direct method and the most common method for measuring ρB and θv in agricultural soils. Soil ρB values were determined using the core method as proposed by Blake and Hartge (1986). Undisturbed soil core samples were collected using a stainless steel coring ring (50 mm internal diameter and 50 mm length) from each plot at 0 - 10 cm, 10 - 20 cm, 20 – 30 cm, 30 – 40 cm, 40 – 50, 50 – 60 cm, 60 – 70 cm, 70 – 80 cm, 80 – 90 cm and 90 – 100 cm depths. Soil cores were oven-dried at 105˚C for 24 hours. Bulk density was calculated by dividing

the mass of oven dried soil by the core volume, and gravimetric moisture content was calculated as the mass of water in the soil sample per mass of the oven dried soil (g g^{-1}). Soil θv (cm^3 cm^{-3}) was obtained by multiplying gravimetric moisture content, θm (g g^{-1}) by soil ρB (g cm^{-3}).

Six soil cores were collected from *Senna* invaded, *Senna* uninvaded forests, and *Senna* managed plots at each depth (0 - 10 cm, 10 - 20 cm, 20 – 30 cm, 30 – 40 cm, 40 – 50, 50 – 60 cm, 60 – 70 cm, 70 – 80 cm, 80 – 90 cm and 90 – 100 cm).

$$Bulk\ density = \frac{\frac{Mass}{Weight\ of soil}}{Voulme\ of\ soil}$$

The clean, dried container was weighed and tared. A soil sample of about 10g was weighed as the weight of moist soil (M) and container and dried in the oven between 105°C and 110°C to a constant weight. After drying, the container was removed from the oven and cooled. The container was weighed with a sample and recorded as the Weight of dry soil (D) (Black, 1965).

$$\%\ Moisture\ content\ (MC) = \frac{\text{Weight of moist soil (M)} - Weight\ of\ dry\ soil\ (D)}{Weight\ of\ dry\ soil\ (D)}$$

3.4.2.2. pH and electrical conductivity

Soil extracts were prepared by dissolving dried soil in distilled water at 1:2 (w/v). The mixture was then stirred on an electric shaker for an hour and left to settle. After 24h, it was filtered using Whatman filter paper 1, and the filtrate was used to determine pH and conductivity. pH of the soil filtrates was analyzed by using pH meter (Ecoscan pH 700, Eutech Instruments Pvt. Ltd., Singapore), and electrical conductivity was determined using a conductivity meter (Ecoscan CON 700, Eutech Instruments Pvt. Ltd., Singapore). Five replicates were maintained for pH and conductivity estimation.

3.4.2.2. Organic Carbon Estimation

Reagents Used:

1. Sodium fluoride (0.2 g)

2. Prepare a 1N potassium dichromate solution by dissolving 49.04 grams of analytical reagent (A.R.) potassium dichromate (previously dried at 105°C) in distilled water, then dilute to the 1-liter mark in a volumetric flask.

3. 0.5 N Ferrous ammonium sulphate (FAS):- Dissolve 196 g of Fe $(NH_4)2(SO_4)$ $26(H_2O)2$in 800 ml distilled water and 20 ml concentrated sulphuric acid and dilute to 1L.

4. Diphenylamine indicator: - Approximately 0.5 g of reagent grade diphenylamine is dissolved in 20ml of distilled water, add 100 ml of Conc. H_2SO_4.

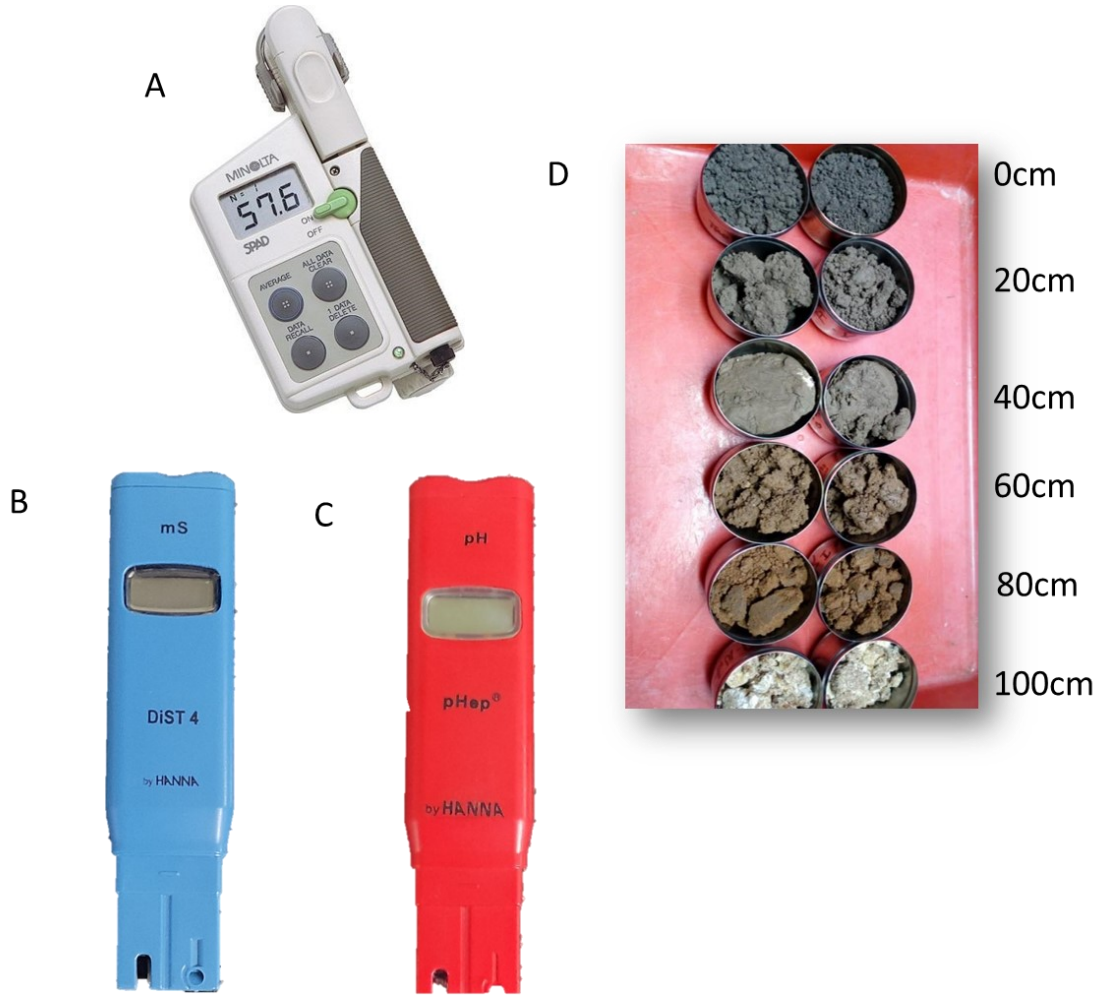


Figure 2. A. SPAD; B. Electrical conductivity: C. pH meter and D. Soil layer from 0 to 100cm

Procedure:

A 1gm soil sample was weighed, passing through a 0.2 mm sieve, and 10 ml of 1NK2Cr2O7 solution was added to it in a 500 ml conical flask. 20ml of concentrated sulfuric acid was added and gently rotated for 1 minute to ensure thorough contact with the soil while preventing soil from splattering onto the sides of the flask. The mixture was then allowed to stand for 30 minutes. A standardization blank, without soil, was prepared using the same procedure. After half an hour, about 200 ml of distilled water, 30 drops of diphenylamine indicator and about 0.2 g of sodium fluoride were added. The solution was back titrated with ferrous ammonium sulphate solution. Initially, the colour was dull green with chromos ion, then shifted to turbid blue as the titration proceeded. At the endpoint, the colour transitions to a brilliant green. If more than 8ml out of

10ml of chromic acid has been utilized during the titration, the analysis is repeated with a reduced quantity of soil or by doubling the amount or treble of $K_2Cr_2O_7$ and H_2SO_4.

$$Organic\ carbon\ (\%) = \frac{(Vblank\ -V\ sample) * MFe2 + *\ 0.003 * 100 * f * mcf}{W}$$

where: $Vblank$ = volume of titrant in blank, mL $Vsample$ = volume of titrant in sample, mL $MFe2+$ = concentration of standardized FeSO4 or (NH4)2 Fe(SO4)2.6H2O solution, molarity 0.003 = carbon oxidised (shown below) = 12 $g\ C\ mole$ × 1 $mole\ K2Cr2O7$ 6 $moles\ FeSO4$ × 3 $moles\ C$ 2 $moles\ K2Cr2O7$ × 1 L 1000 mL f = correction factor, 1.3 W = weight of soil, g mcf = Moisture correction factor)

3.4.3. Isolation and Screening of Plant Growth Promoting Bacteria and Fungi

3.4.3.1. Isolation and enumeration of fungi and bacteria

The spread plate technique was conducted from the collected soil samples (Erin, 2012). 1 gram of the soil sample was dispensed in 10 ml sterile distilled water. Serial dilution was done from 10^{-1} to 10^{-9}. To avoid overcrowding, dilutions of 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6}were used to isolate fungi. To prevent bacterial contamination, 1% of streptopenicillin was added to the PDA medium. Agar plates were incubated at 28ºC for 5-7 days (Raja et al. 2017).

One gram of soil from five sites was weighed and mixed with 9 ml of sterile distilled water. Serial dilution was done from 10^{-4} to 10^{-7} and spread plated into nutrient agar (Sanders, 2012). Three replicas of each soil sample were serially diluted. Then, the plates were incubated at 37°C for 24-48 hours.

3.4.3.2. Pure culturing of fungi and bacterial isolates

Morphologically similar fungal colonies were sub-cultured on Potato Dextrose Agar plates. Individual bacterial colonies were streaked on a nutrient agar medium and incubated at 37°C for 24-48 hours to obtain the pure culture. Identification of bacterial isolates was carried out by

conventional bacteriological methods (Brown, 1939).

The bacteria and fungal isolates were screened for various plant growth-promoting activities, i.e., Ammonia production, IAA production, Potassium solubilization and phosphate solubilization.

1. Ammonia Production

The production of ammonia was detected by the inoculation of bacteria into peptone water and incubation at 37°C for 24-48 hours. The development of brown colour to a deep yellow colour by adding Nessler's reagent indicates maximum production of Ammonia (Cappuccino and Sherman, 1992).

2. Indole Acetic Acid Production

The production of IAA was determined by inoculating the isolates into LB broth supplied with tryptophan. After 24-48 hours of incubation, the cultures were centrifuged at 10,000 rpm for 15 minutes. The 2 ml supernatant was combined with two drops of orthophosphoric acid and 4 ml of Salkowski reagent (1 ml of 0.5M $FeCL_3$ in 50 ml of 35% $HClO_4$). The pink colour indicates the presence of IAA and the production of Auxin (Gordon and Weber, 1951).

3. Phosphate Solubilization

The ability of bacteria to solubilize phosphate was determined by inoculating the isolates on Pikovskaya agar. Following incubation, the plates were observed for a clear hallow zone around the bacterial growth (Pikovskya, 1948). The experiment was done using the spot plate method. The plates were incubated at 28^{o}C for 5-7 days. The formation of a clear zone around the fungal colony indicated phosphate solubilization by the isolates (Varsha et al., 2009).

4. Potassium Solubilization

The bacteria were inoculated on Aleksandrow Agar medium. The development of a clear zone around the bacterial growth indicates a positive result (Hu et al., 2006). The potassium solubilization ability of the isolated fungal species was done in Aleksandrov agar medium (Hu et al., 2006) containing 0.5% Glucose, 0.05% Magnesium sulfate heptahydrate, 0.0005% Iron chloride, 0.01% Calcium carbonate, 0.2% Calcium phosphate, 0.2% Feldspar (Potassium aluminum silicate) and 1.8% Agar. The spot plate method was used for this purpose. The plates were incubated at 28ºC for 5-7 days. The formation of a clear zone around the fungal colony indicated the potassium solubilization by the isolates.

5. Utilization of nitrates

The ability of fungal isolates to utilize nitrates was screened on the semi synthetic medium Czapek Dox Agar, which has Sodium nitrate as the sole nitrogen source. The spot plate method was done on the medium by point inoculating fungi at the centre of the sterile media in the Petri plate. Incubation was done at 28ºC for 5-7 days.

6. Antagonistic activity

The dual culture technique checked the antagonistic activity of fungal isolates (Talapatra, 2017). An agar disc of 6mm diameter of the antagonistic fungi was placed 2 cm away from the periphery of the Petri plate containing sterile PDA medium. The test fungus was similarly placed on the opposite side of the Petri plate. The test fungi were placed on another Petri plate containing sterile PDA as a control. All the pairs were carried out in duplicates. Incubated at 28º C for 7 days. Antagonistic activity was tested by measuring the radius of the test fungi towards the antagonistic fungi (R2) and the radius of the test fungi in the control plate (R1) (Jia, 2016). The readings were converted into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson (1976).

$$PIRG = \frac{R1 - R2}{R1} * 100$$

3.5. DNA extraction, library preparation and metagenome sequencing for microbial diversity

The samples were transported to the laboratory on ice and stored at − 80°C until DNA was extracted from approximately 0.25 g of soil.

Step1 (end-repair/dA tailing)

The DNA was subjected to end-repair/dA tailing using 3 microlitres of UltraII End prep enzyme mix in a 60 microlitre reaction volume in a 0.5 ml PCR tube for 5 minutes at 20 degree celsius and for 5 minutes at 65-degree celcius. The modified DNA fragments were purified using AMPure XP beads. The DNA was eluted in a 25 microlitre volume of MQ water. Recovered DNA was quantified using Qubit reader (ThermoFischer Scientific)

Step 2 Barcode labelling

500 nanogram of end prepped DNA was coupled with a barcode. Each barcode represents one sample. Barcode was ligated using 2X ligation MasterMix supplied by NEB. Ligation reaction was carried out at room temperature for 10 minutes in a 50 microlitre reaction volume. The barcode ligated fragments were purified using AMPure XP beads. The DNA was eluted in a 26 microlitre volume of MQ water. The recovered DNA was quantified using a Qubit reader (ThermoFischer Scientific).

Step 3 Barcode Adapter ligation

The barcode labelled DNA barcode adapter was ligated using 2X ligation MasterMix supplied by NEB. The ligation reaction was carried out at room temperature for 10 minutes. The ligation reaction was carried out using 750ng of pooled samples (750ng/19) in 50 microlitre using 20 microlitres of barcode adapter mix. After the reaction, the DNA fragments were purified using AMPure XP beads. The DNA was eluted in a 15 microlitre volume of elution buffer. The recovered DNA was quantified using a Qubit reader (ThermoFischer Scientific).

Step 4 Priming and Loading the SpotON flow cell

The Sequencer MinION was connected to the SpotON flow cell and primed using 1ml of Primer buffer for 5 minutes at RT, and into the primed flow cell 75 microlitres of pooled library (430ng) was added through the SpotON sample port. Closed the sample port and closed the MinION lid.

Step 5 Sequencing run and basecalling

The MinKNOW program was started after connecting the flow cell to the computer. The sequencing was done at FAST5 mode for 48 hrs. The basecalling was done using the software GUPPY till basecalling was completed.

Step 6 Data analysis

After basecalling the DNA sequence data is obtained as fastq format. The files were uploaded to NCBI website for BLAST analysis

3.6. Statistical analysis

Statistical significance was assessed at $P<0.05$ level. Antioxidant data were statistically analyzed using analysis of variance (ANOVA) using SPSS (Version 16 SPSS Inc., Chicago, IL, USA). Appropriate standard errors of mean (±SE) were calculated for presentation with tables and histograms. The treatment means were analyzed by Duncan's multiple range test (DMRT) at $P<0.05$.

Analysis of similarities (ANOSIM) function was performed in R version 4.3.1 using the Vegan Package based on Bray-Curtis distance (Tian et al., 2020). The "Canonical correlation analysis (CCA)" function of the Vegan Package in R 4.3.1 was used to conduct the CCA of multiple correlation variations among environmental factors and community composition at the genus and phylum level and the environmental factors were fitted with the ordination plots using the Vegan Package in R with 999 permutations.

CHAPTER 4

RESULTS AND DISCUSSION

Chapter 4 Results and Discussion

4.1. To analyse the stress due to invasive tree species Senna spectabilis on selected native plant species

4.1.1. Seed germination and seedling growth

To explore the allelopathic effects of *S. spectabilis* on seed germination and seedling growth in five selected native species *Ailanthus tryphysa* (AT), *Pongamia pinnata* (PP), *Tectona grandis* (TG), *Hopea parviflora* (HP), and *Dendrocalamus strictus* (DS) were treated with different concentrations of *S. spectabilis* leaf extract. The results (Figure 3. A-B) showed that the allelopathic inhibition increased in a concentration dependent manner. When seeds were incubated with extracts in various concentrations, 200 mg ml^{-1} of concentration exerted an extremely significant inhibitory effect on the germination index.

Relative seed germination

The analysis of variance (ANOVA) revealed significant effects of the treatment, plant species, and their interaction on seed germination growth. The relative seed germination growth values varied significantly among species, ranging from 90.47 to 30.35. Post-hoc comparisons indicated significant differences between various species. Additionally, different concentrations of leaf extracts were categorised into four distinct groups based on their impact on seed germination. The highest inhibition of seed germination occurred at 200 mg ml^{-1}, while the control group exhibited the lowest effect.

Furthermore, the interaction effects were found to be significant. Specifically, combinations of certain plant species with specific concentrations of leaf extract resulted in varying degrees of inhibition of seed germination growth. For instance, the combination of PP with 200 mg ml^{-1}, DS with 200 mg ml^{-1}, TG with 100 mg ml^{-1}, and TG with 200 mg ml^{-1} demonstrated higher inhibition, while the combination of PP with 10 mg ml^{-1} exhibited the least inhibition of seed germination

growth.

Relative plumule growth

The analysis of variance (ANOVA) results revealed significant effects of treatment, species, and their interaction on plumule growth. Across different species, relative plumule growth values ranged from 163.78 to 38.4, demonstrating significant differences among species. Post hoc comparisons grouped the various leaf extracts into five distinct groups, with the highest inhibition observed at 200 mg ml^{-1} and the lowest effect at 10 mg ml^{-1}

The combination of PP with 200 mg ml^{-1}, DS with 200 mg ml^{-1}, TG with 100 mg ml^{-1}, and TG with 200 mg ml^{-1} exhibited the highest inhibition of plumule growth. Conversely, the combination of HP with the control showed the least inhibition. These findings underscore the importance of individual factors (treatment, species) and their interaction in influencing plumule growth, providing valuable insights into the nuanced effects of different leaf extracts on this biological response.

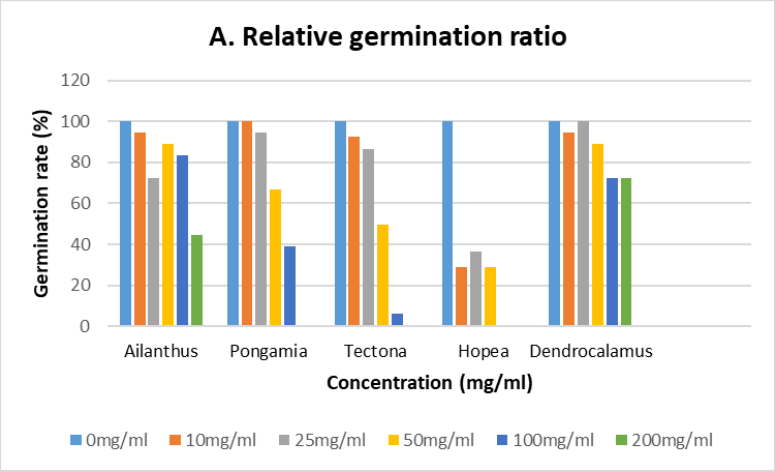
Relative radicle growth

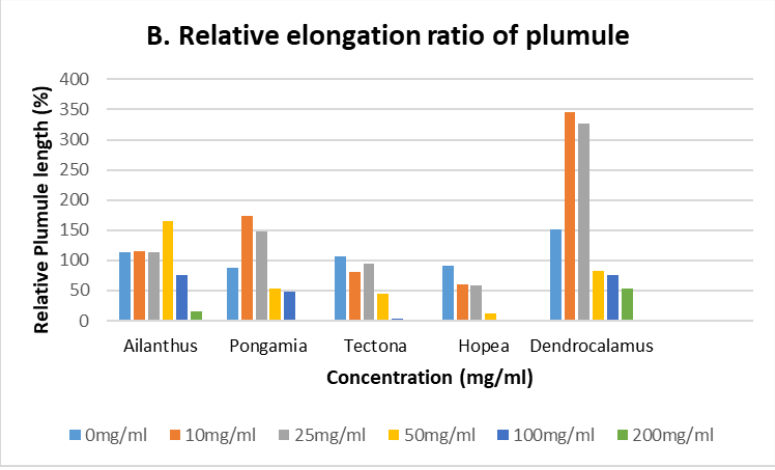
The analysis of variance (ANOVA) revealed significant effects of treatment, species, and their interaction on radicle growth. Across different species, relative radicle growth values ranged from 111.94 to 25.25, demonstrating statistically significant ($p< 0.05$) differences among species. Post hoc comparisons further grouped the various leaf extracts into two distinct groups. The most pronounced inhibition of radicle growth occurred at a concentration of 200 mg ml-1, while the control group exhibited the least effect on radicle growth.

Furthermore, significant interaction effects were identified. The combination of PP with 200 mg ml^{-1}, DS with 200 mg ml^{-1}, TG with 100 mg ml^{-1}, and TG with 200 mg ml^{-1} exhibited the highest inhibition of radicle growth. Conversely, the combination of PP with the control group demonstrated the least inhibition of radicle growth. These findings emphasise the complex interplay between treatment, species, and their interaction in influencing the observed variations in radicle

growth.

The quality and viability of seeds are usually evaluated by seed germination rate, seed germination potential, seed vigour index, and seed germination speed. The seed germination test is one of allelopathy research's most commonly used biological detection methods (Rice, 2012). In the present research, the germination indices of native seeds changed after soaking in *S. spectabilis* extracts. The germination rate decreased with the increase of the aqueous extract concentrations, which is consistent with the results of a previous study, where the germination rate was directly related to extract concentrations (Amare, 2018). However, many studies have found a promotional effect at low concentrations and an inhibitory effect at high concentrations (Yan et al., 2010; Wang et al., 2018)





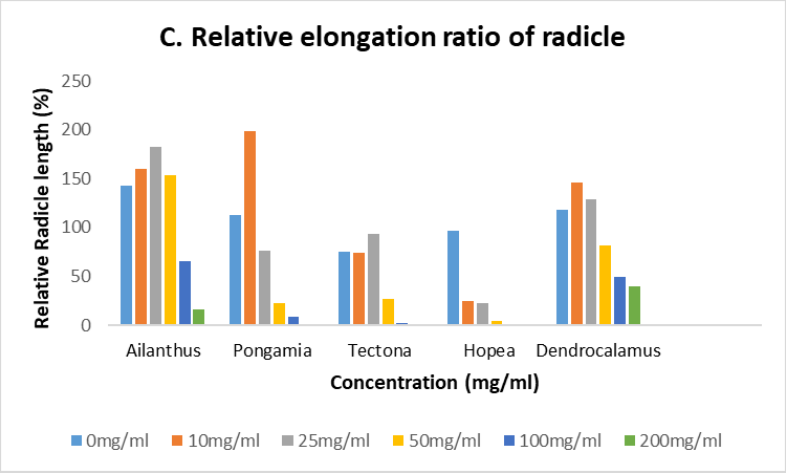


Figure 3. A. Relative germination ratio of seeds of different species in response to aqueous extract of *S. spectabilis*. B. Relative elongation ratio of plumule seeds of different species in response to aqueous extract of *S. spectabilis.* C. Relative elongation ratio of radicle seeds of different species in response to aqueous extract of *S. spectabilis*

Seedling growth

A multivariate analysis of variance (MANOVA) was conducted to evaluate the impact of growth year on four key growth parameters ie., height, girth, leaf area, and chlorophyll contentof *Ailanthus triphysa* over a four-year period, with Year 0 serving as the baseline.

The analysis in *Ailanthus triphysa* revealed a statistically significant multivariate effect of growth year (Pillai's Trace = 1.937, $F(12, 141) = 21.418$, $p < 0.001$), indicating substantial variation in the measured variables over time. Descriptive statistics demonstrated a marked increase in mean height, from 16.60 cm in Year 0 to 42.43 cm in Year 3, and a corresponding rise in girth, from 2.63 cm to 9.51 cm. Leaf area also expanded significantly, increasing from 13.46 cm to 35.80 cm, while chlorophyll content exhibited a notable improvement, rising from 36.37 units in Year 1 to 47.36 units in Year 3. Post hoc Tukey HSD tests further corroborated these findings, revealing that Year 0 consistently showed significantly lower values across all variables compared to subsequent years ($p < 0.001$). However, differences between intermediate years, such as Year 1 and Year 2, were not

statistically significant. These results underscore the substantial growth and physiological development of *Ailanthus triphysa* across the four-year period, emphasizing the influence of temporal progression on its growth parameters.

The analysis in *Tectona grandis* revealed a significant multivariate effect of growth year (Pillai's Trace = 1.692, $F(12, 141) = 15.203$, $p < 0.001$), indicating substantial differences in growth parameters across years. Treatment concentration also exhibited a significant multivariate effect (Pillai's Trace = 0.819, $F(20, 192) = 2.470$, $p = 0.001$), demonstrating the influence of varying concentrations on growth metrics. However, the interaction between Growth Year and Conc. did not yield a significant multivariate effect (Pillai's Trace = 0.899, $F(60, 192) = 0.927$, $p = 0.626$), suggesting that the combined effects of temporal progression and treatment concentration were not statistically significant. Post hoc Tukey HSD tests further revealed significant pairwise differences in height, girth, and chlorophyll content across growth years and treatment concentrations, with notable improvements observed in lower concentrations (e.g., 10 mg/ml and 25 mg/ml), while higher concentrations exhibited diminishing effects. These findings underscore the independent contributions of temporal progression and treatment concentration to the physiological development of *Tectona grandis*, highlighting distinct growth patterns across years and optimal treatment levels for stimulating growth.

The results of *Hopea parviflora* revealed a significant multivariate effect of concentration (Pillai's Trace = 0.813, $F(12, 84) = 6.473$, $p < 0.001$), indicating substantial differences in growth parameters across the concentrations. Concentration significantly affected Height ($F = 4.062$, $p < 0.001$), Girth ($F = 7.236$, $p < 0.001$), and Chlorophyll Content ($F = 2.818$, $p = 0.001$), with substantial variability in these parameters explained by the model ($R^2 = 0.661$, 0.776, and 0.574, respectively). However, the interaction between concentration and growth parameters did not show a significant multivariate effect on Leaf Area ($F = 1.579$, $p = 0.091$), suggesting that concentration had a lesser impact on leaf expansion. Post-hoc analysis using Tukey HSD comparisons confirmed significant differences in

Height between the 0 mg/mL and 50 mg/mL concentrations (mean difference = 22.16, p = 0.020). However, no significant differences were found for Girth or Chlorophyll Content across concentrations, indicating that these traits were less sensitive to the varying concentrations. For Leaf Area, a significant difference was observed only at the 100 mg/mL concentration (mean difference = 5.19, p = 0.027) when compared to the control group (0 mg/mL). These findings highlight the differential response of *Hopea parviflora* to varying concentrations of the substance, with certain parameters, particularly Height, showing significant changes, while others remained unaffected.

In *Pongamia pinnata* results indicated a significant multivariate effect of growth year (Pillai's Trace = 1.540, $F(12, 141) = 12.397$, $p < 0.001$), demonstrating substantial differences in growth parameters across years. Similarly, treatment concentration also showed a significant multivariate effect (Pillai's Trace = 0.819, $F(20, 192) = 2.470$, $p = 0.001$), suggesting that varying concentrations influenced the growth parameters. However, the interaction between growth year and concentration did not yield a significant effect (Pillai's Trace = 0.899, $F(60, 192) = 0.927$, $p = 0.626$), implying that the combined effects of these factors were not statistically significant. Post hoc Tukey HSD tests revealed significant pairwise differences in height, girth, and chlorophyll content across growth years and concentrations, with lower concentrations (e.g., 10 mg/mL and 25 mg/mL) showing greater growth improvements compared to higher concentrations. These findings highlight the independent roles of growth year and treatment concentration in influencing the growth of *Pongamia pinnata*, with specific concentration levels proving more effective for stimulating optimal growth.

The analysis in *Dendrocalamus strictus* revealed a statistically significant multivariate effect of concentration (Pillai's Trace = 1.174, $F(20, 120) = 3.821$, $p < 0.001$), demonstrating substantial differences in the growth parameters of *Dendrocalamus strictus* across concentrations. Post hoc Tukey HSD tests showed significant pairwise differences in chlorophyll content, with the 50 mg/ml concentration exhibiting notably higher values compared to the lower concentrations ($p < 0.05$).

This suggests that higher concentrations have a significant impact on chlorophyll content. However, the analysis found no significant effects of concentration on height, girth, and leaf area ($p > 0.05$). These results highlight the specific role of higher concentrations in improving chlorophyll content, while concentrations at lower levels did not significantly affect the other growth parameters of *Dendrocalamus strictus*.

In our study, the allelopathy of *S. spectabilis* on the germination of native plant seeds showed a promoting effect at low concentrations and an inhibitory effect at higher concentrations. The results demonstrated that compared with the control, aqueous extract of *S. spectabilis* inhibited the viability of the seeds. The results obtained from the petri dish experiment indicate that the plumule length, radicle length, and germination rate were significantly lower in higher concentrations of aqueous extract. The development and progression of the radicle significantly influence plant growth, while the plumule's advancement directly correlates with the growth rate of plants during the seedling stage. Prior investigations have demonstrated that root length is more susceptible to allelopathic effects during seed germination than seedling height (Turk and Tawaha, 2003; Wu et al., 2003).

In the present study, there was a noticeable downward trajectory in both radicle and plumule lengths with escalating concentrations of aqueous extracts. This observation aligns with findings from prior research, which observed a substantial inhibition in root and shoot growth of *Oryza sativa* upon exposure to aqueous extracts derived from *Mikania micrantha* leaves (Sahu and Devakota, 2013). Our results agree with previous reports indicating a stimulatory effect on radicle and plumule growth at lower extract concentrations (Braine et al., 2012; Gatti et al., 2010). Despite the decline in radicle and plumule lengths with increasing extract concentrations, there was a concurrent rise in the ratio of radicle length to plumule length. This elevation in the radicle-to-plumule ratio protects against adverse stress conditions, facilitating enhanced water and nutrient absorption and mitigating damage induced by such stressors (Hou et al., 2016).

Phenols, quinones, coumarins, flavonoids, terpenes, sugars, glycosides, alkaloids and non-protein

amino acids are allelopathic chemicals (Zhang et al 2011). However, many plant produced inhibitory chemicals can stimulate growth at low concentrations (Liu and Lovett, 1993; Pratley, 1996). Sivagurunathan et al. (1997) reported that phenolics' nature and their concentration decide the inhibitory effect. A lower concentration of *S. spectabilis* aqueous extract showed a stimulatory effect on plumule growth of all seedlings, while a higher concentration led to inhibition of plumule length. All treatments caused a significant reduction in the radicle growth.

However, at high concentrations, the growth performance and competitiveness of native species were significantly inhibited, leading to enhanced competitiveness of *S. spectabilis* in the presence of allelochemicals. These findings underscore the concentration-dependent effects of allelopathic compounds on the growth and competitiveness of native species and *S. spectabilis*. Understanding the dynamics of allelochemical interactions between these species is vital for managing and mitigating the impacts of invasive plants on biodiversity. Further research is essential to unravel the underlying mechanisms of allelopathy and its ecological implications.

The evaluation of allelopathic potential within invasive alien plant species necessitates an examination of seed germination and seedling growth dynamics, as established by previous studies (Zheng and Feng, 2005; Huangfu et al., 2010). Seed germination represents a crucial stage in establishing invasive plant populations following their introduction to new environments (Zheng and Feng, 2005). Subsequent effects, such as reduced root length, prolonged germination duration, delayed seedling emergence, decreased root hair development, and instances of senescence or mortality induced by allelopathic compounds produced by invasive alien plants, significantly impede the competitive abilities of native flora for both above-ground and below-ground resources (Zheng and Feng, 2005; Deng et al., 2010).

Our previous investigation unveiled pronounced inhibitory effects on seed germination and root elongation of certain major native plant species, namely *B. pilosa, A. conyzoides, D. sanguinalis,* and *C. virgate*, upon exposure to aqueous extracts (0.00125-0.1 g mL^{-1}) of *S. spectabilis*. The degree

of allelopathic potential in invasive alien plant species is typically influenced by factors such as the species being targeted, extract concentration, and the plant tissues serving as the source of allelochemicals (Tian et al., 2015; Chen et al., 2008; Gao et al., 2012). Generally, higher extract concentrations result in inhibition of seed germination or seedling growth in the target species, while lower concentrations may paradoxically promote these processes, indicating a concentration-dependent stimulatory or inhibitory effect (Deng et al., 2010; Tian et al., 2015; Chen et al., 2008; Gao et al., 2012).

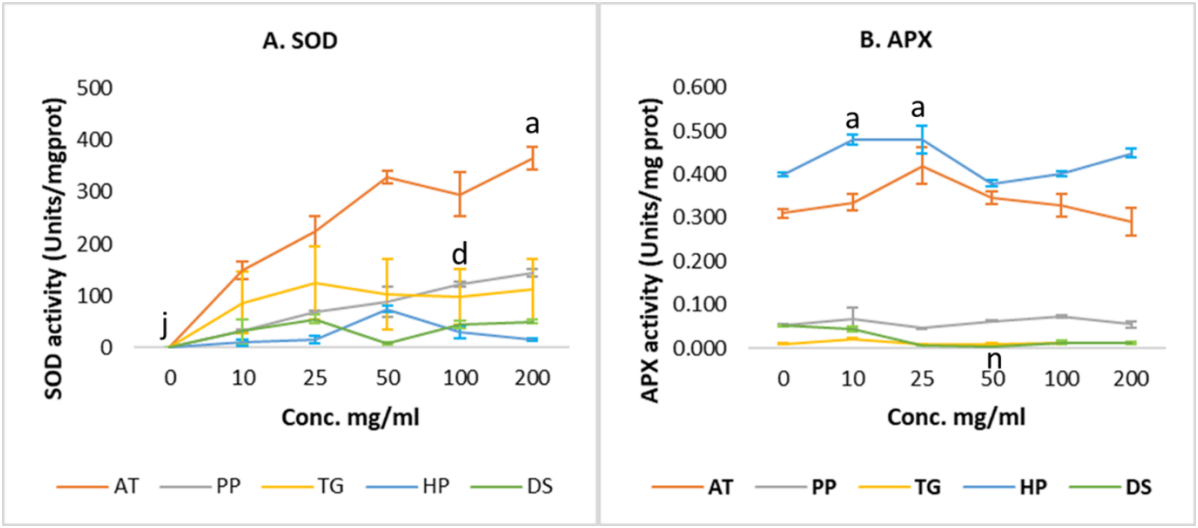
Wang et al. (2022) observed a significant positive influence at lower concentrations and an inhibitory effect at higher concentrations, consistent with the findings of our study. These results corroborate previous findings by Thiebaut et al. (2019) and Masum et al. (2018) regarding the non-uniformly inhibitory nature of allelopathic interactions, with compounds often exhibiting growth-promoting effects at lower concentrations. Conversely, higher concentrations frequently induce inhibition of seed germination and seedling growth, indicative of allelopathic effects—an essential phenomenon in plant interactions within both natural ecosystems and agroecosystems (Piršelová et al., 2019). This concentration-dependent activity, characterised by growth inhibition at higher concentrations and stimulation at lower concentrations, is a ubiquitous feature of allelopathic research and is widely acknowledged in the scientific literature (Islam et al., 2018).

4.1.2. Physiological response

The aqueous extracts of *S. spectabilis* influenced antioxidant enzyme activities differently across native species: superoxide dismutase (SOD) levels peaked at 50 mg ml^{-1} but decreased at higher concentrations, with significant variations among species. Ascorbate peroxidase (APX) activity varied significantly across species and extract concentrations, with notable increases in AT and HP. Catalase (CAT) activity showed species-specific increases in HP and AT, while polyphenol oxidase (PPO) activity varied widely among species and extract concentrations, with AT exhibiting the highest activity.

Superoxide dismutase

The influence of the *S. spectabilis* extracts on antioxidant enzyme activities is shown in Figure 4. A-D. The analysis of variance revealed that the concentration of the extract, the type of native species, and their interactions significantly ($p < 0.05$) influenced the accumulation of superoxide dismutase (SOD) in the leaves of these native species. SOD increases up to a concentration of 50 mg ml^{-1}, and at a higher extract concentration, there is a decrease. The levels of SOD accumulation in the leaves varied across different extract concentrations, ranging from 0 µg $moles^{-1}$ to 127.92 µg $moles^{-1}$. Notably, leaves treated with 200 mg ml^{-1} exhibited a distinct difference compared to the rest, while the other concentrations also showed significant differences ($p < 0.05$) from each other (Figure 4A). Among the native species, AT displayed the highest SOD accumulation at 0.013, while HP had the lowest accumulation at 0.002, indicating a notable variation in SOD levels among the species. Furthermore, the interaction effects were found to be significant ($p < 0.05$) as well. Specifically, the combination of the native species AT with an extract concentration of 200 mg ml^{-1} resulted in the highest SOD accumulation.



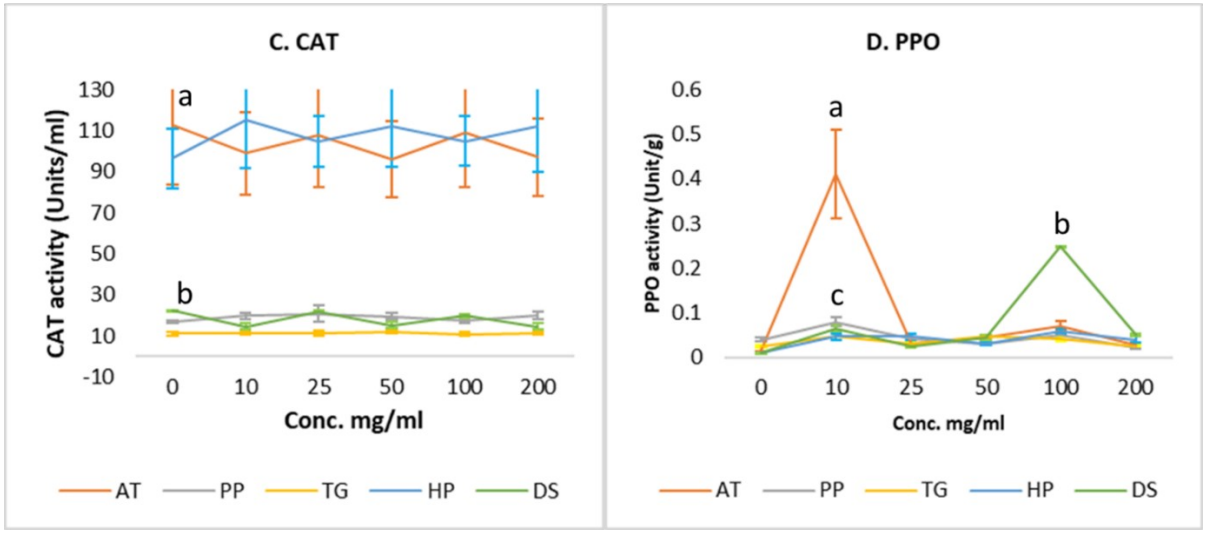


Figure 4. Comparison of antioxidant enzyme activities of native species treated with *S. spectabilis*. (A), Superoxide dismutase (SOD); (B), Ascorbate peroxidase (APX); (C), Catalase (CAT); (D), Polyphenol oxidase (PPO).

The mean values of three parallel experiments are provided in each figure. The error bars represent the Standard Error Mean. *Ailanthus tryphysa* (AT), *Pongamia pinnata* (PP), *Tectona grandis* (TG), *Hopea parviflora* (HP), and *Dendrocalamus strictus* (DS)

Ascorbate peroxidase Activity

Changes in the APX activity due to the allelopathic compounds present in the aqueous extracts of *S. spectabilis* appeared to vary significantly across the native species. We observed a considerable increase in APX activity in AT and HP (Figure 4B). APX activity of TG decreased significantly ($p < 0.05$) compared to control plants. The concentration of the extract, the type of native species, and their interactions were found to significantly impact APX (ascorbate peroxidase) activity in the leaves of native species. APX accumulation in leaves exhibited a range of values, spanning from 0.184 µg moles^{-1} to 0.1551 µg moles^{-1} across various concentrations. Notably, leaves treated with a 200 mg ml^{-1} concentration differed significantly from others. The highest APX accumulation was observed in the HP, reaching 0.431 µg moles^{-1}, while the TG exhibited the lowest accumulation at 0.012 µg moles^{-1}. Furthermore, the interaction effects between extract concentration and native species type were also significant. Specifically, the combination of HP with a concentration of 25 mg ml^{-1} displayed the highest APX accumulation, in contrast to DS, which exhibited the lowest

accumulation at 50 mg ml^{-1}. These findings underscore the intricate interplay between extract concentration, native species, and their combined effects on leaf APX activity.

Catalase Activity

The greatest increase in CAT activity was observed in HP and AT species treated with the aqueous extracts of *S. spectabilis* (Figure 4.C). Different concentrations did not lead to significant variations ($p < 0.05$) in catalase levels, as there were no distinct groupings or patterns. In contrast, significant variations were observed in the catalase levels between species. They are divided into three groups with catalase levels ranging from approximately 107.56 µg $moles^{-1}$ to 11.26 µg $moles^{-1}$.

Polyphenol oxidase Activity

PPO activity was low in the majority of the native species compared to that of the control plants (Figure 4 D). AT showed the highest PPO activity, followed by DS. Significant variation ($p < 0.05$) in PPO activity was observed in both treated and control seedlings of other native species. The analysis of variance revealed significant differences in the concentration of extract, the type of native species, and their interactions, all of which exerted a noticeable influence on the PPO (Polyphenol Oxidase) activity in the leaves of these native species. PPO activity in the leaves exhibited a wide range of values, spanning from 0.28 µg $moles^{-1}$ (at 100 mg ml^{-1} concentration) to 0.23 µg $moles^{-1}$ (at 0 mg ml^{-1} concentration), with statistically significant ($p< 0.05$) distinctions among these concentrations. Among the native species, the highest PPO activity was observed in the case of species AT, reaching a level of 0.26 µg $moles^{-1}$, while species TG displayed the lowest accumulation, with PPO activity measured at 0.03 µg/moles. The interaction effects between different concentrations and native species were also significant. Specifically, the combination of the native species AT with an extract concentration of 15 mg ml^{-1} exhibited the highest PPO activity, reaching 1.41 µg $moles^{-1}$. In contrast, native species DS displayed the lowest PPO activity when subjected to an extract concentration of 0 mg ml^{-1}, registering a value of 0.009 µg $moles^{-1}$.

Plants use enzymatic and non-enzymatic antioxidants to prevent oxidative damage and maintain lower levels of reactive oxygen species (Ozgur et al., 2013). As depicted in Figure 4, different concentrations of *S. spectabilis* affected antioxidant enzyme activities (superoxide dismutase (SOD), ascorbate oxidase (APX), polyphenol oxidase (PPO), catalase (CAT)) of native seedlings; moreover, the effects varied in different native species. Our study showed that in AT, PP and TG, SOD levels were elevated with the treatment concentration, while PPO levels were low except for AT (10 mg ml^{-1}) and DS (100mg ml^{-1}). CAT enzyme was higher in AT and HP species. APX activity was higher for AT and HP than for other species. Furthermore, according to Scandalios (1993), CAT and APX emerge as the most potent antioxidant enzymes in safeguarding cells from damage. Azevedo-Neto et al. (2006) underscore their significance in regulating intracellular levels of H_2O_2. Our results show that both the SOD and APX activities in native plant species increased initially (from 0 to 50 mg ml^{-1}) and then declined (from 50 to 200 mg ml^{-1}) with increasing concentration of the *S. spectabilis* (Fig. 4. A-D) like those found in previous studies (Matters and Scandalios, 1987). The decrease in SOD, APX, CAT and PPO activities at higher concentrations can be correlated with the inhibition of these enzymes caused by the allelochemical extract of *S. spectabilis*. Thus, our findings are in contrast with that of Cai et al. (2011) in the case of SOD activity, who reported that SOD activity increases with increase in the concentration of allelochemicals. Low concentrations of *S. spectabilis* extracts stimulated the growth of native species and activated the metabolism of antioxidant enzymes, thus protecting the growth of seedlings from adverse conditions. Nevertheless, when they were subjected to higher concentrations ($\geq$50 mg ml^{-1}) of *S. spectabilis* extracts, abundant allelochemicals in the extracts and the ROS produced destroyed the antioxidant enzymes and thus the ability of the enzyme (Cai et al., 2015). Antioxidant enzymes have different roles in protecting cells by maintaining membrane structures and neutralising ROS's deleterious effects (Willekens et al., 1995). The recorded increase in antioxidant enzyme activities under allelochemical stress has been previously reported in other plant

species (Oracz et al., 2007). In maize, the H_2O_2 content and the activity of CAT, SOD, and APX enzymes were increased under the allelochemical stress of walnut husk (Ciniglia et al., 2015).

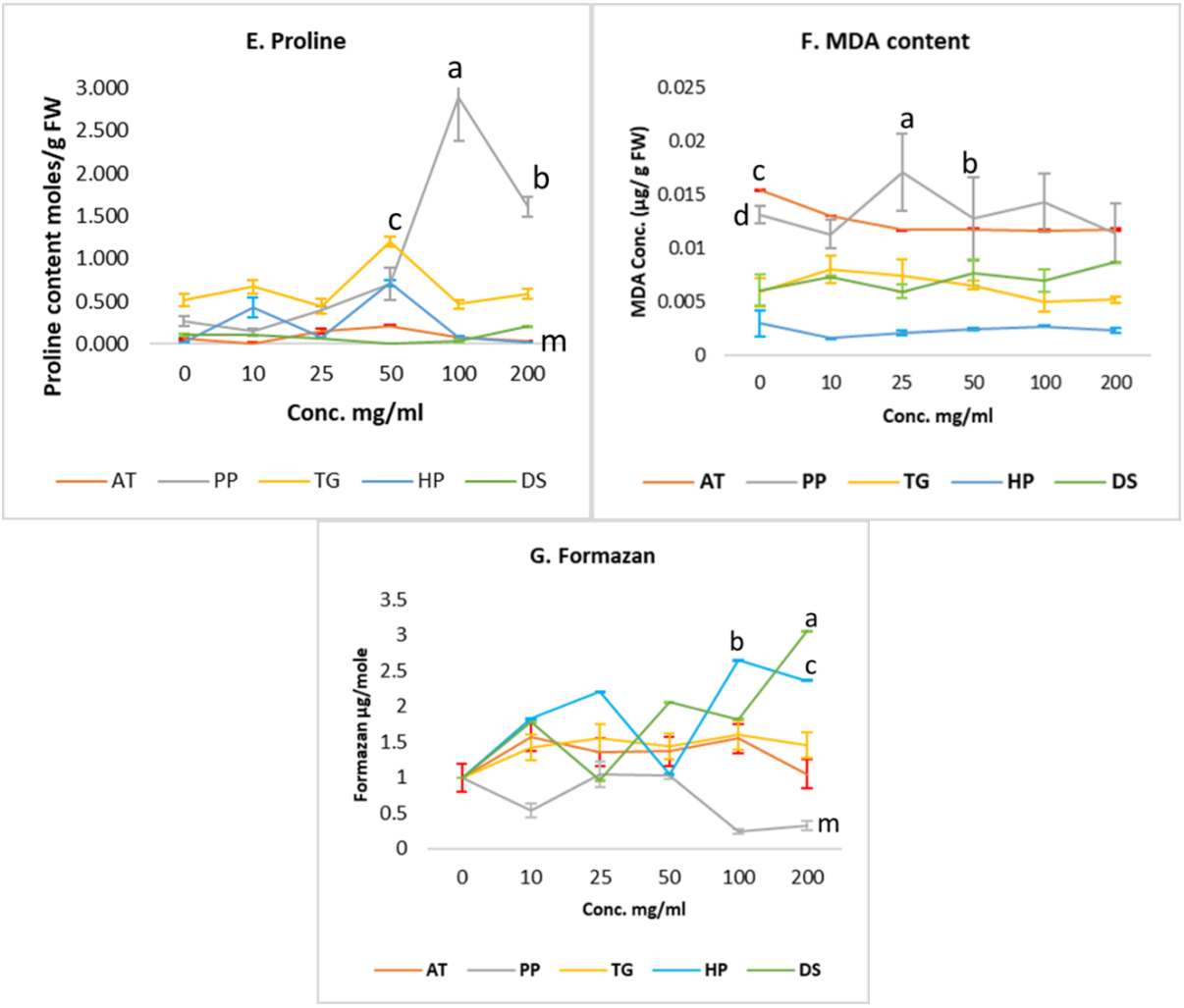


Figure 4. Comparison of antioxidant enzyme activities of native species treated with *S. spectabilis*. (E), Proline content; (F), MDA content; (G), Formazan content.

Values are reported as the mean ± standard deviation of three parallel experiments. The mean values of three parallel experiments are provided in each figure. The error bars represent the Standard Error Mean.

Proline content

Proline accumulation in leaf tissues gradually increased as the concentration increased in *S. spectabilis* extract treated on native species. The results revealed that the concentration of the extract, the type of native species, and their interactions had a significant impact on the accumulation

of L-proline in the leaves of these native species (Figure 4E). Figure 4E displays variations in proline content across various concentrations of *S. spectabilis* extracts. Specifically, for the enzyme PP, an increase in proline content is evident with increasing extract concentration up to the point of saturation at 100 mg ml^{-1}. However, at higher concentrations, there is a notable decline in this enzyme's activity. Other species exhibit relatively stable proline content levels, except at 50 mg ml^{-1} (TG, HP and AT). On the other hand, DS does not show differences in Proline content levels across all extract concentrations when compared to the control group, except at 200 mg ml^{-1}. L-Proline levels in the leaves varied across different extract concentrations, ranging from 0.61 µg $moles^{-1}$ to 0.176 µg $moles^{-1}$. Notably, the 100 mg ml^{-1} concentration significantly differed from the control group. When comparing the various native species, they could be categorised into five distinct groups. Specifically, species AT and DS were grouped due to their similarity, while the others showed significant differences. The highest l-proline accumulation was observed in species PP, with a concentration of 1.003 µg $moles^{-1}$, while DS displayed notably lower levels at 0.08 µg $moles^{-1}$, representing a hundred-fold difference from the highest accumulation. Furthermore, the interaction effects were found to be significant as well. Specifically, combining species PP with an extract concentration of 100 mg ml^{-1} resulted in the highest proline accumulation when contrasted with species DS, which exhibited the lowest accumulation at 50 mg ml^{-1}.

Metabolic activity

Formazan contents reflect the metabolic activity of cells, mainly the activities of dehydrogenase enzymes and, thus, mitochondrial respiration. In this study, PP showed a significant decrease in formazan production above 50 mg ml^{-1} compared to the control. The concentration of extract, the type of native species, and their interactions significantly impacted the formazan activity within the leaves of these native species (Figure 4G). Formazan accumulation in the leaves exhibited a wide range of values, varying from 1.53 µg $moles^{-1}$ (at 200 mg ml^{-1}) to 1.00 µg $moles^{-1}$ (at 0 mg ml^{-1}), and these differences were statistically significant ($p< 0.05$). Among the native species, the highest

formazan accumulation was observed in HP, reaching 1.847 µg moles^{-1}, while PP exhibited the lowest accumulation at 0.694 µg moles^{-1}. Furthermore, the interaction effects between the concentration of the extract and the native species type were also significant. Notably, the combination of DS at a concentration of 200 mg ml^{-1} resulted in the highest formazan accumulation, measuring 3.057 µg/moles. In contrast, PP at 75 mg ml^{-1} exhibited the lowest formazan accumulation among the various combinations studied.

Lipid peroxidation

The analysis of variance conducted in this study revealed significant influences on the accumulation of MDA (malondialdehyde) in the leaves of native plant species. These influences were attributed to three main factors: the concentration of the extract, the type of native species, and their interactions. The MDA accumulation levels in the leaves exhibited values spanning from 0.008 µg moles^{-1} to 0.006 µg moles^{-1}, with variations observed across different extract concentrations (Figure 4F). No statistically significant difference existed between the MDA accumulation in leaves treated with 50 mg ml^{-1} extract and the control group. However, significant differences were observed when comparing these groups with other extract concentrations. Among the native species studied, there were distinct differences in MDA accumulation. Species PP exhibited the highest MDA accumulation, with a concentration of 0.013 µg moles^{-1}, followed by species AT (0.127 µg moles^{-1}). In contrast, species HP displayed the lowest MDA accumulation at a concentration of 0.002 µg moles^{-1}. Furthermore, the interactions between the extract concentration and native species type were significant. Specifically, combining species PP with an extract concentration of 25 mg ml^{-1} resulted in the highest MDA accumulation compared to species HP, which exhibited the lowest MDA accumulation at the same 25 mg ml^{-1} concentration.

Djanaguiraman et al. (2005) demonstrated that allelochemicals found in the leachate of eucalyptus leaves elevated the proline levels in sorghum and mung bean. Likewise, Thapar and Singh (2006)

observed increased proline production in *Parthenium hysterophorus* leaves treated with leachate from *Cassia tora* leaves. Proline accumulation is a general phenomenon in all stressed plants (Parida and Jha, 2010; Shahbaz et al., 2013). Proline acts as an electron acceptor and prevents membrane damage (Ain-Lhout et al., 2001). It also protects photosynthetic perturbations induced by ROS (Hare et al., 1998); this could be due to the induction of specific proteins in response to oxidative damage caused by allelochemical stress (Mishra et al., 2006).

The results indicated a noticeable increase in formazan production in DS and HP when exposed to higher concentrations than the control group, with varying degrees of significance. The decrease in the activity of dehydrogenases could reflect cell damage due to exposure to allelochemicals present in extracts of *S. spectabilis*. The effect of plant extracts or allelochemicals on respiration has been reported in the literature (Sampietro et al., 2006; Rashid et al., 2010). Furthermore, our results showed that membrane damage of native plant species was affected differently depending on the extract concentration.

Malondialdehyde (MDA) is considered a sensitive marker commonly used to assess the lipid peroxidation of the membrane (Goel and Sheoran, 2003). Polyunsaturated fatty acids constitute the primary lipid constituents of membranes that are prone to peroxidation and degradation. Indeed, the increase in membrane permeability under allelochemical stress corresponds to an increase in lipid peroxidation estimated by malondialdehyde (MDA) accumulation (Omezzine et al., (2014) as observed in AT and PP in this study, which gradually decreased above mg ml^{-1}. The other species show lower MDA values. Allelochemicals may potentially harm cell membranes by directly interacting with a membrane constituent or impairing essential metabolic functions required to maintain membrane integrity (Rice, 1984). The increase in lipid peroxidation is also a marker for oxidative stress and is used as a possible explanation of lipid peroxidation during germination (Schopfer et al., 2001). It was reported earlier that allelopathic compounds influenced the cell membranes of cucumber and sorghum roots, and lipid peroxidation was determined as the MDA

content (Zeng et al., 2008). The relationship between lipid peroxidation and enzymatic activity in soybeans was significantly correlated when exposed to the phenolic extract of Brassica napus (Haddadchi and Gerivani, 2009). In the present study, the contents of MDA in native plants steadily increased with increasing concentration of *S. spectabilis* extracts (Fig. 2F). Comparable outcomes were noted in *Palmellococcus miniatus* when exposed to solutions containing the volatile oil of *Artemisia ordosica*. The results indicate an increase in the MDA content and photosynthesis inhibition through oxidative damage, which might negatively affect the development of *P. miniatus* (Yang et al., 2012). These interaction effects between extract concentration and native species underscore the complexity of the relationship in influencing MDA accumulation in the leaves.

	Formazan	**Proline**	**MDA**	**SOD**	**APX**	**Catalase**	**PP0**
Formazan	1.000						
Proline	-0.366	1.000					
MDA	-0.238	0.274*	1.000				
SOD	-0.139	0.099	0.346*	1.000			
APX	0.233**	-0.258	-0.172	0.172	1.000		
Catalase	0.202	-0.290	-0.090	0.231**	0.895	1.000	
PP0	0.096	-0.120	0.192	0.110	0.146	0.161	1.000

Table 1. Pearson's correlation coefficient between antioxidant enzymes * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

Table 1 summarises Pearson's correlation coefficients between antioxidant enzymes present in the aqueous extracts of *S. spectabilis,* indicating that the level of terpenoids or phenolic compounds present in *S. spectabilis* extracts could play an essential role in the antioxidant capacity of the native species. In Table 1, Pearson's correlation coefficient analysis reveals that CAT demonstrates a positive correlation with SOD, and this correlation is statistically significant ($p < 0.01$). As well, APX exhibits a positive correlation with Formazan, and this correlation is also statistically significant ($p < 0.01$). Proline shows a positive correlation with MDA and is statistically significant ($p < 0.05$), while MDA shows a positive correlation to SOD activity which is also statistically significant ($p < 0.05$). It is worth noting that while these correlations are statistically significant,

they are not characterised by a substantial degree of association.

4.2.To identify, isolate and elucidate the structure of allelochemicals of Senna spectabilis

GC-MS is one of the most precise methods to identify various secondary metabolites in the plant extract (Deshpande and Kadam., 2013; Payum., 2016). Phytoconstituents from *S. spectabilis* were extracted from leaves, bark and root using Soxhlet apparatus where five solvents, hexane, diethylether, chloroform, ethanol and methanol, were used. Eighty-six volatile chemicals were found in the extract after GCMS analysis, accounting for approximately 99% of the overall composition. The compounds were identified based on various peaks, retention times and peak areas (Table 2). According to their reducing peak areas, the top ten compounds from the solvent extract of *S. spectabilis* were Cassine, Decane, 1,9-bis[(trimethylsilyl)oxy]-, 2,6-di-Tert-Butyl-4-Methylphenol, 3',5'-Dimethoxyacetophenone, Decyl Chloroacetate, (Cis)-2-Nonadecene, Phenacyl Hexadecanoate, 1,3-Propanediamine, N,N'-Diethyl-, Mome Inositol and 1-Eicosanol.

4.2.1. GC–MS analysis

The GC–MS analysis Table 2.1-2.3 contains the list of phytoconstituents detected in various solvent extracts (hexane, diethylether, chloroform, ethanol, methanol) of *S. spectabilis* along with their respective retention times (R.Time) and relative abundance (Area%) (Figure 5.A-7.E). To identify the detected plant allelochemicals from Table 2.1-2.3, we focused on compounds typically found in S. spectabilis with known allelopathic properties. These allelochemicals can influence the growth and development of neighbouring plants. Based on table 2.1-2.3, 86 phytoconstituents were detected, and the following plant allelochemicals were identified from leaf: γ-Sitosterol (Hexane), Lupeol (Hexane), Phytol (Diethylether), Neophytadiene (Diethylether, Chloroform, Ethanol), Squalene (Hexane, Diethylether, Chloroform), Nerolidol (Ethanol); bark: 2,4-Ditert-Butylphenol (Chloroform) 3',5'-Dimethoxyacetophenone, Hexadecane and Octadecane (Ethanol). These compounds are known to have allelopathic effects on plants and have been previously reported in the literature (Liang et al., 2012, Thang et al., 2023, Luz et al., 2010).

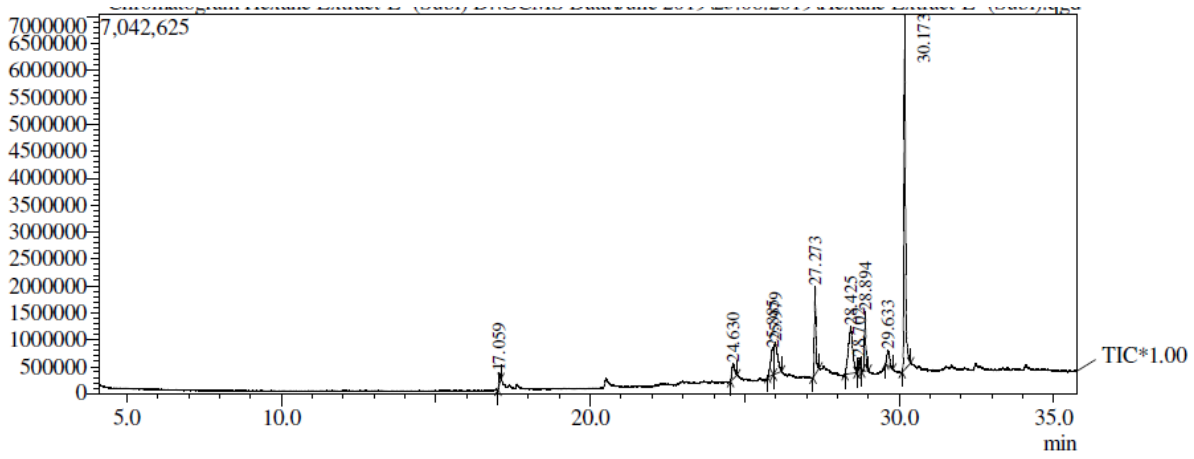


Figure 5.A: Identification of volatile compounds in the leaf hexane extract of *S. spectabilis* through GCMS chromatogram

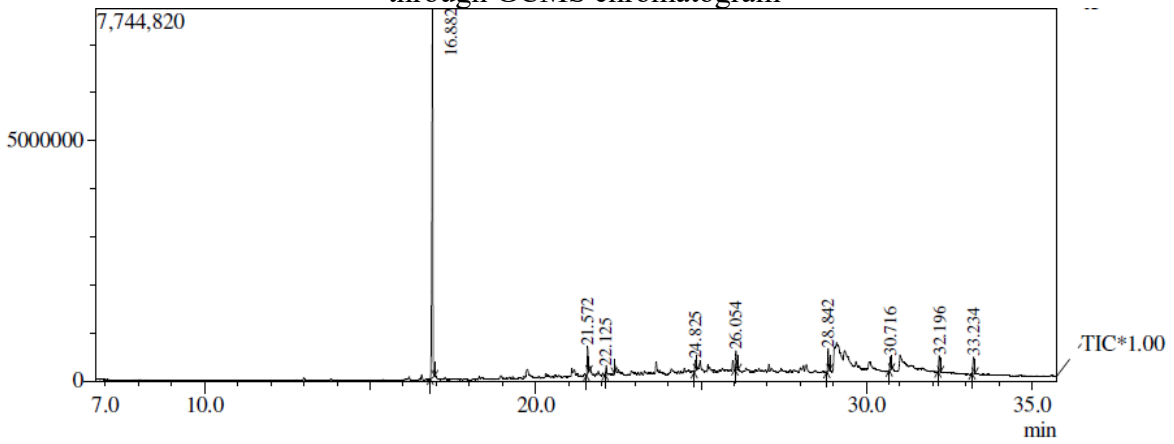


Figure 5.B: Identification of volatile compounds in the leaf Diethylether extract of *S. spectabilis* through GCMS chromatogram

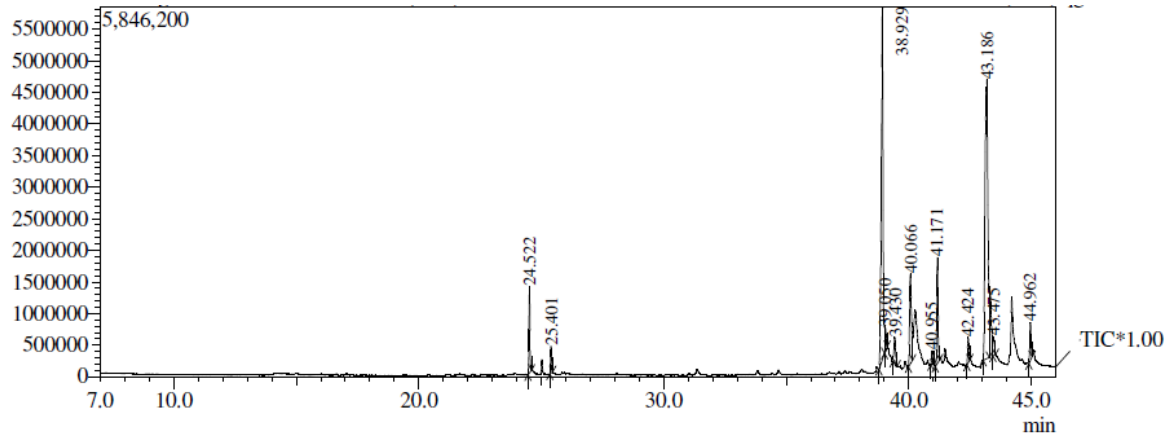


Figure 5.C: Identification of volatile compounds in the leaf Chloroform extract of *S. spectabilis* through GCMS chromatogram

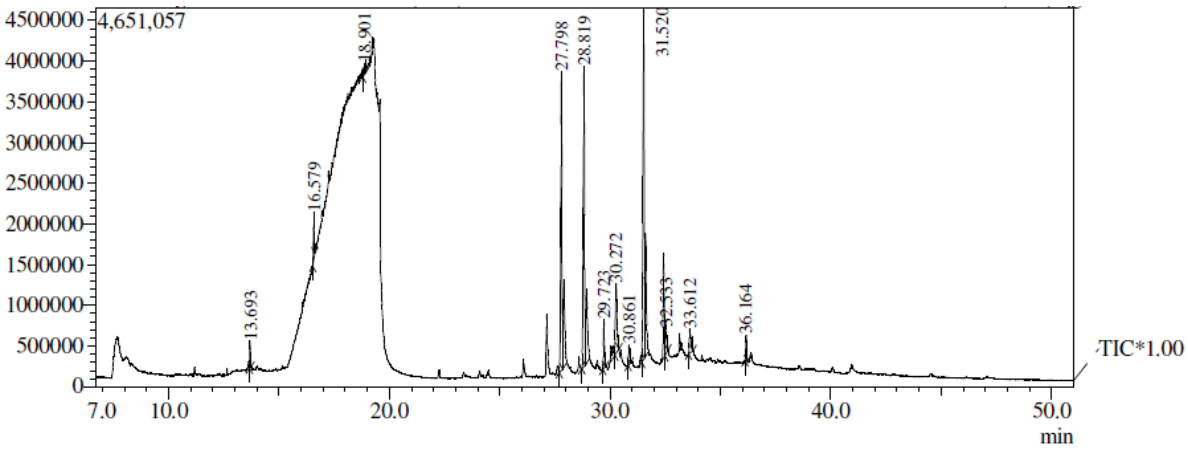


Figure 5.D: Identification of volatile compounds in the leaf Ethanol extract of *S. spectabilis* through GCMS chromatogram

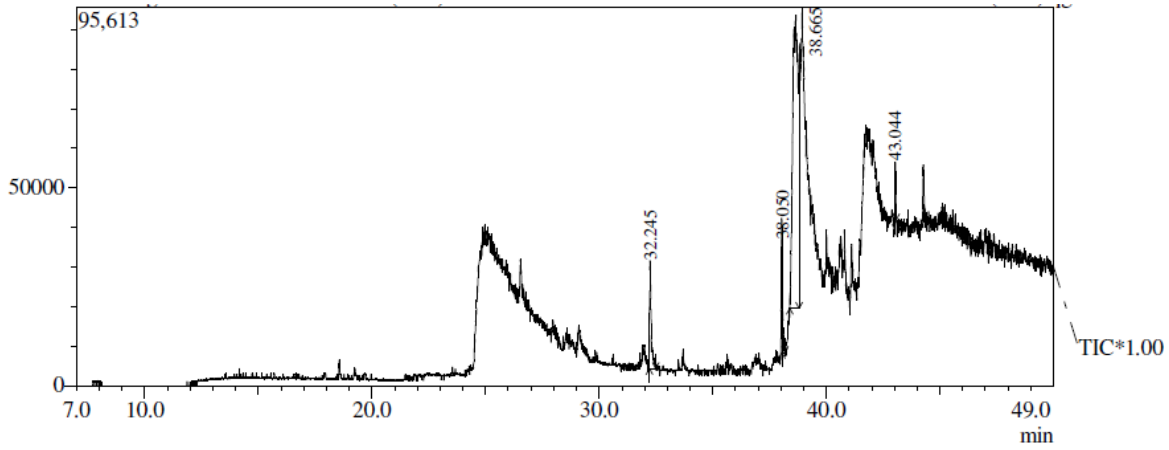


Figure 5.E: Identification of volatile compounds in the leaf Methanol extract of *S. spectabilis* through GCMS chromatogram

Peak#	R.Time	Area %	Name of compound	Molecular Formula	Molecular weight
A. HEX					
1	17.059	1.16	Phytol, acetate	$C_{22}H_{42}O$	338.5677
2	24.630	3.31	Piperazine-3,5-dione, 1-tetradecanoyl-	$C_7H_{12}N_2O$	156.1824
3	25.885	5.68	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7067
4	25.979	9.21	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	278.4
5	27.273	10.36	2-methyloctacosane	$C_{29}H_{60}$	408.8
6	28.425	15.89	(-)-Globulol	$C_{15}H_{26}O$	222.37
7	28.702	1.77	Heneicosane	$C_{21}H_{44}$	296.58
8	28.894	6.88	Squalene	$C_{30}H_{50}$	410.72
9	29.633	3.31	Lupeol	$C_{30}H_{50}O$	426.72
10	30.173	2.42	Tetratetracontane	$C_{44}H_{90}$	618.25
B. DE					

1	16.882	74.81	2,6-di-Tert-Butyl-4-Methylphenol	$C_{15}H_{24}O$	220.35
2	21.572	4.63	Neophytadiene	$C_{20}H_{38}$	278.524
3	22.125	0.81	(E)-Phytol	$C_{20}H_{40}O$	296.531
4	24.825	2.19	Nonadecane	$C_{19}H_{40}$	268.52
5	26.054	4.04	Hexacosyl acetate	$C_{32}H_{64}O_2$	480.85
6	28.842	4.20	1,3,5-Trisilacyclohexane	$C_6H_{15}Si_3$	177.46
7	30.716	2.44	4,4'-((p-Phenylene)diisopropylidene)diphenol	$C_{21}H_{22}O_2$	310.40
8	32.196	3.17	Squalene	$C_{30}H_{50}$	410
9	33.234	3.71	Pentacosane	$C_{25}H_{52}$	352.68
C. CHL					
1	24.522	4.13	Neophytadiene	$C_{20}H_{38}$	278.524
2	25.401	1.32	(E)-Phytol	$C_{20}H_{40}O$	296.531
3	38.929	31.02	Cassine	$C_{18}H_{35}NO_2$	297.5
4	39.050	1.56	Naphtho[1,8-de]-1,3-dioxin-2-one	$C_{11}H_6O_3$	186.17
5	39.430	1.95	N-[4-Cyclooctylaminobutyl]aziridine	$C_{12}H_{22}N_2$	194.32
6	40.066	8.33	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol	$C_{15}H_{31}NO$	241.41
7	40.955	0.74	1,3,5-Trisilacyclohexane	$C_3H_6Si_3$	126.33
8	41.171	6.42	l-Valine, N-allyloxycarbonyl-, hexyl ester	$C_{15}H_{29}NO_3$	271.40
9	42.424	1.51	2-(2H) Carvomenthylacetate	$C_{12}H_{20}O_2$	196.29
10	43.186	39.57	Cassine	$C_{18}H_{35}NO_2$	297.5
11	43.475	1.16	5-Nitrocytosine	$C_4H_4N_4O_3$	160.10
12	44.962	2.29	N-(.beta.-Hydroxyethyl)-4-(.gamma.-hydroxypropyl)piperidine	$C_{10}H_{21}NO_2$	187.27
D. ETH					
1	13.693	1.03	(+)-Nerolidol	$C_{15}H_{26}O$	222.37
2	16.579	2.39	Neophytadiene	$C_{20}H_{38}$	278.524
3	18.901	0.75	Mome Inositol	$C_7H_1O_6$	194
4	27.798	24.12	Cassine	$C_{18}H_{35}NO_2$	*297.5*
5	28.819	26.34	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol	$C_{15}H_{31}NO$	241.41
6	29.723	2.99	N-(.beta.-Hydroxyethyl)-4-(.gamma.-hydroxypropyl)piperidine	$C_{10}H_{21}NO_2$	187.27
7	30.272	6.97	Glycerol .Beta.-Palmitate	$C_{19}H_{38}O_4$	330.50
8	30.861	1.19	5-Nitrocytosine	$C_4H_4N_4O_3$	156.10
9	31.520	27.26	Cassine	$C_{18}H_{35}NO_2$	*297.5*
10	32.533	2.69	(2S,4R,6R)-2-Methyl-6-nonylpiperidin-4-ol	$C_{15}H_{31}NO$	241.41
11	33.612	2.85	Glycerol .alpha.-monostearate	$C_{21}H_{42}O_4$	370.56
12	36.164	1.43	Pentacosane	$C_{25}H_{52}$	352.69
E. MET					

1	32.245	8.88	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, undecyl ester	$C_{18}H_{35}NO_5$	345.48
2	38.050	4.35	2-(Acetylamino)-2-Cyanoacetamide	$C_5H_6N_2O_2$	128.11
3	38.665	85.43	Decane, 1,9-bis[(trimethylsilyl)oxy]-	$C_{22}H_{52}O_2Si_2$	424.89
4	43.044	1.33	R(-)3,7-Dimethyl-1,6-octadiene	$C_{10}H_{18}$	138.25

Table 2.1. List of phytoconstituents detected in leaf of *S. spectabilis* in various solvent extracts (A. HEX (Hexane); B. DE (Diethylether); C. CHL (Chloroform); D. ETH (Ethanol); E. MET (Methanol) provided by GC-MS analysis along with their respective retention times (RT) relative abundance (Area%), Molecular formula and weight.

Allelochemicals extracted from the leaves of *S. spectabilis*

A total of 6 allelochemicals were extracted and identified from GC-MS analysis. Among the allelochemicals identified by GC–MS, (+)-nerolidol is a well-studied sesquiterpene with documented phytotoxic properties. It has been identified as a potential allelochemical in several studies. Landi et al. (2020) and Kamalrul et al. (2022) reported its presence as a phytotoxic compound. Singh et al. (2023) and Abd et al. (2018) also support these findings, highlighting its role in allelopathy. Neophytadiene, isolated from *Nepeta* species, has been shown to inhibit the shoot growth of ragweed (*Ambrosia artemisiifolia*) shoots (Dmitrovi´c et al., 2015). Another allelochemical identified, lupeol, is a triterpene compound naturally occurring in various plant species, and it has garnered significant attention due to its allelopathic properties. The compound has been reported to exert phytotoxic effects on weeds and crops, disrupting processes such as seed germination and plant growth. The allelopathic potential of extracts from the leaves and branches of *Machaerium eriocarpum* and *Machaerium hirtum*, as well as the lupeol compound itself, was studied in sorghum (*S. bicolor* L.) seed germination and seedling growth (Tahira et al., 2021). Experimental studies have demonstrated the phytotoxicity of lupeol and lupenone when applied to weeds such as *Mimosa pudica* and *Senna obtusifolia* (Luz et al., 2010). Furthermore, lupeol was found to completely inhibit the elongation of etiolated wheat coleoptiles at a concentration of 10-3 M, and this bioactivity remained effective even upon dilution (Nebo et al., 2015). Squalene, a

triterpene compound, has been the subject of research regarding its allelopathic effects, particularly its impact on seed germination and plant growth. Notably, it has been observed that pearl millet, a specific plant species, contains elevated levels of squalene and inhibits the germination of seeds belonging to the epiphytic plant *Tillandsia recurvata* (Flores-Palacios et al. in 2015). γ-Sitosterol, a type of phytosterol identified in GC–MS, has potential allelopathic properties, especially in its ability to suppress the growth of weeds. It has demonstrated the capacity to hinder the germination of weed seeds and the early growth of weed seedlings. Additionally, within the group of triterpenoids, certain allelochemicals, such as stigmasterol, γ-sitosterol, and lupeol, have been identified, which may also contribute to the phytotoxic response observed in *A. pintoi* (Thang et al., 2023). Another noteworthy allelopathic compound is phytol, a diterpene alcohol known for its ability to inhibit the germination and growth of various plant species. In the context of the current study, the inhibitory effects of the essential oil from Egyptian *C. procera* can be attributed to the presence of oxygenated terpenoid compounds, with a particular emphasis on major components such as phytol (Al-Rowaily et al., 2020). Moreover, the essential oil derived from *Euphorbia heterophylla* is rich in phytol, and this oil has exhibited allelopathic activity on *Cenchrus echinatus* (Elshamy et al., 2019).

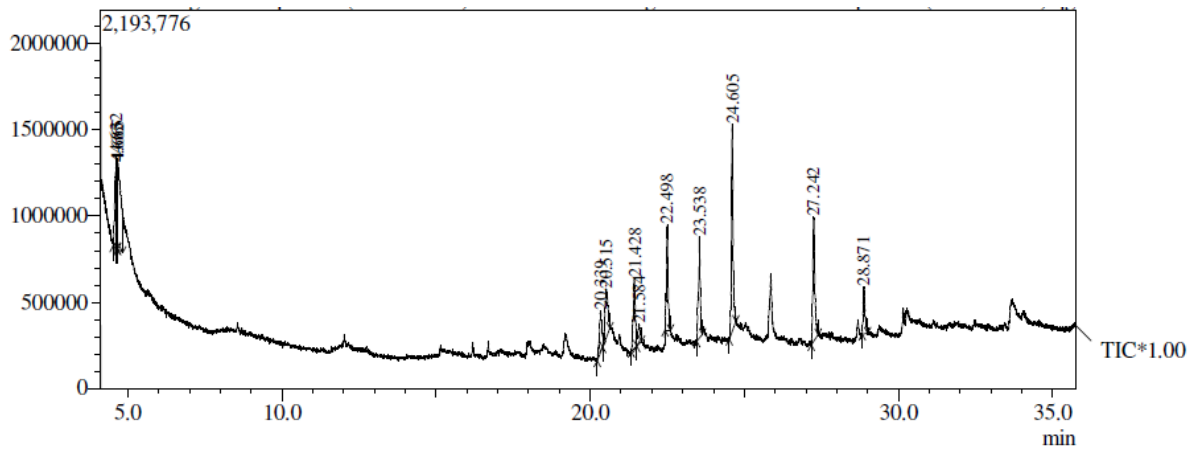


Figure 6.A: Identification of volatile compounds in the bark Hexane extract of *S. spectabilis* through GCMS chromatogram

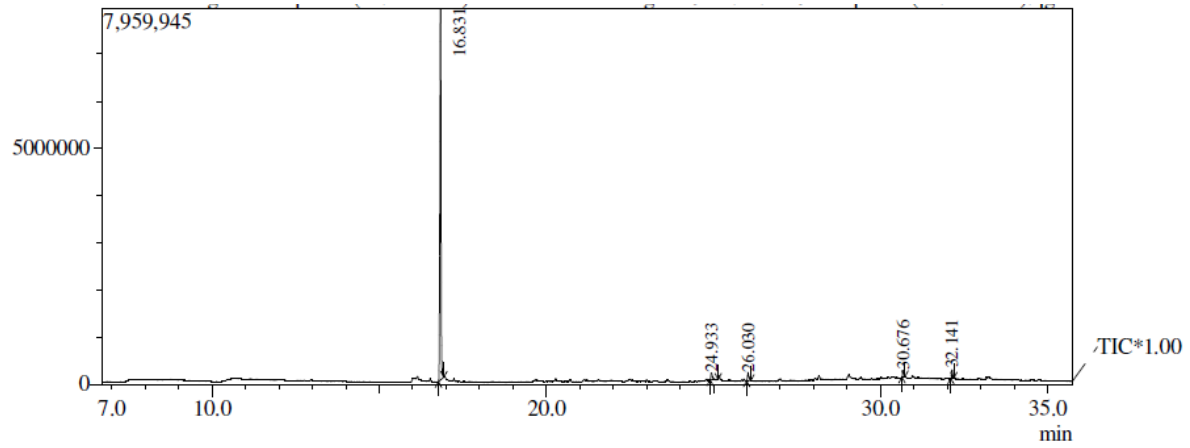


Figure 6.B: Identification of volatile compounds in the bark Diethylether extract of *S. spectabilis* through GCMS chromatogram

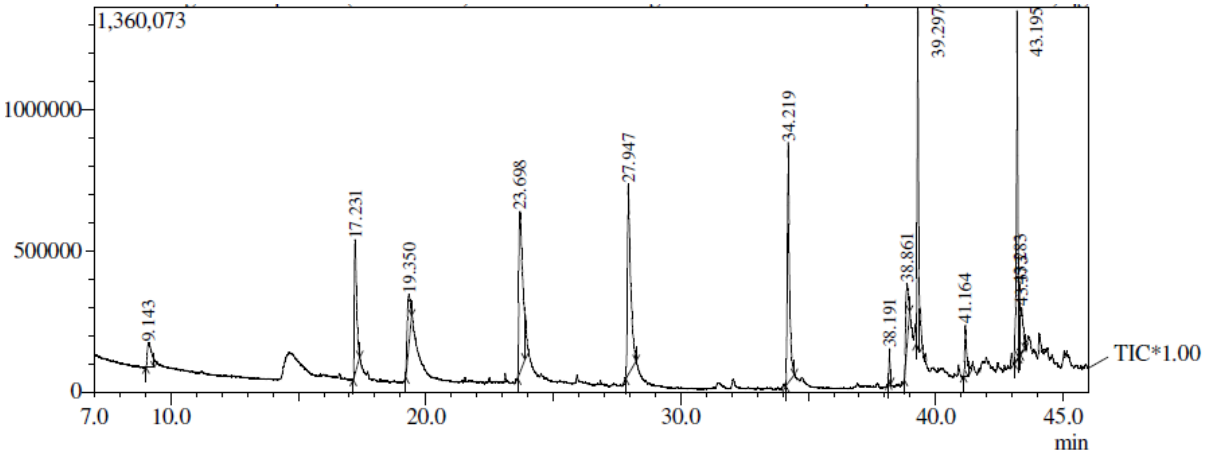


Figure 6.C: Identification of volatile compounds in the bark Chloroform extract of *S. spectabilis* through GCMS chromatogram

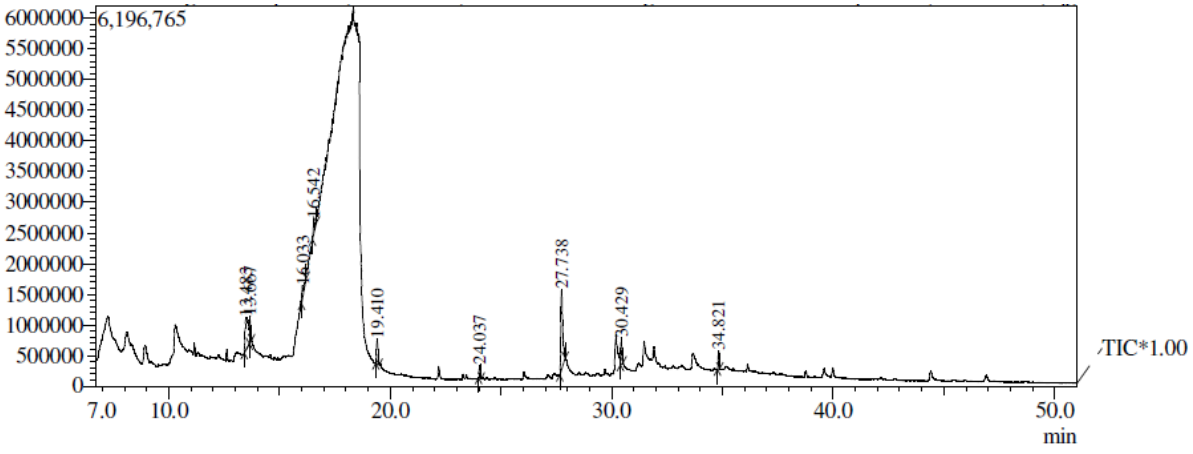


Figure 6.D: Identification of volatile compounds in the bark Ethanol extract of *S. spectabilis* through GCMS chromatogram

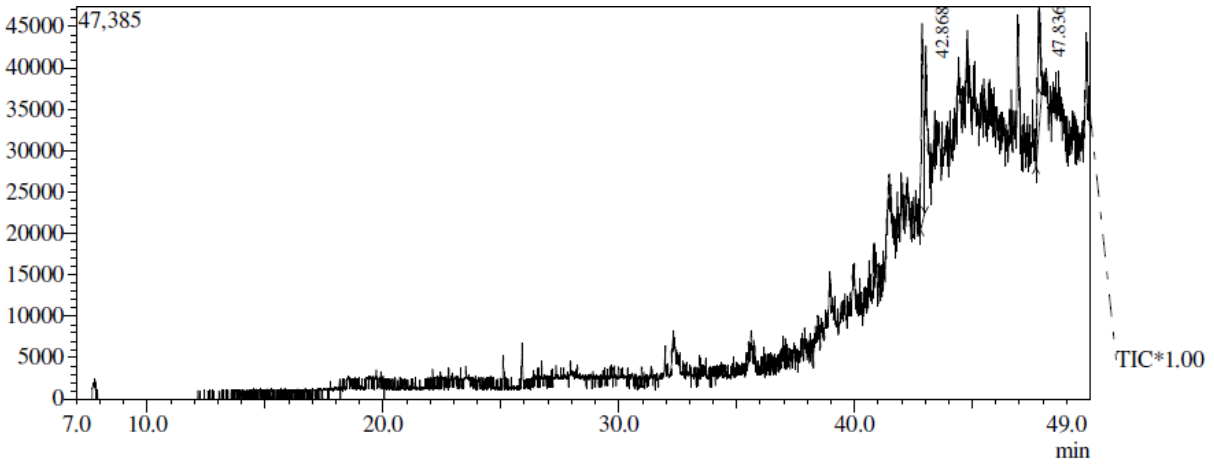


Figure 6.E: Identification of volatile compounds in the bark Methanol extract of *S. spectabilis* through GCMS chromatogram

Peak#	R.Time	Area%	Name of compound	Molecular Formula	Molecular weight
A. HEX					
1	4.632	9.12	Ethylhexanol	$C_8H_{18}O$	130.23
2	4.665	3.61	6,10-Dimethyl-4-Undecanol	$C_{13}H_{28}O$	200.36
3	4.685	13.58	Dianhydromannitol	$C_6H_8O_4$	144.13
4	20.339	5.22	Tritetracontane	$C_{43}H_{88}$	604.17
5	20.515	7.65	Phytol, Acetate	$C_{22}H_{42}O$	338.5677
6	21.428	7.56	Tritetracontane	$C_{43}H_{88}$	604.17
7	21.584	2.32	Lauryl Acetate	$C_{14}H_{28}O_2$	228.37
8	22.498	8.58	Heneicosane	$C_{21}H_{44}$	296.57
9	23.538	9.01	Tetratetracontane	$C_{44}H_{90}$	616.23
10	24.605	16.06	Heneicosane	$C_{21}H_{44}$	296.57
11	27.242	12.41	Tetratetracontane	$C_{44}H_{90}$	616.23
12	28.871	4.88	Squalene	$C_{30}H_{50}$	410
B. DE					
1	16.831	90.32	2,6-Di-Tert-Butyl-4-Methylphenol	$C_{15}H_{24}O$	220.35
2	24.933	2.43	Phytol	$C_{20}H_{40}O$	296.53
3	26.030	3.00	Heptadecyl Acetate	$C_{19}H_{38}O_2$	298.50
4	30.676	1.81	4,4'-((P-Phenylene)Diisopropylidene) Diphenol	$C_{21}H_{22}O_2$	306.40
5	32.141	2.43	Squalene	$C_{30}H_{50}$	410
C. CHL					
1	9.143	2.93	Nonylcyclopropane	$C_{12}H_{24}$	168.32
2	17.231	8.03	2,4-Ditert-Butylphenol	$C_{14}H_{22}O$	206.33
3	19.350	4.32	1-Pentadecen	$C_{15}H_{30}$	210.40
4	23.698	15.32	E-15-Heptadecenal	$C_{17}H_{32}O$	256.44
5	27.947	16.10	Heneicosyl Alcohol	$C_{21}H_{44}O$	316.59
6	34.219	13.95	1-Heneicosanol	$C_{21}H_{44}O$	312.58

7	38.191	0.96	O O'-Biphenol, 4,4',6,6'-Tetra-T-Butyl-	$C_{26}H_{36}O_2$	376.56
8	38.861	4.47	Cassine	$C_{18}H_{35}NO_2$	*297.5*
9	39.297	12.35	1-Heneicosanol	$C_{21}H_{44}O$	312.58
10	41.164	2.55	1,2,4-Triazaspiro[5.5]Undec-2-Em-5-One, 3-Mercapto-	$C_2H_4N_4S$	116.15
11	43.195	13.56	Lignocerol	$C_{24}H_{50}O$	354.65
12	43.283	2.69	Acetamide, 2-[[4-Methyl-5-(4-Morpholinylmethyl)-4h-1,2,4-Triazol-3-Yl]Thio]-	$C_{12}H_{11}ClF_3N_5OS$	365.76
13	43.373	2.76	2-Amino-N-Caprylic Acid, Di-Tms	$C_8H_{17}NO$	159.22
D. ETH					
1	13.483	26.35	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180.20
2	13.667	4.99	Hexadecane	$C_{16}H_{34}$	226.44
3	16.033	4.53	Octadecane	$C_{18}H_{38}$	254.49
4	16.542	4.45	Neophytadiene	$C_{20}H_{38}$	278.524
5	19.410	8.26	Ethyl Nonadecanoate	$C_{20}H_{40}O_2$	312.54
6	24.037	3.41	Octadecanoic Acid, Ethyl Ester	$C_{20}H_{40}O_2$	312.54
7	27.738	36.83	Cassine	$C_{18}H_{35}NO_2$	*297.5*
8	30.429	7.19	1,2-Benzenedicarboxylic Acid	$C_8H_6O_4$	166.13
9	34.821	3.99	Squalene	$C_{30}H_{50}$	410
E. MET					
1	42.868	58.11	Decyl Chloroacetate	$C_{12}H_{23}ClO_2$	234.77
2	47.836	41.89	(Cis)-2-Nonadecene	$C_{19}H_{38}$	266.50

Table 2.2. List of phytoconstituents detected in the bark of *S. spectabilis* in various solvent extracts (A. HEX (Hexane); B. DE (Diethylether); C. CHL (Chloroform); D. ETH (Ethanol); E. MET (Methanol) provided by GC-MS analysis along with their respective retention times (RT) relative abundance (Area%), Molecular formula and weight.

Allelochemicals extracted from the bark of *S. spectabilis*

Zhang et al. (2011) detected the presence of 2,4-Ditert-Butylphenol (2,4-DTBP) in rhizosphere soil extracts of hops (*Humulus lupulus* L.). Their findings indicated that autotoxicity induced by this compound in rhizosphere soil might contribute to the deterioration in crop quality. Additionally, 2,4-DTBP extracted from *Imperata cylindrica* (L.) P. Beauv (cogongrass) rhizomes demonstrated allelopathic effects on the germination and seedling growth of weedy plants in soil-less conditions. For instance, 100 µg mL–1 of 2,4-DTBP completely hindered *I. cylindrica* germination and exhibited 78-95% inhibition on the root and shoot growth of beggar's tick (*Bidens pilosa* L.), Leucaena (*Leucaena leucocephala* (Lam.) de Wit), and barnyard grass (*Echinochloa crus-galli* (L.)

Beauv) (Xuan et al. 2009). More recently, Chuah et al. (2014) identified 2,4-DTBP in the culm and leaf extracts of *Pennisetum purpureum*. Their study revealed that 2,4-DTBP displayed potent herbicidal activity, completely inhibiting the germination of *Leptochloa chinensis* at a concentration of 500 µg mL–1 and preventing root growth of L. chinensis at an application rate as low as 0.60 kg ai ha–1 under soil conditions. Further investigations by Chuah et al. (2015) demonstrated that the herbicidal action of this compound is attributed to its ability to induce oxidative stress, leading to the generation of reactive oxygen species. ROS species, in turn, cause lipid peroxidation and membrane damage in root tissues and chloroplasts in leaf tissues. *Leptochloa chinensis* and *Hedyotis verticillata*, when cultivated in the presence of 50 or 200 µg mL-1 of 2,4-di-tert-butyl phenol (2,4-DTBP), displayed evident symptoms such as wilting and necrosis of leaf blades, along with impaired root growth and abnormal root hairs (Norhafizah et al., 2016). The impact of 2,4-di-tert-butyl phenol can stimulate various physiological activities in plants by enhancing the activity of antioxidant enzymes. Additionally, it was found to modulate the behaviour of soil fauna by increasing the activity of oxidative stress enzymes, particularly superoxide dismutase (SOD). Notably, these effects intensified with higher concentrations of 2,4-di-tert-butyl phenol and prolonged exposure times (Zhiqun et al., 2017).

Phenylpropanoid-type compounds released by injured plants, such as 3,5-dimethoxyacetophenone (acetosyringone) and hydroxyacetosyringone, represent classic examples of chemoattractant for soil-borne pathogens. These compounds attract Agrobacterium tumefaciens, the causal agent of crown gall disease (Stachel et al., 1985; Hess et al., 1991; Dixon, 1995). Culturing other plant species has proven challenging in regions with a substantial presence of *S. alterniflora*. This difficulty has been attributed to the allelopathic effects of chemical substances such as hexadecane and octadecane in the surrounding soil (Liang et al., 2012).

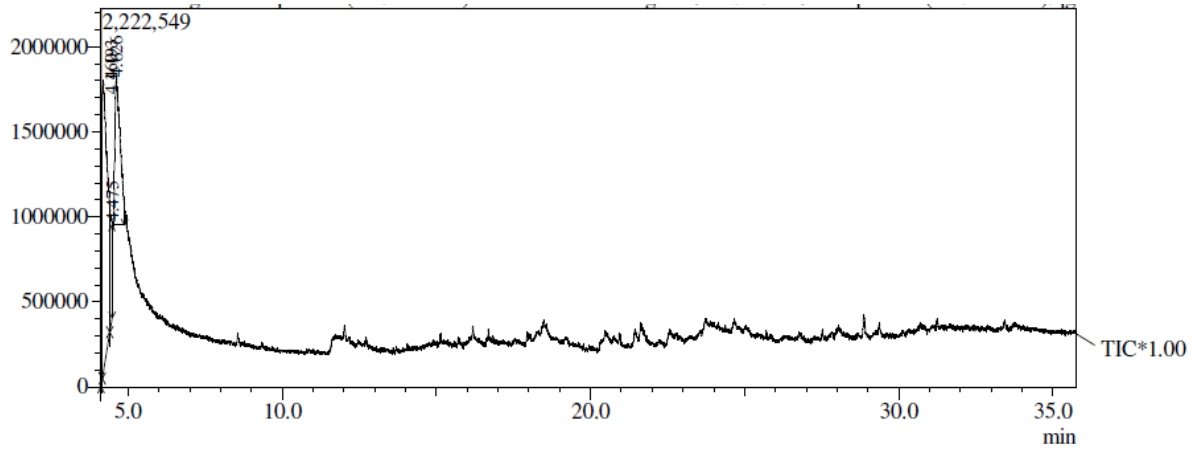


Figure 7.A: Identification of volatile compounds in the root Hexane extract of *S. spectabilis* through GCMS chromatogram

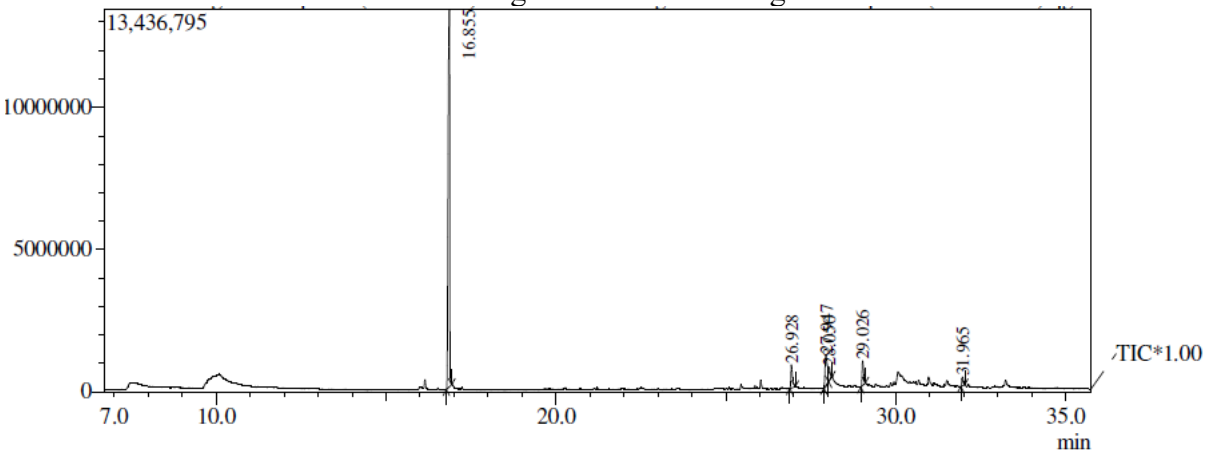


Figure 7.B: Identification of volatile compounds in the root Diethylether extract of *S. spectabilis* through GCMS chromatogram

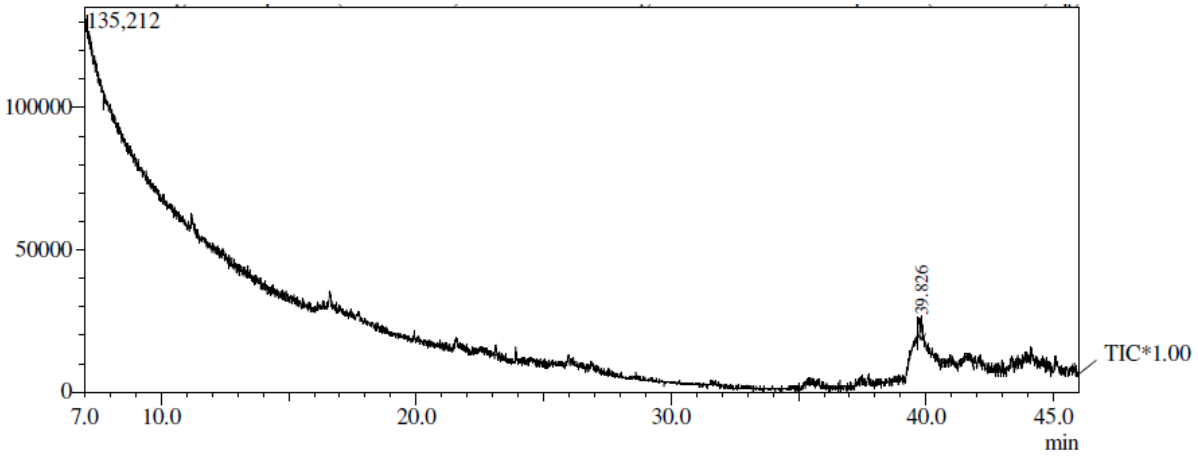


Figure 7.C: Identification of volatile compounds in the bark Chloroform extract of *S. spectabilis* through GCMS chromatogram

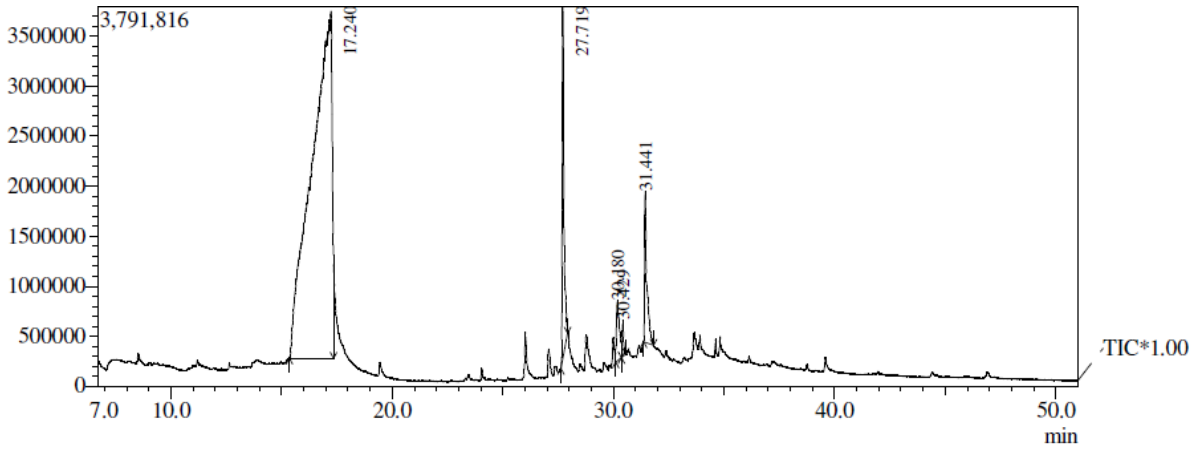


Figure 7.D: Identification of volatile compounds in the root Ethanol extract of *S. spectabilis* through GCMS chromatogram

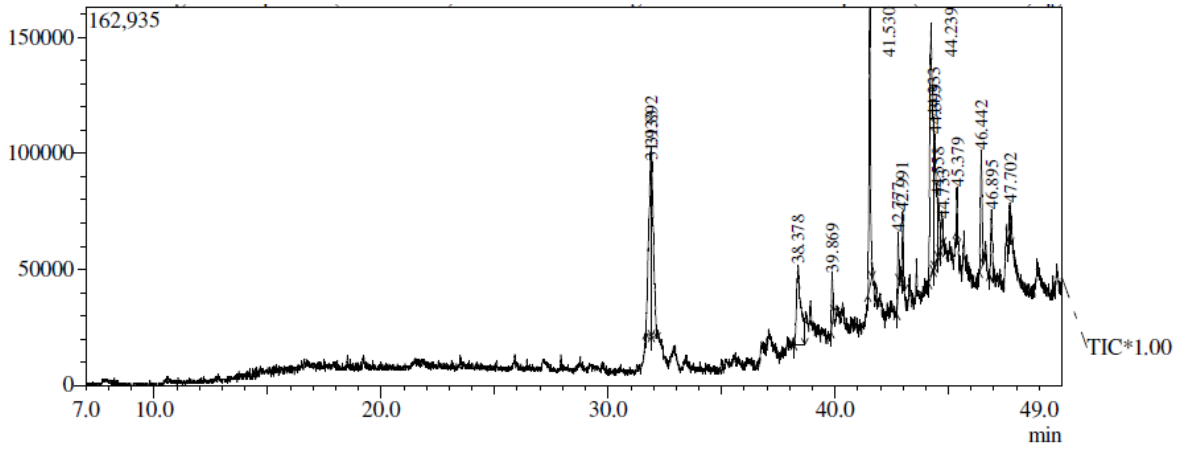


Figure 7.E: Identification of volatile compounds in the root Methanol extract of *S. spectabilis* through GCMS chromatogram

Peak#	R.Time	Area%	Name of compound	Molecular Formula	Molecular weight
A. HEX					
1	4.160	11.86	2,5-Dimethyl-1-Hepten-4-Ol	$C_{10}H_{20}O$	156.27
2	4.193	51.31	Phenacyl Hexadecanoate	$C_{22}H_{28}O_2$	328.46
3	4.475	7.91	2,2-Dimethyl-1,3-Butanediol	$C_6H_{14}O_2$	118.18
4	4.626	28.91	1-Hexanol, 2-Ethyl-	$C_8H_{18}O$	130.23
B. DE					
1	16.855	75.83	2,6-Di-Tert-Butyl-4-Methylphenol	$C_{15}H_{24}O$	220.35
2	26.928	4.89	1-Heneicosanol	$C_{21}H_{44}O$	312.58
3	27.947	9.86	Cassine	$C_{18}H_{35}NO_2$	*297.5*
4	28.050	2.58	Tridecane	$C_{13}H_{28}$	184.37
5	29.026	5.05	Lignocerol	$C_{24}H_{50}O$	354.65

6	31.965	1.79	Octyl Sebacate	$C_{18}H_{36}O_4$	316.47
C. CHL					
1	39.826	100.00	1,3-Propanediamine, N,N'-Diethyl-	$C_7H_{18}N_2$	130.23
D. ETH					
1	17.240	85.89	Mome Inositol	$C_7H_1O_6$	194
2	27.719	7.24	Cassine	$C_{18}H_{35}NO_2$	*297.5*
3	30.180	2.18	Carbonic Acid, Monoamide, N-Methallyl-, Allyl Ester	$C_{13}H_{25}NO_2$	227.34
4	30.429	0.68	1,2-Benzenedicarboxylic Acid	$C_8H_6O_4$	166.13
5	31.441	4.01	Cassine	$C_{18}H_{35}NO_2$	*297.5*
E. MET					
1	31.892	14.37	1-Pentadecanol	$C_{15}H_{32}O$	228.42
2	31.933	10.99	1-Heptacosanol	$C_{27}H_{56}O$	400.74
3	38.378	10.35	1-Eicosanol	$C_{20}H_{42}O$	298.56
4	39.869	1.75	(Cis)-2-Nonadecene	$C_{19}H_{38}$	266.50
5	41.530	11.76	Octadecyl Trifluoroacetate	$C_{20}H_{37}F_3O_2$	376.51
6	42.777	1.72	(Trans)-2-Nonadecene	$C_{19}H_{38}$	266.50
7	42.991	1.59	Farnesol Isomer A	$C_{15}H_{26}O$	222.37
8	44.239	20.06	1-Eicosanol	$C_{20}H_{42}O$	298.56
9	44.333	4.35	Myristyl Chloride	$C_{14}H_{29}Cl$	243.83
10	44.399	7.13	Fukinane	$C_{15}H_{28}$	208.38
11	44.558	2.38	Cytisine, Tetrahydro-12-Methyl-	$C_{11}H_{16}N_2$	168.26
12	44.733	1.61	9-Octadecenoic Acid (Z)-	$C_{18}H_{34}O_2$	282.47
13	45.379	1.31	Silicone Oil	$C_6H_{18}OSi_2$	*162.38*
14	46.442	5.62	1-Heptacosanol	$C_{27}H_{56}O$	396.7
15	46.895	3.47	Ethyl Iso-Allocholate	$C_{27}H_{45}NO_4$	451.66
16	47.702	1.54	Cetylol	$C_{16}H_{34}O$	242.45

Table 2.3. List of phytoconstituents detected in root of *S. spectabilis* in various solvent extracts (A. Hexane; B. Diethylether; C. Chloroform; D. Ethanol; E. Methanol) provided by GC-MS analysis along with their respective retention times (RT), relative abundance (Area%), Molecular formula and weight.

Allelochemicals extracted from the root of *S. spectabilis*

In root extract of *S. spectabilis* potentially active allelochemicals 2,6-Di-Tert-Butyl-4-Methylphenol (BHT) were identified.

The amount of these allelochemically bioactive phytoconstituents ranged from 0.81% (Phytol) to 26.35% (3',5'-Dimethoxyacetophenone) along with the other tracer components like squalene, phytol, neophytadiene, which proved themselves as the allelochemical compound along with other

medicinal attributes. According to previous studies, out of 86 bioactive phytochemicals identified in the present study, about ten phytoconstituents exhibited allelochemical activity and could have played an important role in inducing oxidative stress and elevating antioxidant activity in native species. Further investigation is needed to confirm the specific effect of these allelochemicals in native plants.

According to previous studies, out of 86 bioactive phytochemicals identified in the present study, about ten phytoconstituents exhibited bioactive properties like antioxidant, anti-inflammatory, anti-cancerous, antimicrobial, hypoglycaemic, hepatoprotective, anti-coronary, antiandrogenic, antiarthritic, etc. (Table 2.4).

Sl. No.	Name of compound	Nature	Biological activity	Reference
1	γ-Sitosterol	Triterpenoid	Antiviral, antioxidative, antidiabetic, antimicrobial, anticancer, antidiarrhoeal, anti-inflammatory and anti-angeogenic	Raman et al (2012)
2	Lupeol	Triterpenoid	Antiprotozoal, Anti-inflammatory, Antitumor, Antimicrobial	Gallo and Sarachine, (2009).
3	Phytol	Diterpene	Antioxidative, antimicrobial, hypocholesterolemic, anticancerous, antiinflammatory, diuretic and immunostimulatory	Dhanalakshmi and Manavalan (2014), Santos et al (2013)
4	Neophytadiene	Terpenoid	Antifungal, antioxidant, antipyretic, anti-inflammatory, analgesic and antimicrobial.	Raman et al (2012)
5	Squalene	Triterpene	Antioxidative, antibacterial, pesticide, antitumor, anti-cancerous, antiinflammatory, chemoprotective, stimulates immune system, antiaging, xenobiotic neutraliser, antiatherosclerotic,	Raman et al (2012), Nishanthini et al (2014), Dhanalakshmi and Manavalan (2014)

			diuretic, analgesic, pesticide and hypoglycaemic.	
6	Nerolidol	Sesquiterpene	Antioxidative, Antibacterial, Anti-biofilm, Antifungal, Antimalarial, anticancer	Vinholes et al (2014a), Vinholes et al (2014b), Hada et al (2003), Inoue et al (2004), Lee et al (2014), Lee et al (2007), Lopes et al (1999), Ryabchenko et al (2008)
7	3',5'-Dimethoxyacetophenone	Phenyl propanoid	Anticancer	Chu and Xiao (2023)
8	2,4-Ditert-Butylphenol	Phenol	Antioxidant property	Santos et al (2013)
9	Hexadecane	Alkane hydrocarbon	Antidiarrheal activity	Majumde et al (2021).
10	Octadecane	Alkane hydrocarbon	Lubricants, Anticorrosion agents	Arora et al (2017).

Table 2.4. Bioactive properties of potent phytochemicals of *S. spectabilis* analysed via GC-MS

Based on the comprehensive analysis conducted, it is evident that *Senna spectabilis* possesses a rich repository of allelochemicals, mainly concentrated within its leaves. Various research studies have demonstrated that the foliage of plants typically exerts a more pronounced allelopathic influence than other plant organs (Zheng and Feng, 2005; Huangfu et al., 2010).

4.3.Analysing the microbial diversity in Senna spectabilis invaded areas.

4.3.1. GCMS

The GC–MS analysis Table 3.1 contains the list of phytoconstituents detected in various solvent extracts (hexane and diethylether) of *S. spectabilis,* along with their respective retention times (R.Time) and relative abundance (Area%). To identify the detected soil allelochemicals from Table 3.1, we focused on compounds known for their allelopathic properties. These allelochemicals can influence the growth and development of neighbouring plants. Based on the table 3.1, 9

phytoconstituents were detected. These compounds are known to have allelopathic effects on plants and have been previously reported in the literature (Zhang et al., 2015).

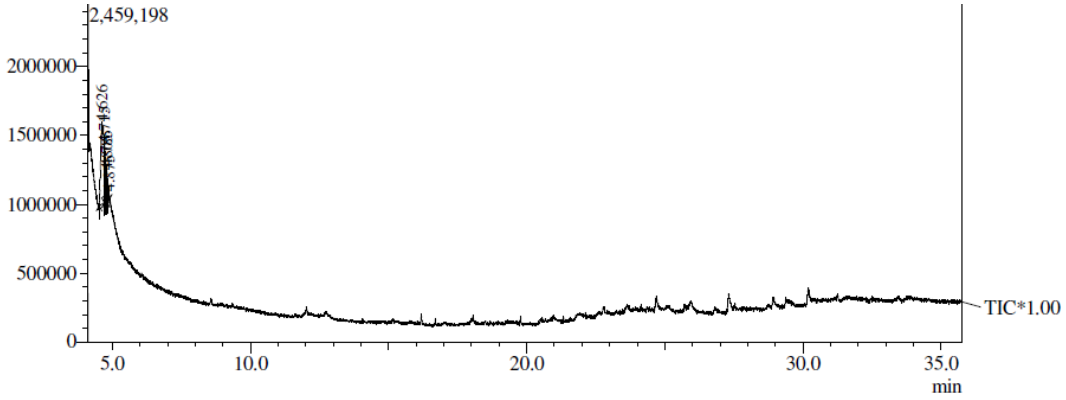


Figure 8.A: GC-MS chromatogram of *S. spectabilis* invaded forest soil in Hexane solvent.

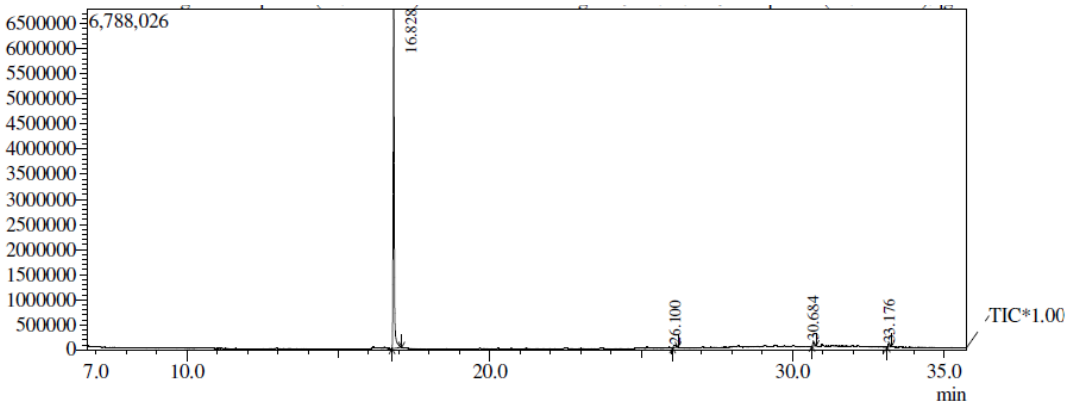


Figure 8.B. GC-MS chromatogram of *S. spectabilis* invaded forest soil in Diethylether solvent

Peak#	R.Time	Area%	Name of compound	Molecular Formula	Molecular weight
1	4.626	68.05	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.22
2	4.715	17.76	2-Undecene, 6-methyl-, (E)-	$C_{12}H_{24}$	168.31
3	4.785	4.25	Ethenyl tert-butyl sulfoxide	$C_6H_{14}OS$	134.24
4	4.805	7.59	1-Hexene, 2,5-dimethyl-	C_8H_{16}	112.21
5	4.875	2.35	cis-2,3-Epoxyoctane	$C_8H_{16}O$	128.21
6	16.828	95.25	2,6-Di-Tert-Butyl-4-Methylphenol	$C_{15}H_{24}O$	220.35
7	26.100	1.79	Heptadecyl acetate	$C_{19}H_{38}O_2$	298.50
8	30.684	1.60	4,4'-((p-Phenylene)diisopropylidene)diphenol	$C_{24}H_{26}O_2$	346.47
9	33.176	1.37	2-Bromotetradecane	$C_{14}H_{29}Br$	277.28

Table 3.1: List of names of compounds detected in the root of *S. spectabilis* provided by GC-

MS analysis along with their respective retention times (RT), relative abundance (Area%), Molecular weight and formula.

2,6-Di-Tert-Butyl-4-Methylphenol (BHT) is the major compound identified from the rhizospheric soil of *S. spectabilis.* Subsequent analysis revealed that introducing BHT markedly decreased the soil urease and catalase activity, indicating a discernible inhibition of soil metabolic enzyme activity. This inhibition intensified with increasing concentrations of BHT addition, as documented by Zhang et al. (2015).

4.3.2. Soil physiochemical properties

Comparison of soil properties

There were no differences in electrical conductivity and soil organic carbon of invaded forest soil with that of uninvaded forest soil and managed soil (Table 3.2). Invaded forest soil tended to have higher bulk density and was statistically significant (p value < 0.05) from others (Table 3.2). Moisture content and organic carbon were higher in uninvaded forest soil. Although organic carbon content was higher in uninvaded forest soil, the difference was not statistically significant compared to invaded and managed soil.

	Bulk density	**Moisture content**	**pH**	**Electrical conductivity**	**Organic carbon**
Invaded	0.36±0.06 a	1.83±0.45 b	5.30±0.55 b	0.10±0.05 a	0.84±0.67 a
Uninvaded	0.25±0.03 c	2.30±0.32 a	6.25±.54 a	0.13±0.10 a	1.19±1.29 a
Managed	0.34±0.06 b	1.74±0.34 b	6.51±.22 a	0.16±0.06 a	0.84±0.40 a

Table 3.2 Soil physiochemical properties of three different soil samples (Invaded forest soil, Forest soil and Managed soil)

Analysis of soil characteristics revealed significant differences in soil bulk density between invaded, uninvaded forest and managed soils (Table 3.2), with the invaded forest soil exhibiting higher

density (P< 0.05). Conversely, the pH levels were significantly higher (P<0.05) in uninvaded forest soil than in invaded soil. Previous studies have highlighted the importance of soil pH in shaping bacterial diversity across various environments (Amoo and Babalola, 2017; Cordero et al., 2020), as it influences nutrient availability and, consequently, the physiology and growth of bacterial communities. Further study showed that predominant phyla varied along the pH gradient, with acidic soils favouring plant growth due to increased micronutrient availability, while alkaline soils enhanced macronutrient abundance (Gentili et al., 2018).

Moreover, the rhizosphere harboured distinct microbial communities relative to bulk soils, attributed to elevated levels of organic exudates from plant roots (Vieira et al., 2020). Consistent with previous findings, our study underscores the influence of plants on microbial diversity and selection in the rhizosphere, potentially through the provision of carbohydrates, aromatic compounds, and amino acids.

4.2.3. Metagenomics

Microbial diversity in *Senna spectabilis* invaded area

In the current study, we address the changes in microbial communities caused by *Senna spectabilis* by performing a metagenomics analysis of three sample areas: *S. spectabilis* invaded land, forest land, and managed land.

From the *S. spectabilis* invaded land, the heterogenous metagenomics resulted in 7,941 reads with an average sequence length of 758bp. From this, 12.88% were classified using the One Codex database. The abundance of *Trypanosoma rangeli* and *Candidatus koribacterversatilis* has been identified from the Targeted Loci database. At the species level, the abundance of the following was estimated using the One Codex database: *Candidatus rokubacteria bacterium* (1.67%), *Pseudonocardia cytotoxica* (0.89%), *Betaproteobacteria bacterium* (0.89%), *Gemmatirosakal amazoonensis* (0.69%), *Rhizophagus irregularis* (0.59%), *Betaproteobacteria bacterium* (0.59%),

Blastocatellia bacterium (0.59%), *Staphylococcus aureus* (0.49%), *Acidobacteria bacterium* (0.49%) and *Betaproteobacteria bacterium* (0.49%) (Figure 10.A-D).

From the Forest land, 1,988 reads were obtained and 20.17% of them where classified using the One Codex database. The following species: *Piromyces sp*. E2 (2.99%), *Saccharopolyspora dendranthemae* (2.74%), *Staphylococcus aureus* (2%), *Streptomyces durmitorensis* (2%), *Fusarium oxysporum*(1.5%), *Diversisporaepigaea* (1%), *Saccharopolyspora rhizosphaerae* (1%), *Halomonas azerbaijanica* (1%), *Aeromonas hydrophila*(0.75%), *Purpureocilliumlilacinum* (0.75%) were abundant in this sample (Figure 10.A-D).

From the managed land, the heterogenous metagenomics resulted in 8,475 reads with an average sequence length of 1051bp. The most abundant species identified from this sample were *Pseudonocardiacytotoxica* (1.19%), *Staphylococcus aureus* (1.12%), *Alphaproteobacteria bacterium* (1.05%), *CandidatusRokubacteria bacterium* (1.05%), *Halomonasazerbaijanica* (1.05%), *Xanthomonas phage* (0.84%), *Piromyces sp.* E2 (0.77%), *Spartobacteria bacterium* (0.56%), *Streptomyces durmitorensis* (0.49%) and *Thermoleophilia bacterium* (0.49%) (Figure 10.A-D).

OTUs clustered sequences at 97% similarity, and the abundance of different OTUs in all samples was obtained. The Venn diagram (Figure 9) demonstrated that the Invaded Forest soil, Uninvaded Forest soil and Managed samples shared 11.4% of OTUs.

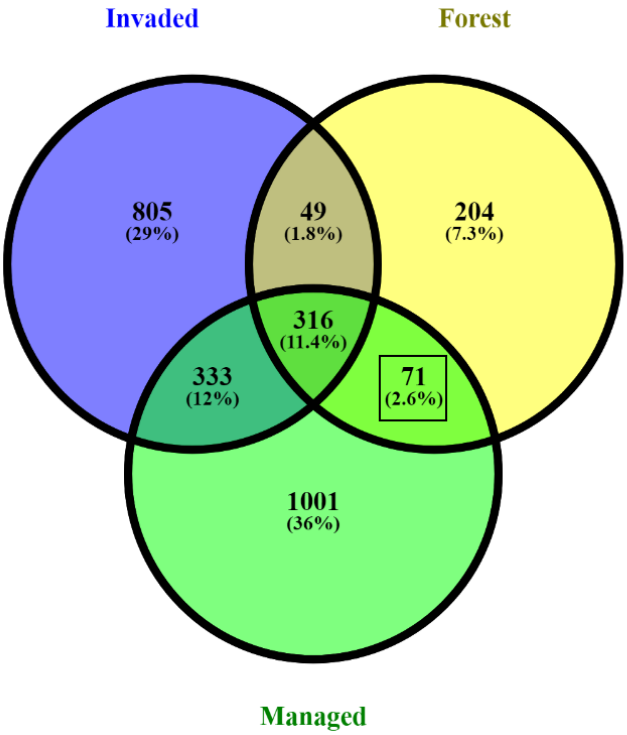


Figure 9: Venn diagram of exclusive and shared bacterial species in three soil samples (Invaded Forest soil, uninvaded Forest soil and Managed soil)

At the phylum level, as shown in (Figure 10.C-D), Pseudomonadota was the dominant phylum with the highest average relative abundance of 42.22%, 34.66% and 33.29% in invaded, uninvaded forest and managed soils, respectively, followed by Actinomycetota, with an average relative abundance of 14.37%, 31.67% and 21.44%. Among the fungi, Ascomycota and Mucoromycota were the dominant phyla in invaded, uninvaded forests and managed soils. The average relative abundance of Ascomycota was 1.08%, 3.24% and 1.54% in invaded, uninvaded forest and managed soils, respectively. The average relative abundance of Mucoromycota was 0.79%, 1% and 0.56% in invaded, uninvaded forest and managed soils.

At the genus level (Figure 10. A-B), Bradyrhizobium had the highest average relative abundance of 3.44% and 3.43% in invaded and managed soils, respectively, while in uninvaded forest soil, Saccharopolyspora showed a high relative abundance of 10.22%. Streptomyces followed

this, with an average relative abundance of 3.44%, 9.23% and 2.38%, respectively. Among the fungi Piromyces were the dominant genus in both uninvaded Forest and managed soils. The average relative abundance of Piromyces was 41% and 28% in uninvaded forest and managed soil respectively. Next to it was Fusarium (24%) in uninvaded forest soil and Rhizophagus which was 23% and 15% respectively in invaded and managed soil.

The analysis of similarities (ANOSIM) method assessed significant differences in the bacterial community composition between invaded, forest and managed rhizosphere soils. It was observed that bacterial genus level community patterns significantly varied between Invaded Forest soil and uninvaded forest soil (ANOSIM R = 0.1155, *p* value = 0.029), although invaded and managed soil did not show any significant difference (ANOSIM R = -0.02937). In the Fungal genus level community, no significant difference was observed between invaded, and forest invaded soil (ANOSIM R = -0.1636) and among invaded soil with that of managed soil (ANOSIM R = -0.07465)

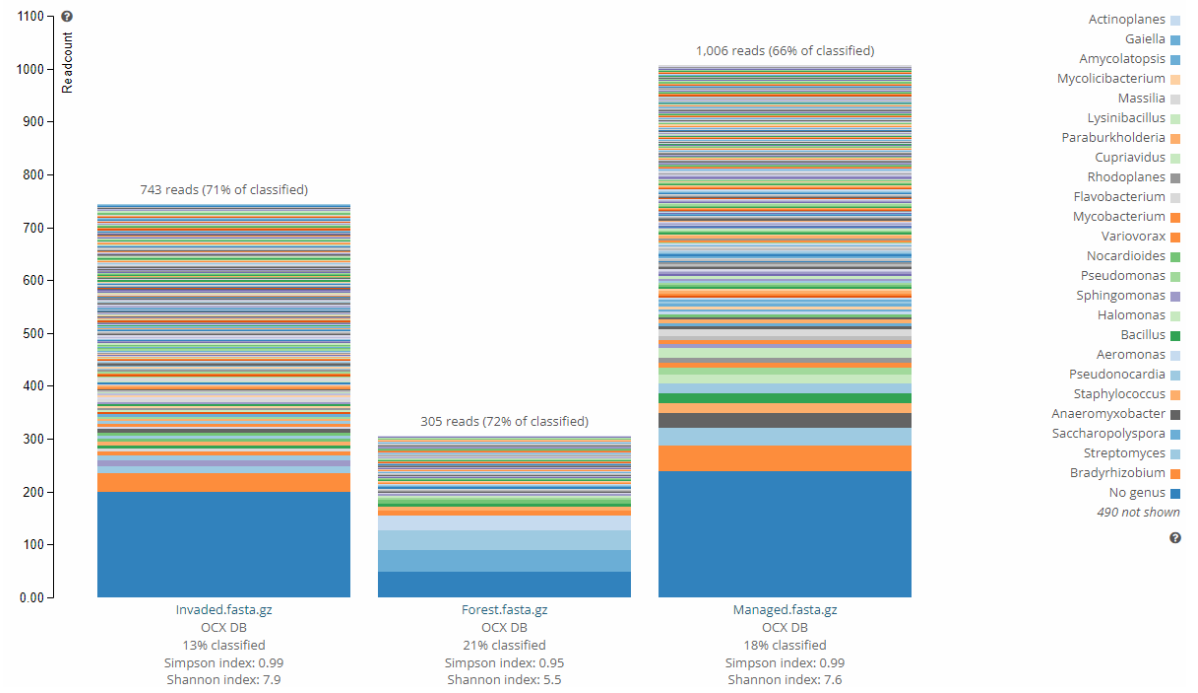


Figure 10.A: Comparison of Genus level bacterial diversity in three samples analysed. Abundance estimation and identification were based on One codex database.

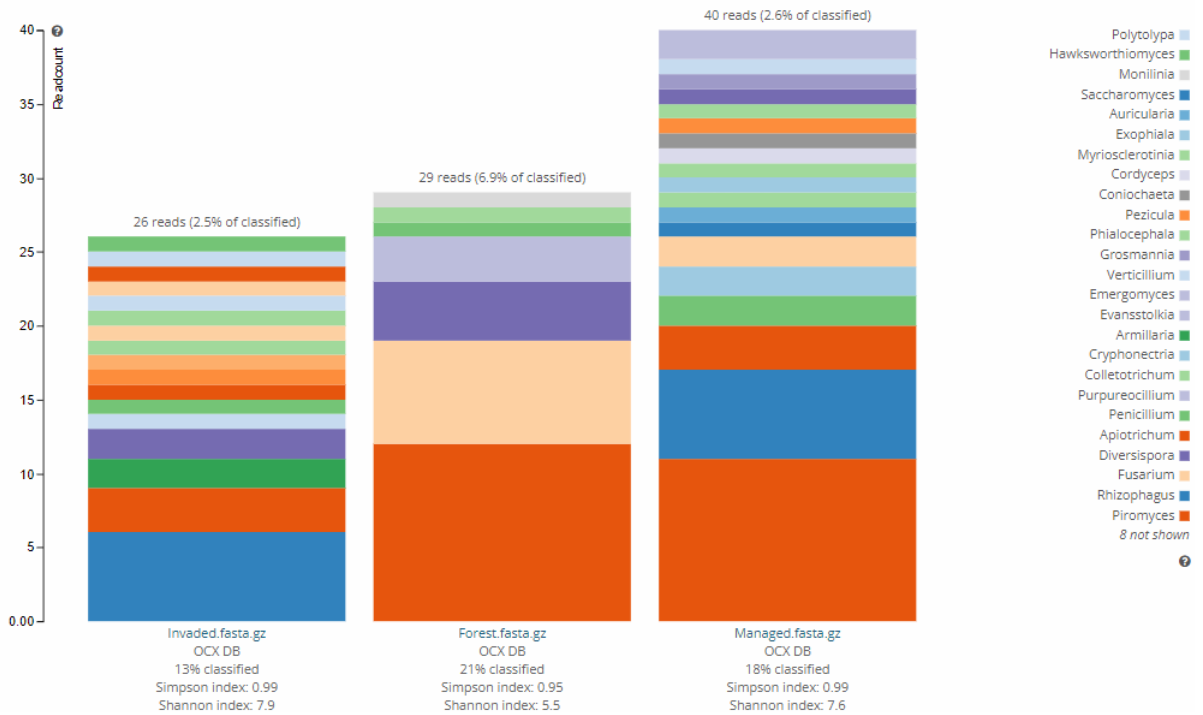


Figure 10.B: Comparison of Genus level fungal diversity in three samples analysed. Relative abundance estimation and identification was based on One codex database.

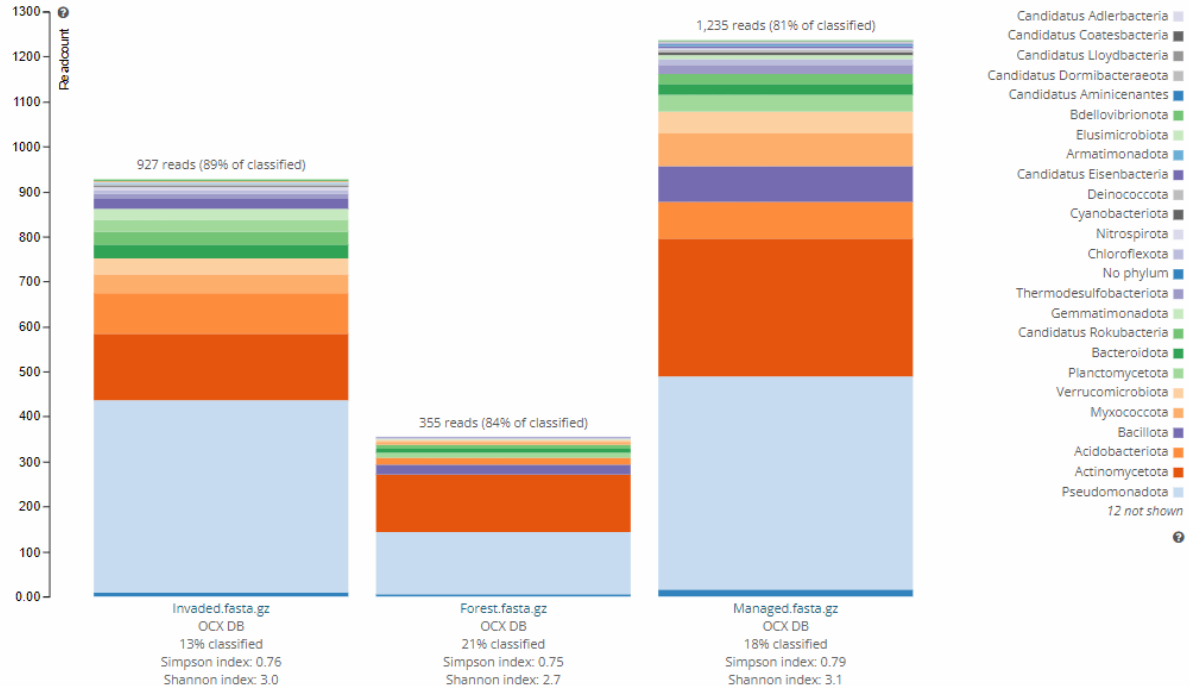


Figure 10.C: Comparison of Phylum level bacterial diversity in three samples analysed. Relative abundance estimation and identification were based on the One Codex database.

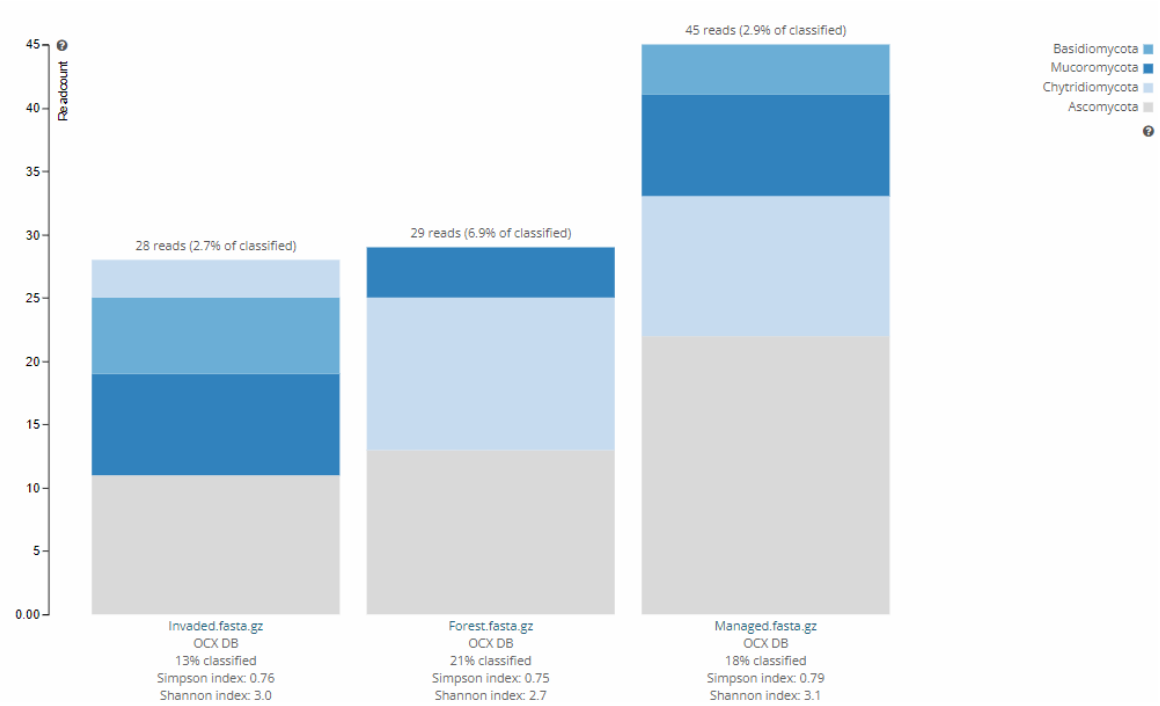


Figure 10.D: Comparison of Phylum level fungal diversity in three samples analysed. Relative abundance estimation and identification were based on the One Codex database.

Plant invasions notably impact soil microbial composition, altering carbon biomass to promote its growth and survival (Elsheikh et al., 2021). In turn, soil microbes affect the growth and survival o f invasive plants by influencing nutrient availability and uptake (Morgan et al., 2016). The microbi al composition of invaded, uninvaded forest, and managed soils exhibited noteworthy variations in the relative abundance of various microorganisms across different taxonomic levels, although the overall microbial compositions were largely similar. At the phylum level, the dominant phyla in al l three soil types were Pseudomonadota and Actinomycetota, consistent with findings from diverse habitats, including alpine soils, indicating their ecological prominence and adaptability (Hollister e t al., 2010; Wang et al., 2021). However, significant disparities were observed in specific taxa's rel ative abundance and dominance at lower taxonomic levels. Particularly, Pseudomonadota exhibite d significantly higher relative abundance in invaded soil, likely due to the lower pH of such soil en vironments, which favour the proliferation of Pseudomonadota (Yang et al., 2021; Zhang et al., 20 20).

In the fungal community, Ascomycota and Mucoromycota emerged as the predominant phyla, playing vital roles in decomposing organic matter and lignocellulose from plant residues (Frey et al., 2004; Beimforde et al., 2014). The higher relative abundances of Ascomycota and Mucoromycota in uninvaded forest soil were attributed to the abundance of dead leaf litter requiring degradation in the rhizosphere zone.

Diversity analysis revealed that microbial richness and diversity were significantly higher in invaded soil than uninvaded forest soil. This disparity was presumed to be influenced by root secretions, which selectively promote the growth of specific soil microflora (Grayston et al., 1998; Marilley and Aragno, 1999). The physicochemical properties of soil, notably pH and moisture content, were identified as crucial determinants shaping microbial community diversity, influenced by plant root secretions and microbial metabolism (Liu et al., 2020; Liu et al., 2021).

Significant differences in soil bulk density, moisture content, and pH were observed between invaded and uninvaded forest soil, impacting microbial diversity and richness. Moreover, low moisture content and organic carbon levels in managed soils impeded microbial growth and activity, reducing microbial diversity (Liu et al., 2020; Liu et al., 2021; Zhang et al., 2017). The variation in pH between invaded soils may be attributed to the root activity of *Senna spectabilis,* which alters rhizosphere pH, affecting nutrient availability and subsequently influencing microbial community structure and diversity (Yang and Liu, 2015; Wand and Jiang, 2022).

Relationships between soil microbial communities and environmental variables

We analysed the relationships between soil microbial communities and the environmental variables using canonical correspondence analysis (CCA). Bulk density, Moisture content, pH, Electrical conductivity and soil organic carbon were the environmental variables considered for the study. Analysis of ecological distance by ordination was conducted to understand the species composition at the genus level of bacteria in 3 different sites that were invaded (*Senna*), forest, and managed.

Unimodal distribution of species is assumed by CCA, which is more common in nature.

Influence of environmental factors on the bacterial community Canonical Correspondence analysis (CCA) (Figure 11.A-C) was used to determine the effects of physical and chemical parameters (Table 3.2) on the bacterial community distribution. All the soil physical and chemical properties (Table 3.2) were used for the CCA plot (Figure 11.A-C). The CCA plot indicated that the soil properties affected the composition of the bacterial genus level communities. The vector length of Bulk density (g/cm^3) (on axis 1) positively correlated with *Flavihumibacter* and *Pseudomonas*. On axis 2, the vector length of moisture content positively correlated with *Sphingomonas, Bradyrhizobium,* but negatively correlated with *Streptomyces, Saccharopolyspora* and *Aeromonas*. The vector length of Electrical conductivity positively related with *Bradyrhizobium* and for pH with *Staphylococcus* and *Pseudomonas*. Bulk density and organic carbon concentration increased gradually, while the influence of electrical conductivity, pH on quadrat distribution increasingly weakened. Invaded soil was affected by organic carbon.

In phylum level bacterial community, the vector length of Bulk density (g/cm^3) (on axis 1) positively correlated with Actinomycetota. On axis 2, the vector length of moisture content positively correlated with Pseudomonadota, Chloroflexota. The vector length of Electrical conductivity is positively related to Pseudomonadota and for pH with Chloroflexota.

In the genus-level fungal community, moisture content and electrical conductivity correlate positively with r*hizophagus irregularis*. The vector length of pH positively correlated with *Penicillium digitatum*. Organic carbon positively correlated with *Rhizophagus irregularis, Hymenoscyphus fructigenus, Exidia glandulosa* and *Polytolypa hystricis*

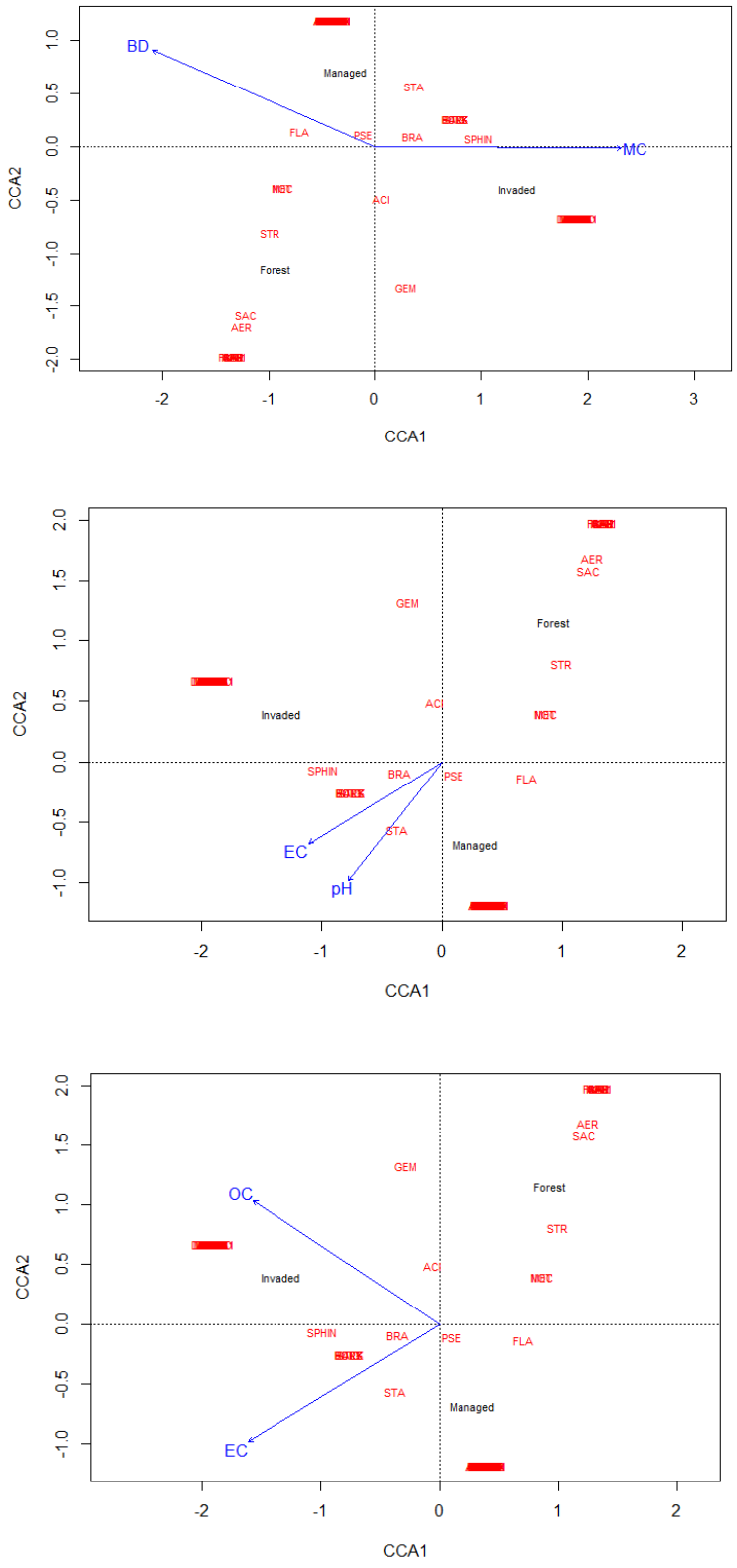


Figure 11.A: Canonical correspondence analysis (CCA) of the bacterial genus community distribution and soil properties (BD: Bulk density; MC: Moisture content; EC: Electrical conductivity; pH; OC: Organic carbon) of invaded, forest and managed soil

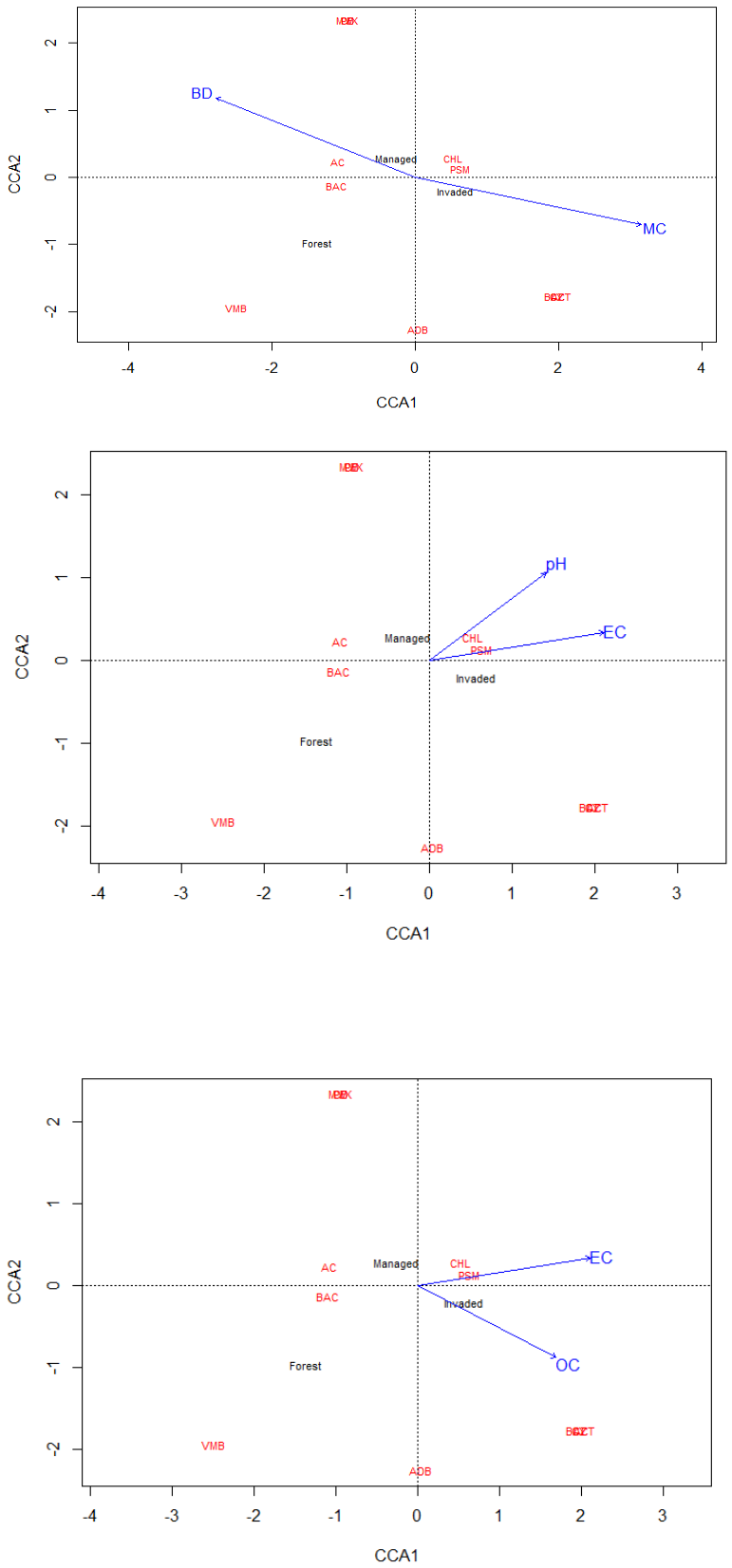


Figure 11.B: Canonical correspondence analysis (CCA) of the bacterial phylum community distribution and soil properties (BD: Bulk density; MC: Moisture content; EC: Electrical

conductivity; pH; OC: Organic carbon) of invaded, forest and managed soil

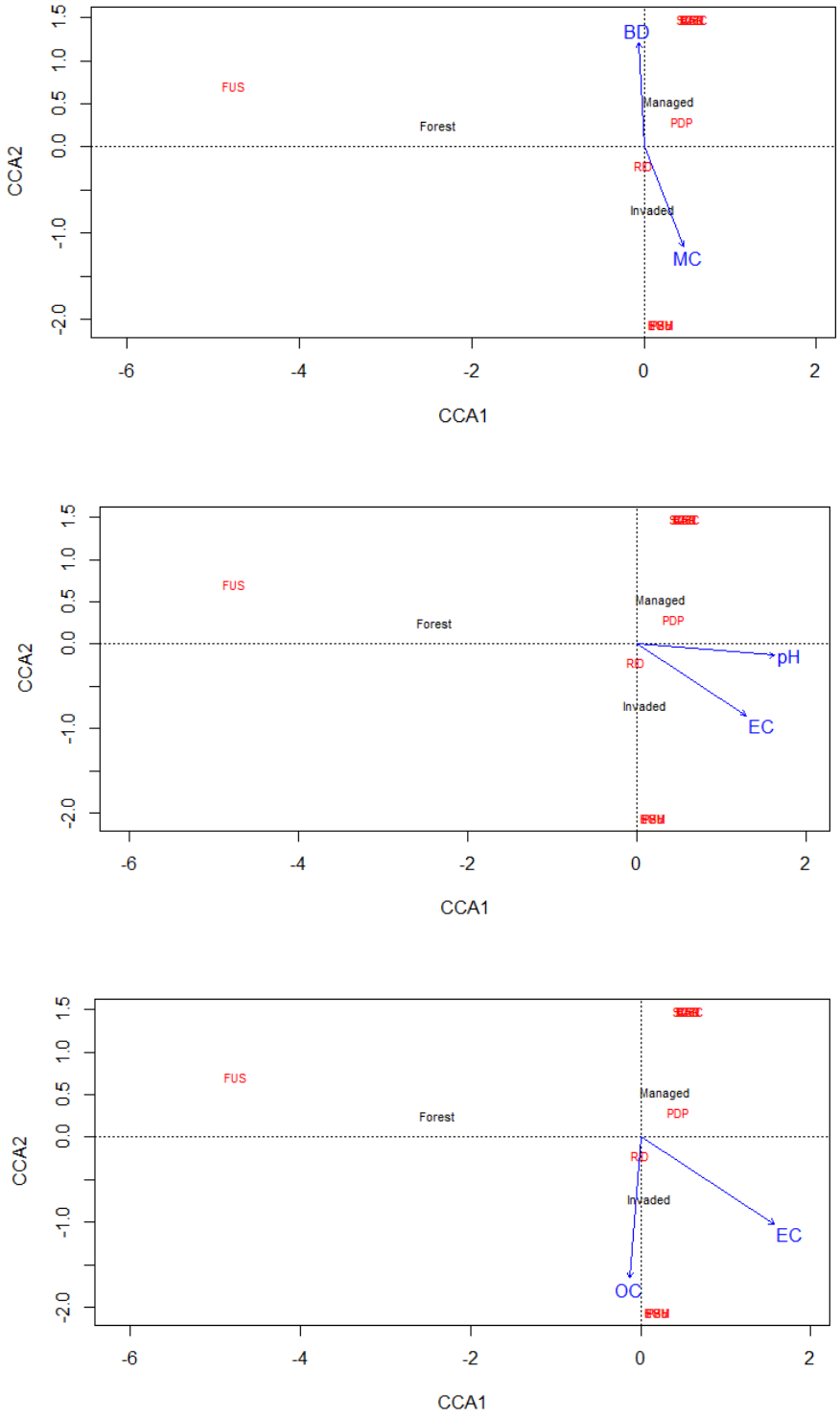


Figure 11.C: Canonical correspondence analysis (CCA) of the Fungal genus community distribution and soil properties (BD: Bulk density; MC: Moisture content; EC: Electrical conductivity; pH; OC: Organic carbon) of invaded, forest and managed soil

Our findings reveal significant differences in the structural diversity of the rhizosphere bacterial microbiome among invaded, uninvaded forest, and managed soils. Canonical Correspondence Analysis (CCA) indicates that soil pH, bulk density and moisture content exert greater influence on the microbial community structure of control soil. Prior research (Kalayu, 2019) indicates that soil pH is primarily influenced by the activity of Phosphate Solubilizing Microbes (PSMs), which enhance phosphate availability through the production of organic acids, consequently lowering soil pH (Kalayu, 2019). Studies have documented the potential for bacterial phosphate solubilization in rhizosphere soils (Chaudhari et al, 2020; Rahi et al., 2020) while the pH of control soil remains relatively stable. Our observations suggest that pH fluctuations are influenced by microbes and plant species, displaying a stronger association with managed soil. Additionally, moisture content in the soil has been reported to determine microbial community structure (Steres et al., 2008). Interestingly, our study identifies moisture as a significant determinant of bacterial community structure in the rhizosphere of invaded and uninvaded forest soil.

Our results reveal that the rhizosphere of the invasive *S. spectabilis* showed selective enrichment of bacterial communities from diverse phyla like Acidobacteria compared to its non-invasive congener and the managed soil. Acidobacteria are gram-negative bacteria which constitute important drivers of rhizosphere ecology and nutrient cycling with vast potential to alter plant growth and fitness through mechanisms like phosphate solubilization, secondary metabolite production, and antimicrobial synthesis, including chitinase production to control fungal phytopathogens (Sathya et al., 2017). Likewise, Chlorofexi, filamentous bacteria, are capable of producing a wide variety of exoenzymes, including chitinase (Kragelund et al., 2007), suggesting their likely role as fungal antagonists in the rhizosphere of *S. spectabilis*. Moreover, members of Firmicutes and Acidobacteria, with three- to four-fold increased abundance, respectively, in *S. spectabilis*, are known to enhance plant growth through heavy metal tolerance (Qamar et al., 2017) and phytohormone production (Kielak et al., 2016). Actinomycetota phylum bacteria rank among the

top five most prevalent organisms documented in soil samples. Actinomycetota plays a significant role in inhibiting *Rhizoctonia solani* in soil (Hou et al, 2018). Furthermore, from the soil with Rhizoctonia-suppressive properties, we isolated *Paenarthrobacter ureafaciens,* a member of the Actinomycetota phylum (Roy et al., 2020; Qi et al., 2022). *P. ureafaciens* is recognized as a producer of indole acetic acid and siderophores (Antenozio et al., 2021), and it possesses the capability to degrade herbicides (Zhang et al., 2022; Nunes et al., 2020). Consequently, Actinomycetota, particularly *P. ureafaciens,* could influence plant growth and microbial interactions within soil environments.

4.2.4. Isolation of bacterial and fungal isolates and their growth promoting factor

Total 12 bacteria and 9 fungi were isolated from the invaded, uninvaded forest and managed soil (Figure 12).

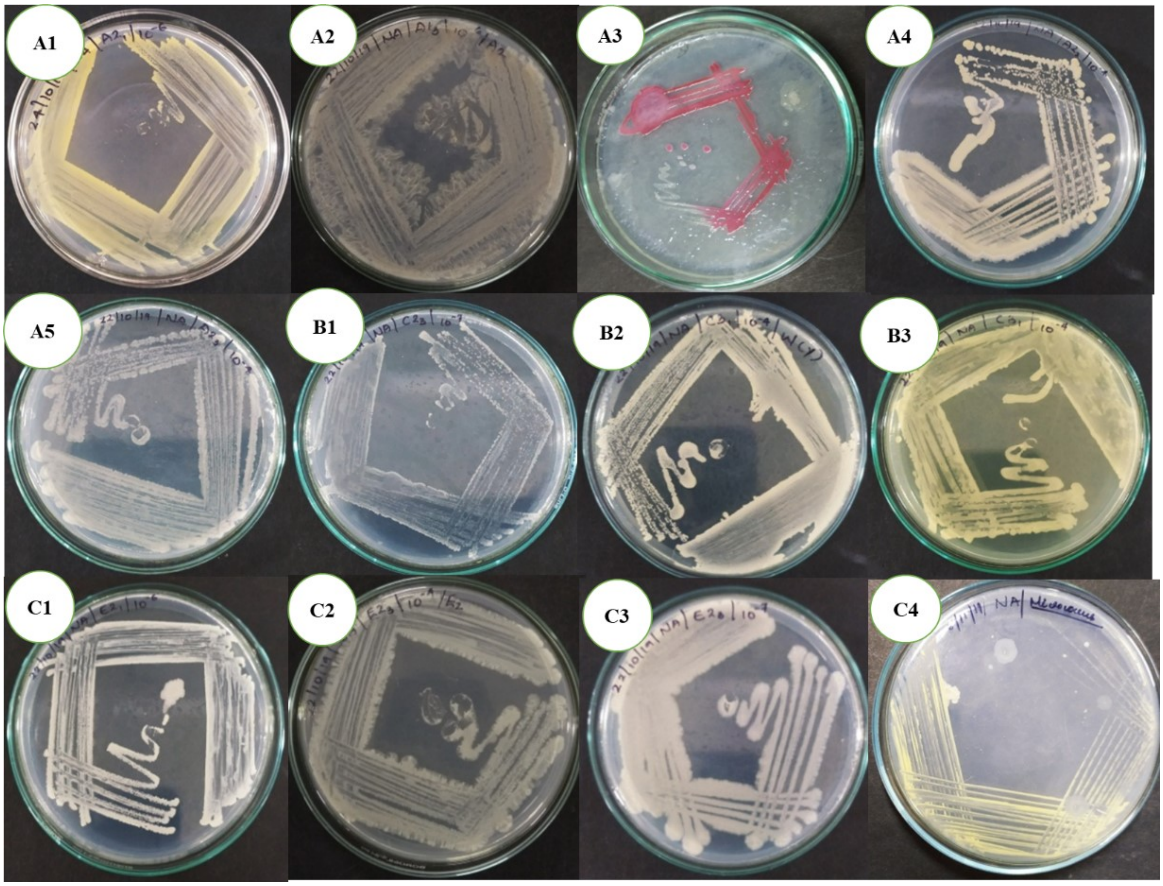


Figure 12: Streak plates showing isolated colonies from A1-4: *S. spectabilis* invaded forest soil; B1-3: Forest soil (uninvaded forest soil); C1-4: Managed soil

Bacterial isolate	Gram staining
A1	Gram positive cocci
A2	Gram negative short rods
A3	Gram negative short rods
A4	Gram positive rods
A5	Gram positive rods
B1	Gram positive cocci
B2	Gram negative short rods
B3	Gram negative short rods
C1	Gram positive cocci
C2	Gram positive long rods
C3	Gram positive rods in chains
C4	Gram variable cocci in tetrads

Table 3.3: Gram staining characteristics

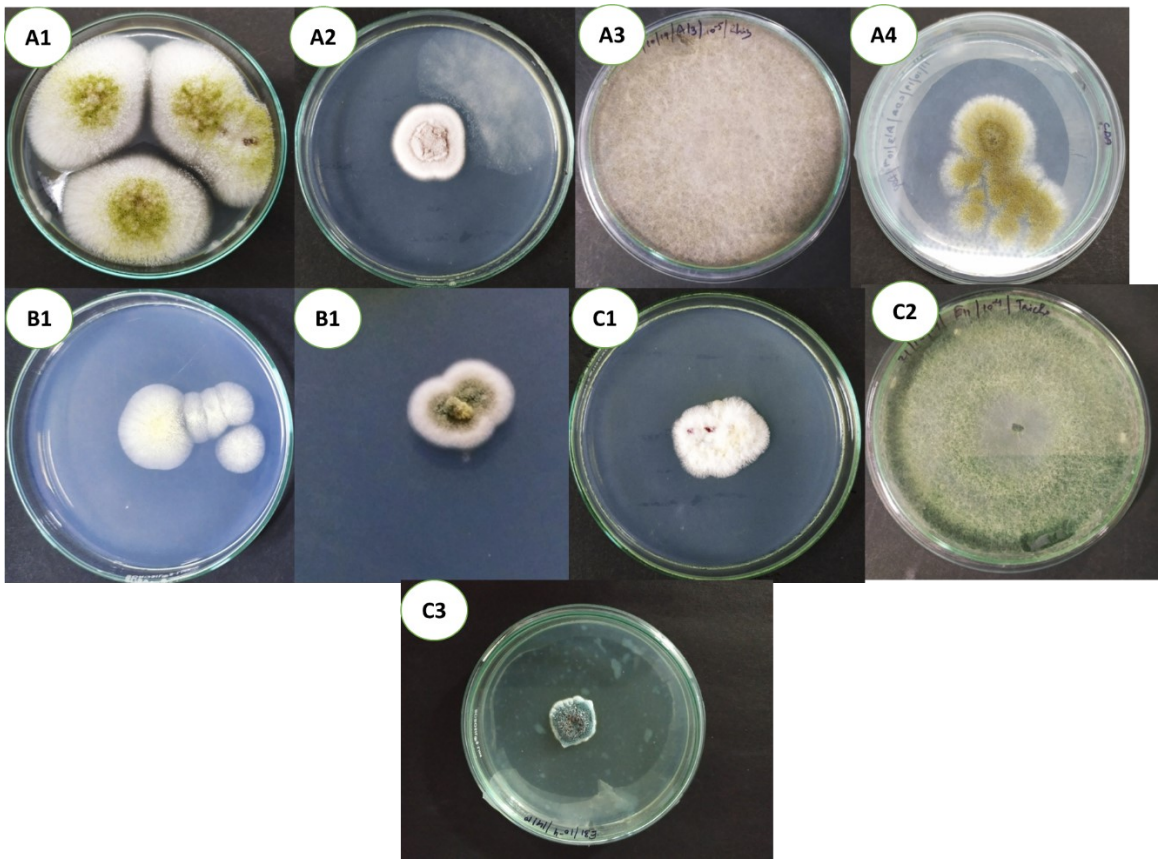


Figure 13: Fungal isolates from soil (Invaded forest soil - A1, A2, A3, A4 and A5; Uninvaded Forest soil – B1, B2 and B3; Managed soil – C1, C2, C3 and C4)

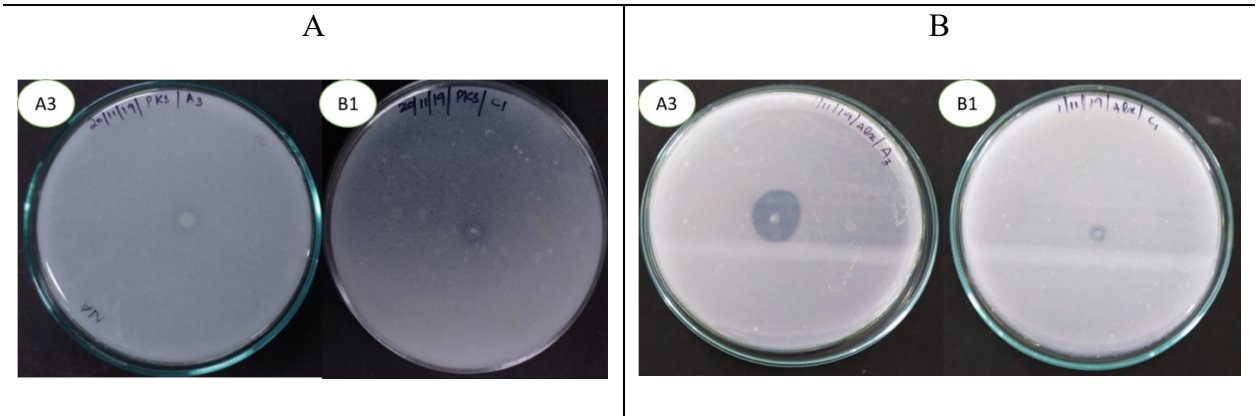


Figure 14: Bacteria isolates A. Phosphate solubilization and B. Potassium solubilization in A3 isolate (*Invaded forest soil*) and B1 (Uninvaded forest soil)

Screening of Plant Growth Promoting Bacteria and Fungi

All bacterial strains were screened for plant growth promoting activities, i.e., Ammonia production, IAA production, Potassium solubilisation and Phosphate solubilisation. The fungal isolates were screened for their following growth promoting factors, ie., Potassium solubilisation, Nitrate utilisation and Phosphate solubilisation

Bacterial isolate	Ammonia production	Potassium solubilisation	IAA production	Phosphate solubilisation
A1	+	-	+	-
A2	+	-	-	-
A3	+	+	+	+
A4	-	-	-	-
A5	-	-	-	-
B1	-	+	-	+
B2	-	-	-	-
B3	-	-	-	-
C1	-	-	-	-
C2	-	-	-	-
C3	-	-	-	-
C4	-	-	+	-

Table 3.4: Screening for plant growth promoting rhizobacteria

Fungal isolate	Potassium solubilisation	Utilisation of nitrates	Phosphate solublisation
A1 (*Aspergillus flavus*)	-	+	-
A2 (*Pencillium sp.)*	-	+	-
A3 (*Rhizopus sp.*)	-	+	-
A4 *(Aspergillus sp.)*	-	+	-
B1 *(Aspergillus sp.)*	-	+	-
B2 *(Aspergillus sp.)*	-	+	-
C1 *(Mycelia sterilia)*	-	-	-
C2 (*Trichoderma sp.)*	-	+	-
C3 (*Pencillium sp.)*	-	+	-

Table 3.5: Screening of plant growth promoting fungi

The Plant growth promoting rhizobacteria (PGPR) were identified from previously pure cultures bacterial isolates. The isolates were screened for ammonia production, phosphate solubilising activity, potassium solubilising activity and ability to produce plant growth hormone Indole-3-acetic acid (Auxins). Three bacterial isolates from invaded soil ie., A1, A2 and A3 exhibited ammonium production. Potassium solubilizing activity and Phosphate solubilizing activity was shown by both A3 (invaded soil) and B1 (uninvaded forest soil) bacterial isolates. A1, A3 (invaded soil) and C4 (managed soil) showed ability to produce plant growth hormone Indole-3- acetic acid (Auxins).

The plant growth promoting fungi (PGPF) were identified from previously pure cultured fungal isolates. The isolates were screened for phosphate solubilising activity, potassium solubilising activity and ability to produce plant growth hormone Indole-3- acetic acid (Auxins). No fungal isolates showed Phosphate and Potassium solubilisation. The fungus that can utilise nitrate can only grow on a medium that contains sodium nitrate as the only nitrogen source. All the fungal isolates showed visible growth on CDA except C1, which showed no growth on CDA, indicating a negative result for nitrate utilisation. The symbiotic relationship between plants and microbes is widely

observed in ecology. Host plants derive various advantages from this symbiosis, including enhanced nutrition availability (Ngwene et al., 2016; Bertolazi et al., 2019), increased yields (Xia et al., 2016), and improved tolerance to both abiotic and biotic stresses (Daneshkhah, Grundler & Wieczorek, 2018; Song et al., 2015).

The rhizosphere microorganisms are important in producing bioactive substances like antibiotics, enzymes, plant growth promoting factors (PGPF) etc. Some of the rhizosphere microorganisms, including bacteria and fungi, can promote plant growth by providing plant growth promoting factors, and they are called plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR). The PGPF and PGPR can also help to escape from the plant from soil borne pathogens. Plant growth promoting fungi are sets of heterogeneous organisms that are non- pathogenic and promote plant growth and establishment. Nitrogen, phosphorous and potassium are the major macronutrients involved in the growth and development of plants. Certain fungal species can fight against phytopathogens, solubilise Phosphorous, Potassium etc., and produce Indole-3- acetic acid (Auxin), thus promoting plant growth. Plants and fungi produce auxins, which are important as plant growth hormones. There is a great role in plant-microbe interaction by IAA. The plant growth promoting fungi can control the plant pathogens through antibiosis or antagonism, mycoparasitism, predation etc. In this study, PGPR and PGPF screening was carried out on all the bacterial and fungal isolates obtained from five soil samples.

Ammonia production plays an important role in the nitrogen fixation process. As ammonia is useful for plants directly or indirectly, the ammonia production by plant growth promoting bacteria influences plant growth indirectly (Geetha et al., 2014). The ability of bacteria to produce ammonia was observed in 3 bacterial isolates. As a secondary metabolite product of the plant growth promoting bacteria, this compound has a role in antagonistic effect (Jacques et al., 1993).

Potassium is the third important plant nutrient. Potassium is an essential macronutrient for plant growth and plays a significant role in activating several metabolic processes including Protein

synthesis, Photosynthesis, Enzymes, and Resistance to diseases and insects. A3 bacterial isolate exhibited three of the growth promoting factors.

This study found that three isolates tested positive for their ability to synthesize IAA, a capability observed in certain rhizospheric microbes. This discovery aligns with previous findings by Palazzini et al. (2018) and Bereika et al. (2020), suggesting a potential correlation between bacteria-produced IAA and enhanced plant growth following colonization (Shi et al. 2009).

4.3.4. Antagonistic activity

The *in vitro* antagonistic effect of the invaded forest soil isolates was evaluated against uninvaded forest soil, and soil isolates were managed by dual culture, investigating the activity of diffusible compounds. The percentage inhibition of radial growth was recorded in Table 3.6 according to the observed results. An interesting inhibitory effect was observed for one of the five fungi. The C1 showed no inhibitory effect or moderate inhibition (<25 mm). Table 3.6 presents the results recorded for antagonistic activity with efficacy against at least two fungi isolated from invaded soil. The invaded forest soil isolates exhibit an inhibitory effect on uninvaded forest soil and managed soil, with some exceptions. B1, B2 and C2 against A4 exhibited strong antagonistic activity.

C2 showed the highest antagonistic activity in vitro, inhibiting A4 tested with an inhibition diameter >25 mm. Moderate inhibition (15–25 mm) against most invaded soil isolates was observed for B2, except for A3 (low inhibition, 5–15 mm). B1 was observed with only low antagonist activity against A1, A2 and A3, while for A4, moderate inhibitory activity was observed. In most cases, soil fungi did show antagonism for space but not because of the release of toxic substances or competition.

The inhibition percentages calculated for fungal isolates ranged from 0% to 70.6% (Table 3.6), showing that all fungal isolates from invaded soil had some inhibitory action over the growth of fungal isolates from uninvaded forest and managed soil. As expected from the interaction types found, C2, B1 and B2 demonstrated a greater ability to inhibit the growth of A4 in the direct inhibition test, with percentages of 70.6%, 66.6% and 61.1%, respectively.

Fungal isolates	Percentage inhibition of radial growth (PIRG)			
	A1(control 8cm)	**A2(control 6.5cm)**	**A3 (control 8cm)**	**A4 (control 8cm)**
B1	0	0	0	66.6
B2	25	55.4	0	61.1
C1	46.25	36	0	57.7
C2	58.75	43.75	37.5	70.6
C3	37.5	10.1	0	55.5

Table 3.6. Antagonistic activity between invaded forest soil isolates with uninvaded forest soil and managed soil

	A1	**A2**	**A3**	**A4**
B1	-	-	-	++
B2	++	++	-	++
C1	++	++	-	++
C2	++	++	++	+++
C3	+	-	-	++

Table 3.7: In vitro antagonistic activity between invaded forest soil isolates with uninvaded forest soil and managed soil. (+++, high inhibition, > 25 mm; ++, moderate inhibition, (15–25 mm); + low inhibition, (5–15 mm); −, no inhibition.)

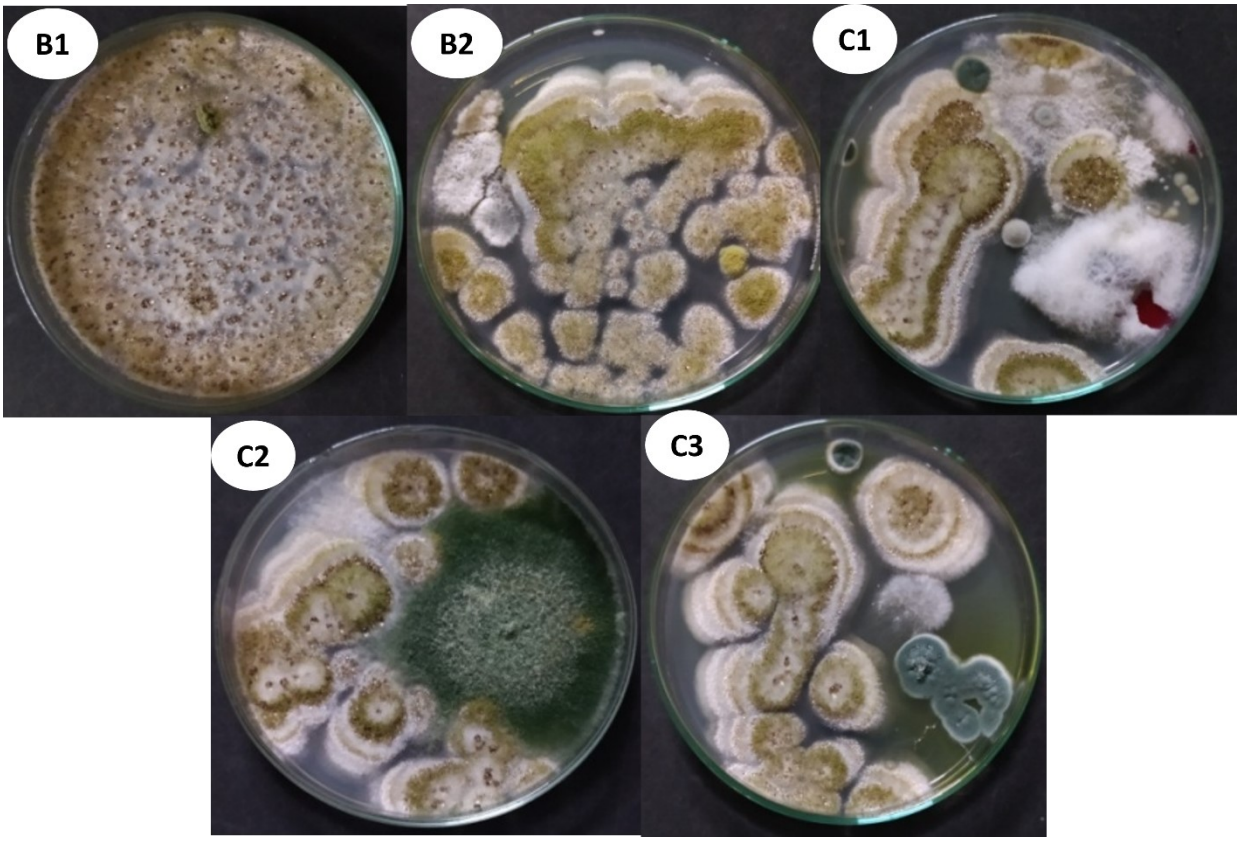


Figure 15.A: Antagonistic activity checked for fungal isolates against A1

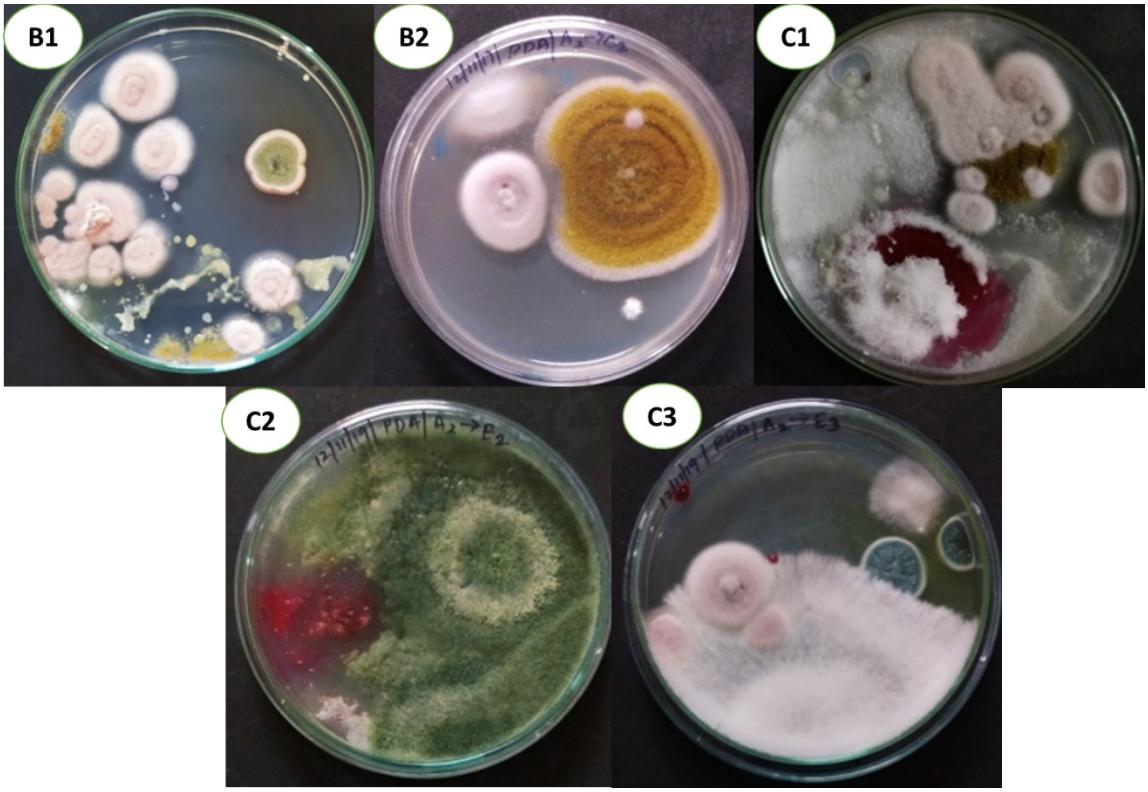


Figure 15.B: Antagonistic activity checked for fungal isolates against A2

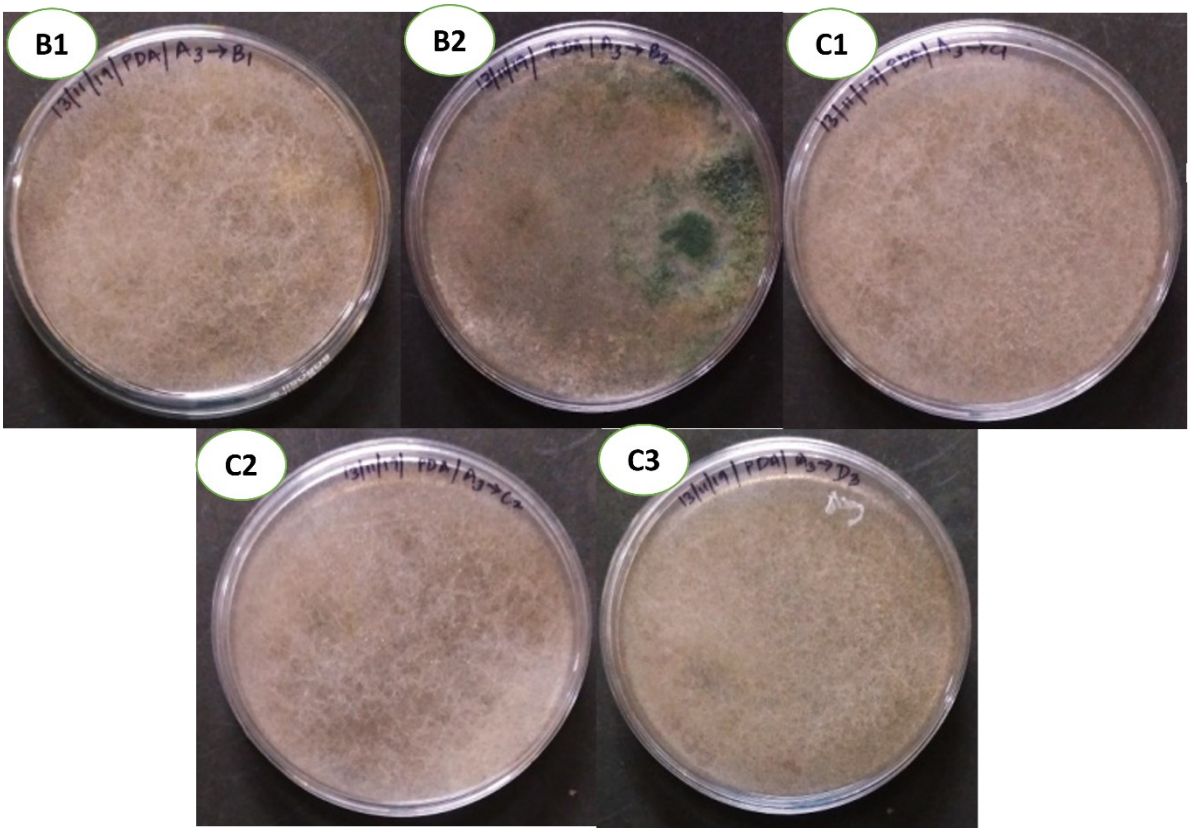


Figure 15.C: Antagonistic activity checked for fungal isolates against A3

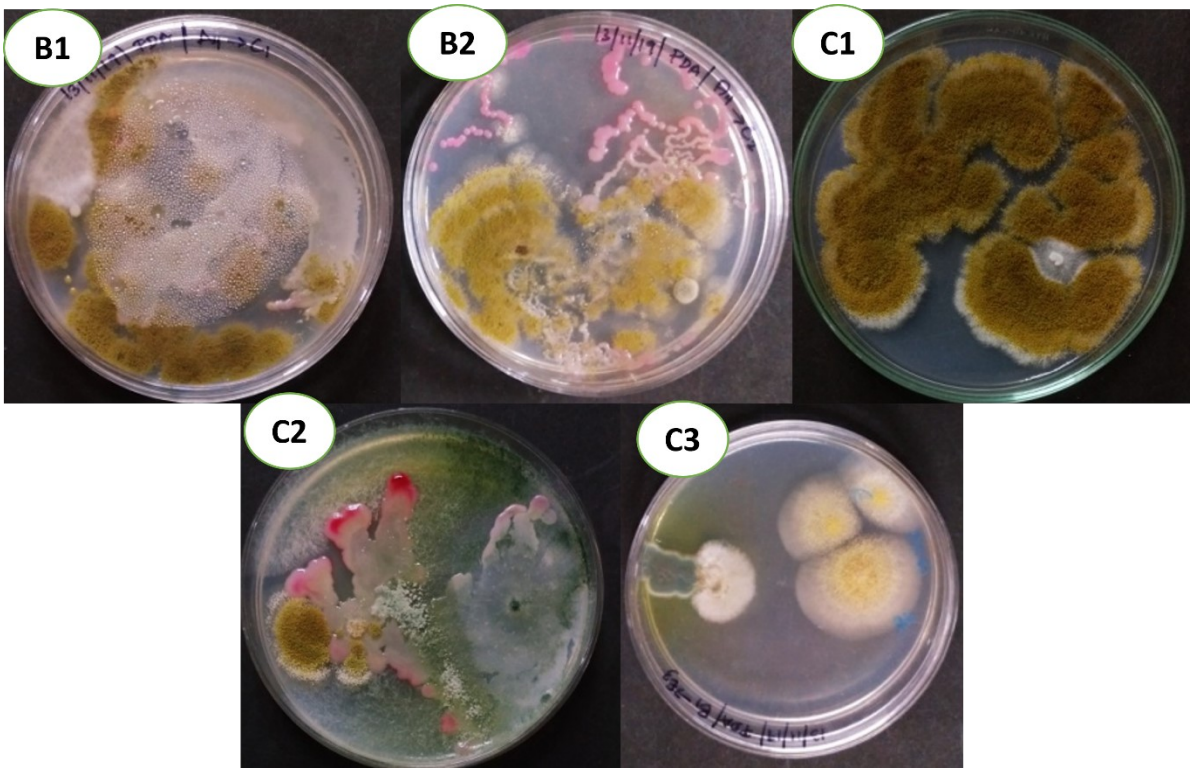


Figure 15.D: Antagonistic activity checked for fungal isolates against A4

Plants frequently host numerous fungi that do not induce disease symptoms. Some of these fungi can positively impact plant growth by supplying vital nutrients, thereby indirectly bolstering plant resilience against pathogens. Others may directly shield plants from pathogens through antagonistic interactions (Wang et al., 2013), as noted by Harrison (2005). Inter-microbial competition is prevalent in various natural ecosystems, often stemming from limited nutrients and space. This competition can lead to the diminished growth of certain species and a shift in microbial community composition (Bell, Callender, Whyte, & Greer, 2013), which, in turn, can influence plant growth due to varying effects exerted by different components of the microbial community (Bever, Platt, & Morton, 2012). The study conducted by Gabriel Berg and Kornelia (2009) showed that there are high levels of bacteria and fungi associated with the roots of invaded plants that can act as antagonistic to other microbes in the soil, giving protection to the plant and possibly resulting in increased level of invasion by plants. The rhizosphere microbes are important in producing bioactive substances like enzymes and antibiotics, which gives them antagonistic activity against other microbes.

CHAPTER 5

SUMMARY AND CONCLUSION

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Invasive plant species represent a significant challenge to global biodiversity conservation, and understanding the mechanisms that underlie their success is essential for effective management and control. While the hypotheses discussed provide valuable insights into the factors that contribute to the success of invasive plants, the complex and dynamic nature of invasive species means that a multifaceted approach is necessary for their effective management and control. Understanding the mode of action and physiological pathways of allelochemicals produced by invasive alien plants can aid in developing effective management strategies to control the spread of these plants and minimize their impact on natural ecosystems and agricultural production.

Effect of allelochemicals of *S. spectabilis* on seed germination and seedling growth of native plant species

The study aimed to investigate the allelopathic effects of *S. spectabilis* leaf extract on seed germination and seedling growth of five native species: *Ailanthus tryphysa* (AT), *Pongamia pinnata* (PP), *Tectona grandis* (TG), *Hopea parviflora* (HP), and *Dendrocalamus strictus* (DS). Different concentrations of *S. spectabilis* leaf extract were used in the study.

The results indicated a concentration-dependent increase in allelopathic inhibition on seed germination. Particularly, 200 mg ml^{-1} concentration significantly inhibited the germination index. The analysis revealed significant effects of treatment, plant species, and their interaction on seed germination growth. Different concentrations of leaf extracts were categorized into distinct groups based on their impact on seed germination, with the highest inhibition observed at 200 mg ml^{-1}. The interaction effects were also significant, with specific combinations of plant species and leaf extract concentrations resulting in varying degrees of inhibition of seed germination growth.

Similarly, significant effects of treatment, species, and their interaction were observed on plumule

and radicle growth. The highest inhibition occurred at 200 mg ml^{-1} concentration for plumule and radicle growth. Specific combinations of plant species with certain concentrations of leaf extract exhibited the highest inhibition of plumule and radicle growth.

The study also highlighted the importance of seed germination tests in assessing allelopathy, with the germination rate decreasing with increasing extract concentrations. Additionally, it was observed that lower extract concentrations stimulated plumule growth while higher concentrations inhibited it. Radicle growth was significantly reduced in all treatments.

Overall, the study emphasized the concentration-dependent effects of allelopathic compounds on seed germination and seedling growth, with higher concentrations inhibiting growth performance and competitiveness of native species. Understanding these dynamics is crucial for managing the impacts of invasive plants on biodiversity. The findings align with previous research indicating a stimulatory effect at lower concentrations and an inhibitory effect at higher concentrations of allelopathic compounds.

Response of antioxidant activity to the allelochemicals of *S. spectabilis*

The study investigated the impact of aqueous extracts from *S. spectabilis* on the antioxidant enzyme activities across various native species, revealing intricate dynamics in enzyme responses. Superoxide dismutase (SOD) levels peaked at 50 mg ml^{-1} but declined at higher concentrations, with significant variations among species. Ascorbate peroxidase (APX) activity varied notably across species and extract concentrations, increasing AT and HP. Catalase (CAT) activity displayed species-specific increases in HP and AT, while polyphenol oxidase (PPO) activity varied widely among species and extract concentrations, with AT exhibiting the highest activity. The findings elucidate the differential responses of native species to *S. spectabilis* extracts, indicating complex interactions between extract concentration, native species, and antioxidant enzyme activities.

The study revealed significant influences of extract concentration, native species, and their interactions on the accumulation of SOD in native species' leaves. SOD levels increased up to 50

mg ml-1 but decreased at higher concentrations, with notable variations among species. AT displayed the highest SOD accumulation, while HP exhibited the lowest, underscoring species-specific responses. Similarly, APX activity varied significantly across species and extract concentrations, with notable increases in AT and HP. CAT activity showed species-specific increases in HP and AT, with no significant variations across concentrations. In contrast, PPO activity exhibited wide variations among species and concentrations, with AT displaying the highest activity.

Furthermore, the study explored the influence of *S. spectabilis* extracts on proline accumulation, metabolic activity, and lipid peroxidation. Proline accumulation increased with extract concentration, reaching saturation at 100 mg ml-1, followed by a decline at higher concentrations. Different species exhibited distinct proline accumulation patterns, indicating species-specific responses. Metabolic activity, reflected by formazan production, varied significantly across species and extract concentrations, with notable increases observed in DS and HP at higher concentrations. Lipid peroxidation, assessed through MDA accumulation, exhibited variations across species and concentrations, with higher values observed in PP and AT. The findings underscore the complex interplay between extract concentration, native species, and physiological responses, highlighting the diverse strategies native species employ to cope with allelochemical stress.

Additionally, Pearson's correlation coefficient analysis revealed significant correlations between antioxidant enzymes and other physiological parameters. CAT showed a positive correlation with SOD, while APX exhibited a positive correlation with formazan. Proline positively correlated with MDA, indicating potential associations between antioxidant enzyme activities and other physiological responses. These findings provide insights into the underlying mechanisms of antioxidant defence and oxidative stress tolerance in native species exposed to allelochemicals, emphasizing the importance of considering multiple physiological parameters in assessing plant responses to environmental stressors.

Changes in soil physical, chemical and microbial community due to *S. spectabilis*

Invasive plant species can have significant impacts on soil microbial communities, altering microbial diversity, structure, and function. These impacts can in turn influence microbial chemotaxis, potentially providing invasive species with a competitive advantage in the ecosystem. However, the study of these interactions may also provide opportunities for developing novel control strategies for invasive species and insights into the mechanisms by which ecosystems adapt to novel conditions. Despite the potential applications of enhancing the antagonistic activity of microbes in response to invasive plant species, there are several limitations and challenges to using this phenomenon in managing invasive species. One of the limitations is the specificity of the microbial community to the invasive plant species. The enhancement of antagonistic activity may not occur in response to all invasive plant species, and the microbial community may not be effective against all invasive species. Another challenge is the potential negative effects of the microbial community on non-target species. The use of microbial communities as biocontrol agents may have unintended consequences, such as the suppression of beneficial microbes or the alteration of the ecological balance of the ecosystem. Invasive plant species can have significant effects on microbial communities in soil, leading to the enhancement of the antagonistic activity of microbes. Various mechanisms, including the production of antifungal compounds, alteration of microbial community structure, and production of chitinases can mediate the enhancement of antagonistic activity. The enhancement of antagonistic activity has the potential to be applied in the management of invasive plant species using biocontrol agents. However, further research is needed to investigate the specificity of the microbial community to invasive plant species and to address the potential negative effects of the microbial community on non-target species.

The analysis of phytoconstituents in the rhizospheric soil of *S. spectabilis* using Gas Chromatography-Mass Spectrometry (GC-MS) provides valuable insights into the chemical composition of the soil and its potential effects on neighbouring plants. The identified

phytoconstituents, particularly those known for their allelopathic properties, can influence the growth and development of surrounding vegetation. Among these compounds, 2,6-Di-Tert-Butyl-4-Methylphenol (BHT) emerged as a major component in the rhizospheric soil, indicating its prevalence and potential significance in mediating allelopathic interactions.

The allelopathic effects of these compounds, including BHT, were further elucidated through subsequent analyses, which revealed a marked decrease in soil urease and catalase activity upon BHT introduction. These findings suggest a discernible inhibition of soil metabolic enzyme activity, indicating the potential for these compounds to alter soil microbial communities and nutrient cycling processes. Such alterations in soil enzymatic activity can have cascading effects on soil fertility, plant nutrient uptake, and overall ecosystem functioning.

Additionally, comparing soil physiochemical properties between invaded and uninvaded forest soils revealed significant differences, particularly in bulk density and pH levels. The invaded forest soil exhibited higher bulk density, indicative of potential soil compaction from invasive plant species like *S. spectabilis*. Soil pH variations were also observed, with invaded forest soil displaying lower pH levels than uninvaded forest soil. These differences in soil pH have important implications for microbial diversity and community composition, as soil pH influences the availability of nutrients and shapes microbial community structure.

Previous studies have highlighted the importance of soil pH in shaping bacterial diversity across various environments. Soil pH influences nutrient availability and, consequently, the physiology and growth of bacterial communities. The observed variations in bacterial community composition between invaded and uninvaded forest soils underscore the influence of invasive plant species on soil microbial communities. The rhizosphere, the region of soil influenced by plant root exudates, harbours distinct microbial communities compared to bulk soils, attributed to elevated levels of organic exudates from plant roots. This underscores the role of plants in shaping microbial diversity and selection in the rhizosphere by providing carbohydrates, aromatic compounds, and amino acids.

Metagenomic analysis revealed significant differences in microbial communities among invaded, uninvaded forest, and managed soils. The invaded soil exhibited higher microbial richness and diversity than uninvaded forest soil, likely influenced by root secretions selectively promoting the growth of specific soil microflora. Soil physicochemical properties such as pH and moisture content were identified as crucial determinants shaping microbial community diversity, influenced by plant root secretions and microbial metabolism. Additionally, soil microbial communities exhibited selective enrichment of specific taxa in response to invasive plant species like *S. spectabilis*, highlighting the complex interplay between plants and soil microbes.

Canonical Correspondence Analysis (CCA) was employed to elucidate the relationships between soil microbial communities and environmental variables. The analysis considered bulk density, moisture content, pH, electrical conductivity, and soil organic carbon as environmental variables. The CCA plots indicated that soil properties significantly influenced the composition of bacterial genus-level communities, with bulk density positively correlated with certain bacterial taxa and moisture content showing positive and negative correlations with different bacterial groups.

Furthermore, *in vitro* antagonistic assays demonstrated the inhibitory effects of invaded forest soil isolates on fungi isolated from uninvaded forest and managed soils. These findings highlight the potential role of rhizosphere microbes in mediating plant-microbe interactions and ecosystem dynamics. The antagonistic activity observed suggests that rhizosphere microorganisms, including bacteria and fungi, play a crucial role in producing bioactive substances such as enzymes and antibiotics, which can inhibit the growth of other soil microbes, including pathogens.

In conclusion, the comprehensive analysis of soil physiochemical properties, microbial diversity, and plant-microbe interactions in invaded and uninvaded forest soils provides valuable insights into the ecological impacts of invasive plant species like *S. spectabilis.* These findings contribute to our understanding of soil dynamics and resilience in the face of biological invasions, highlighting the importance of considering soil microbial communities in invasive species

management and ecosystem restoration efforts. Further research in this area is warranted to unravel the complex interactions between invasive plants, soil microbes, and ecosystem processes, ultimately informing more effective management strategies for invasive species control and conservation of native biodiversity.

REFERENCES

References

Abd El-Gawad, A. M., El-Amier, Y. A., & Bonanomi, G. (2018). Essential oil composition, antioxidant and allelopathic activities of *Cleome droserifolia* (Forssk.) Delile. *Chemistry & biodiversity*, 15(12), e1800392.

Abhilasha, D., Quintana, N., Vivanco, J., & Joshi, J. (2008). Do allelopathic compounds in invasive Solidago canadensis sl restrain the native European flora?. *Journal of Ecology*, *96*(5), 993-1001.

Ábrahám, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. *Plant stress tolerance: methods and protocols*, 317-331.

Adams, Sheherezade N., Engelhardt, Katharina A. M. (2009). Diversity declines in *Microstegium vimineum* (Japanese stiltgrass) patches. *Biological Conservation*, 142(5), 1003-1010.

Aebi, H. (1984). Catalase in vitro. *Methods Enzymol*. Academic Press 105, 121–126.

Agbodjato, N. A., Noumavo, P. A., Baba-Moussa, F., Salami, H. A., Sina, H., Sèzan, A., Bankolé, H., Adjanohoun, A. & Baba-Moussa, L. (2015). Characterization of potential plant growth promoting rhizobacteria isolated from Maize (Zea mays L.) in central and Northern Benin (West Africa). *Applied and Environmental Soil Science*, *2015*(1), 901656.

Aguilera, A. G., Alpert, P., Dukes, J. S., & Harrington, R. (2010). Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. *Biological Invasions*, 12(5), 1243-1252.

Aguilera, A. G., Alpert, P., Dukes, J. S., & Harrington, R. (2010). Impacts of the invasive plant Fallopia japonica (Houtt.) on plant communities and ecosystem processes. *Biological invasions*, *12*, 1243-1252.

Ain-Lhout, F., Zunzunegui, F.A., Diaz Barradas, M.C., Tirado, R., Clavijio, A., Garcia Novo, F. (2001). Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. *Plant Soil.* 230, 175–183.

AJ, Walkley & Black, I.. (1934). An Examination of the Degtjareff Method for Determining Soil Organic Matter, and A Proposed Modification of the Chromic Acid Titration Method. Soil Science. 37. 29-38.

Ajayi, O. E., Balusu, R., Morawo, T. O., Zebelo, S., & Fadamiro, H. (2015). Semiochemical modulation of host preference of *Callosobruchus maculatus* on legume seeds. *Journal of Stored Products Research*, *63*, 31-37.

Alam, S., Khalil, S., Ayub, N., & Rashid, M. (2002). In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *Int J Agric Biol*, *4*(4), 454-458.

Aliferis, K. A., & Jabaji, S. (2011). Metabolomics–A robust bioanalytical approach for the discovery of the modes-of-action of pesticides: A review. *Pesticide Biochemistry and physiology*, *100*(2), 105-117.

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, 105:11512-11519.

Allison, S. D., & Vitousek, P. M. (2004). Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. *Biotropica*, *36*(3), 285-296.

Allison, V. J., Yermakov, Z., Miller, R. M., Jastrow, J. D., & Matamala, R. (2007). Using landscape and depth gradients to decouple the impact of correlated environmental variables on soil microbial community composition. *Soil Biology and Biochemistry*, *39*(2), 505-516.

Al-Rowaily, S. L., Abd-ElGawad, A. M., Assaeed, A. M., Elgamal, A. M., Gendy, A. E. N. G. E., Mohamed, T. A., ... & Elshamy, A. I. (2020). Essential oil of *Calotropis procera*: Comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. *Molecules*, 25(21), 5203.

Alshehri, M. M., Quispe, C., Herrera-Bravo, J., Sharifi-Rad, J., Tutuncu, S., Aydar, E. F., ... & Cho, W. C. (2022). A review of recent studies on the antioxidant and anti-infectious properties of Senna plants. *Oxidative Medicine and Cellular Longevity*, *2022*.

Amare, T. (2018). Allelopathic effect of aqueous extracts of parthenium (Parthenium hysterophorus L.) parts on seed germination and seedling growth of maize (Zea mays L.). *Journal of Agriculture and Crops*, *4*(12), 157-163.

Amoo, A. E., & Babalola, O. O. (2017). Ammonia-oxidizing microorganisms: key players in the promotion of plant growth. *Journal of soil science and plant nutrition*, *17*(4), 935-947.

Anjanadevi, I.P., John, N.S., John, K.S., Jeeva, M.L., Misra, R.S., (2016). Rock inhabiting potassium solubilizing bacteria from Kerala, India: characterization and possibility in chemical K fertilizer substitution. *J. Basic Microbiol.* 56, 67–77.

Anoop, N. R., & Ganesh, T. (2020). The forests and elephants of Wayanad. *Current Science*, *118*(3), 362-367.

Anoop, N. R., Sen, S., Vinayan, P. A., & Ganesh, T. (2022). Native mammals disperse the highly invasive *Senna spectabilis* in the Western Ghats, India. *Biotropica*, *54*(6), 1310-1314.

Antenozio, M. L., Giannelli, G., Marabottini, R., Brunetti, P., Allevato, E., Marzi, D., Capobianco, G., Bonifazi, G., Serranti, S., Visioli, G. and Stazi, S.R (2021). Phytoextraction efficiency of *Pteris vittata* grown on a naturally As-rich soil and characterization of As-resistant rhizosphere bacteria. *Scientific Reports*, *11*(1), 6794.

Antoun, H. (2012). Beneficial microorganisms for the sustainable use of phosphates in agriculture. *Procedia Engineering*, *46*, 62-67.

Aon, M. A., & Colaneri, A. C. (2001). II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied soil ecology*, *18*(3), 255-270.
Aon, M. A., & Colaneri, A. C. (2001). II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied soil ecology*, *18*(3), 255-270.
Apel, K.,& Hirt, H., (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol*., 55, 373-399.

APFISN KFRI (2014) Final report on control and management of destructive forest invasive species in South Asian Natural and plantation forests. TCP/RAS/3408

Arianoutsou M, Bazos I, Christopoulou A, Kokkoris Y, Zikos A, Zervou S, Delipetrou P, Cardoso AC, Deriu I, Gervasini E, & Tsiamis K (2021) Alien plants of Europe: introduction pathways, gateways and time trends. PeerJ 9:e11270.

Aronson, M. F. J., & Handel, S. N. (2011). Deer and invasive plant species suppress forest herbaceous communities and canopy tree regeneration. Natural Areas Journal, 31(4), 400-407.

Arora, S., Kumar, G., & Meena, S. (2017). GC-MS analysis of bioactive compounds from the whole plant hexane extract of *Cenchrus setigerus* Vahl. *Pharma Science Monitor,* 8(4), 137-46.

Ashton, I. W., Hyatt, L. A., Howe, K. M., Gurevitch, J., & Lerdau, M. T. (2005). Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. *Ecological Applications*, 15(4), 1263-1272.

Awasthi, R., Tewari, R., Nayyar, H., (2011). Synergy between plants and P-solubilizing microbes in soils: effects on growth and physiology of crops. Int. Res. J. Microbiol. 2, 484–503.

Azevedo Neto, A. D., Prisco, J. T., Enéas Filho, J., Abreu, C. D., & Gomes Filho, E. (2006).

Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. 87-94.

Babalola, O. O., Molefe, R. R., & Amoo, A. E. (2021). Metagenome assembly and metagenome-assembled genome sequences from the rhizosphere of maize plants in Mafikeng, South Africa. *Microbiology Resource Announcements*, *10*(8), 10-1128.

Babalola, O. O., Molefe, R. R., & Amoo, A. E. (2021). Revealing the active microbiome connected with the rhizosphere soil of maize plants in Ventersdorp, South Africa. *Biodiversity Data Journal*, *9*.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57:233-266.

Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M., & Vivanco, J. M. (2003). Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, *301*(5638), 1377-1380.

Bais, H. P., Walker, T. S., Stermitz, F. R., Hufbauer, R. A., & Vivanco, J. M. (2002). Enantiomeric-dependent phytotoxic and antimicrobial activity of (±)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiology*, *128*(4), 1173-1179.

Bais, H.P., Walker, T.S., Kennan, A.J., Stermitz, F.R.1 & Vivanco, J.M., (2003). Structure-dependant phytotoxicity of catechins and other flavonoids: flavonoid conversions by cell-free protein extracts of *Centaurea maculosa* (spotted knapweed). J. Agri. Food Chem. 51, 897-901.

Baker, H. G. (1974). The evolution of weeds. *Annual review of ecology and systematics*, *5*(1), 1-24.

Balasubramanian,V. & L. Sekanaynge (1991) Effects of tree legumes. on soil fertility changes and crop performance in semi-arid highlands of Rwanda. *Biological. Agriculture and Horticulture* 8: 1-32

Barney, J. N., & Tekiela, D. R. (2020). Framing the concept of invasive species "impact" within a management context. *Invasive Plant Science and Management*, *13*(2), 37-40.

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*, *13*(3), 1140.

Bates, L. S., Waldren, R. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and soil, 39, 205-207.

Batish, D.R., Singh, H.P., Kaur, S., Kohli, R.K., Yadav, S.S., (2008). Caffeic acid affects early growth, and morphogenetic response of hypocotyl cuttings of mung bean (*Phaseolus aureus*). *J. Plant Physiol.* 165, 297–305.

Batten, K., Scow, K., Davies, K., & Harrison, S. (2006). Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biological Invasions*, 8(2), 217-230.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276-287.

Becerra, P. I., Catford, J. A., Inderjit, Luce McLeod, M., Andonian, K., Aschehoug, E. T., Montesinos, D. & Callaway, R. M. (2018). Inhibitory effects of *Eucalyptus globulus* on understorey plant growth and species richness are greater in non-native regions. *Global Ecology and Biogeography*, *27*(1), 68-76.

Beimforde, C., Feldberg, K., Nylinder, S., Rikkinen, J., Tuovila, H., Dörfelt, H., ... & Schmidt, A. R. (2014). Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. *Molecular phylogenetics and evolution*, *78*, 386-398.

Bell, T. H., Callender, K. L., Whyte, L. G., & Greer, C. W. (2013). Microbial competition in polar soils: a review of an understudied but potentially important control on productivity. *Biology*, *2*(2), 533-554.

Bereika, F. F. M., Sallam, N. M. A., Alamri, S. A. M., Abo-Elyousr, K. A. M., Hashem, M., & Mostafa, Y. S. (2020). Approving the biocontrol strategy of potato wilt caused by *Ralstonia solanacearum* on field scale using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. *Egypt J Biol Pest Control*, *30*(1), 61.

Bereika, M. F. F., Moharam, M. H. A., Abo-elyousr, K. A. M., & Asran, M. R. (2020). Control of potato brown rot and wilt disease caused by Ralstonia solanacearum using some water plant extracts. *Journal of Sohag Agriscience (JSAS)*, *5*(1), 30-47.

Bereika, M. F. F., Moharam, M. H. A., Abo-elyousr, K. A. M., & Asran, M. R. (2020). Control of potato brown rot and wilt disease caused by Ralstonia solanacearum using some water plant extracts. *Journal of Sohag Agriscience (JSAS)*, *5*(1), 30-47.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in plant science*, *17*(8), 478-486.

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology*, *68*(1), 1-13.

Bertin, C., Weston, L. A., Huang, T., Jander, G., Owens, T., Meinwald, J., & Schroeder, F. C. (2007). Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. *Proceedings of the National Academy of Sciences*, *104*(43), 16964-16969.

Bertolazi, A. A., De Souza, S. B., Ruas, K. F., & de Rezende, C. E. (2019). Inoculation with *Piriformospora indica* is more efficient in wild-type rice than in transgenic rice over-expressing the vacuolar H+-PPase. *Frontiers in Microbiology*, *10*, 454146.

Bever, J. D. (2002). Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society of London. Series B: Biological Sciences, 269(1509), 2595-2601.

Bever, J. D. (2003). Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New phytologist*, *157*(3), 465-473.

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual review of microbiology,* 66, 265-283.

Bi, J., Blanco, J. A., Seely, B., Kimmins, J. P., Ding, Y., & Welham, C. (2007). Yield decline in Chinese-fir plantations: a simulation investigation with implications for model complexity. *Canadian Journal of Forest Research*, *37*(9), 1615-1630.

Black, C. A. (1965). Method of soil analysis part 2. *Chemical and microbiological properties*, *9*, 1387-1388.

Blake, G.R. and Hartge, K.H. (1986) Bulk density. In: Klute, A., Ed., Methods of Soil Analysis, Part 1—Physical and Mineralogical Methods, 2nd Edition, Agronomy Monograph 9, American Society of Agronomy—Soil Science Society of America, Madison, 363-382.

Blank, R. R., & Young, J. A. (2004). Influence of three weed species on soil nutrient dynamics. *Soil Science*, *169*(5), 385-397.

Blossey, B., & Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology*, 83(5), 887-889.

Blumenthal, D. M. (2009). Carbon addition interacts with water availability to reduce invasive forb establishment in a semi-arid grassland. *Biological Invasions*, *11*, 1281-1290.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144(1), 1-11.

Bradley, B. A., Blumenthal, D. M., Early, R., Grosholz, E. D., Lawler, J. J., Miller, L. P., ... & Olden, J. D. (2012). Global change, global trade, and the next wave of plant invasions. *Frontiers in Ecology and the Environment*, *10*(1), 20-28.

Braine, J. W., Curcio, G. R., Wachowicz, C. M., & Hansel, F. A. (2012). Allelopathic effects of Araucaria angustifolia needle extracts in the growth of Lactuca sativa seeds. *Journal of forest research*, *17*(5), 440-445.

Broadbent, P., Baker, K. F., & Waterworth, Y. (1971). Bacteria and actinomycetes antogonistic to fungal root pathogens in Australian soils. *Australian Journal of Biological Sciences*, *24*(4), 925-944.
Broadbent, P., Baker, K. F., & Waterworth, Y. (1971). Bacteria and actinomycetes antogonistic to fungal root pathogens in Australian soils. *Australian Journal of Biological Sciences*, *24*(4), 925-944.
Brondizio, E. S., Settele, J., Diaz, S., & Ngo, H. T. (2019). Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Brooks, M. L., D'antonio, C. M., Richardson, D. M., Grace, J. B., Keeley, J. E., DiTomaso, J. M., ... & Pyke, D. (2004). Effects of invasive alien plants on fire regimes. *BioScience*, *54*(7), 677-688.

Brown, J.H., (1939). Bergey's manual of determinative bacteriology. *American Public Health Association.*

Buckley, Y. M., & Catford, J. (2016). Does the biogeographic origin of species matter? Ecological effects of native and non-native species and the use of origin to guide management. *Journal of Ecology*, 104(1), 4-17.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E. and Peplies, J. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91-95.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E. and Peplies, J., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), pp.91-95.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual review of plant biology*, *64*, 807-838.

Cai, F., Mei, L., An, X., Gao, S., Tang, L., & Chen, F. (2011). Lipid peroxidation and antioxidant responses during seed germination of Jatropha curcas. *International Journal of Agriculture and Biology*, *13*(1), 25-30.

Cai, M., Li, Y., Li, Y., & Du, K. (2015). Physiological and biochemical responses and microscopic structure changes of Populus tomentosa Carr seedlings to 4-BDE exposure. *Environmental Science and Pollution Research*, *22*, 14258-14268. *International journal of clinical and experimental pathology*, *8*(7), 8655.

Caldwell, B. A. (2005). Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia*, *49*(6), 637-644.

Caldwell, B. A. (2006). Effects of invasive scotch broom on soil properties in a Pacific coastal prairie soil. *Applied Soil Ecology*, *32*(1), 149-152.

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, *290*(5491), 521-523.

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, 290(5491), 521-523.

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, *2*(8), 436-443.

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, 2(8), 436-443.

Callaway, R. M., Ridenour, W. M., Laboski, T., Weir, T., & Vivanco, J. M. (2005). Natural selection for resistance to the allelopathic effects of invasive plants. *Journal of ecology*, 576-583.

Callaway, R. M., Thelen, G. C., Rodriguez, A., & Holben, W. E. (2004). Soil biota and exotic plant invasion. *Nature*, 427(6973), 731-733.

Camel, V. (2006). Extraction methodologies in plants: General introduction. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*, 1-26.

Cao, C., Sun, S., Wang, X., Liu, W., & Liang, Y. (2011). Effects of manganese on the growth, photosystem II and SOD activity of the dinoflagellate Amphidinium sp. *Journal of applied phycology*, *23*, 1039-1043.

Cappuccino, J. C., & Sherman, N. (1992). Negative staining. *Microbiology: a laboratory manual*, *3*, 125-179.

Cappuccino, N., & Arnason, J. T. (2006). Novel chemistry of invasive exotic plants. *Biology letters*, *2*(2), 189-193.

Carvalhais, L. C., Dennis, P. G., & Schenk, P. M. (2014). Plant defence inducers rapidly influence the diversity of bacterial communities in a potting mix. *Applied soil ecology*, *84*, 1-5.

Castro-Díez, P., Godoy, O., Alonso, A., Gallardo, A., and Saldaña, A. (2014). What explains variation in the impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. *Ecology Letters*, 17, 1-12.

Catford, J. A., Baumgartner, J. B., Vesk, P. A., White, M., Buckley, Y. M., & McCarthy, M. A. (2016). Disentangling the four demographic dimensions of species invasiveness. *Journal of Ecology*, *104*(6), 1745-1758.

Catford, J. A., Daehler, C. C., Murphy, H. T., Sheppard, A. W., Hardesty, B. D., Westcott, D. A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C & Hulme, P. E. (2012). The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. *Perspectives in plant ecology, evolution and systematics*, 14(3), 231-241.

Catford, J. A., Jansson, R., & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and distributions*, 15(1), 22-40.

Chandrashekar, K.R., Sandhyarani, S., (1996). Salinity induced chemical changes in *Crotalaria striata* DC plants. *Indian J. Plant Physiol.* 1, 44–48.

Chaneton, E. J., Mazia, C. N., Machera, M., Uchitel, A., and Ghersa, C. M. (2004). Establishment of Honey Locust (*Gleditsia triacanthos*) in Burned Pampean Grasslands1. *Weed Technology*, 18, 1325-1329.

Charles, H., & Dukes, J. S. (2007). Impacts of invasive species on ecosystem services. *Biological invasions*, 217-237.

Chaudhari, D., Rangappa, K., Das, A., Layek, J., Basavaraj, S., Kandpal, B. K., ... & Rahi, P. (2020). Pea (*Pisum sativum* L.) plant shapes its rhizosphere microbiome for nutrient uptake and stress amelioration in acidic soils of the North-East region of India. *Frontiers in Microbiology*, *11*, 538448.

Chen, L. C., Wang, S. L., Wang, P., & Kong, C. H. (2014). Autoinhibition and soil allelochemical (cyclic dipeptide) levels in replanted Chinese fir (Cunninghamia lanceolata) plantations. *Plant and Soil*, *374*, 793-801.

Chen, L., Fan, X., & Ma, D. (2008). Allelopathic effects of *Galinsoga parviflora* Cav. on antioxidant enzyme systems in rape seedlings. *Southwest China Journal of Agricultural Sciences*, *21*(2), 332-334.

Cheng F, Cheng Z (2015) Research Progress on the use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. *Front Plant Sci* 6:1020

Chu, Y., & Xiao, J. (2023). 2′-Hydroxy-4′, 5′-dimethoxyacetophenone exhibit collagenase, aldose reductase inhibition, and anticancer activity against human leukemic cells: an in vitro, and in silico study. *Molecular Biotechnology*, *65*(6), 881-890.

Chuah, T. S., Norhafizah, M. Z., & Ismail, B. S. (2015). Evaluation of the biochemical and physiological activity of the natural compound, 2, 4-ditert-butylphenol on weeds. *Crop and Pasture Science*, *66*(2), 214-223.

Chuah, T. S., Norhafizah, M. Z., & Ismail, S. (2014). Phytotoxic effects of the extracts and compounds isolated from Napiergrass (Pennisetum purpureum) on Chinese Sprangletop (Leptochloa chinensis) germination and seedling growth in aerobic rice systems. *Weed science*, *62*(3), 457-467.

Ciniglia, C., Mastrobuoni, F., Scortichini, M., & Petriccione, M. (2015). Oxidative damage and cell-programmed death induced in Zea mays L. by allelochemical stress. *Ecotoxicology*, *24*, 926-937.

Cipollini, K., Titus, K. Y. L. E., & Wagner, C. (2012). Allelopathic effects of invasive species (*Alliaria petiolata*, *Lonicera maackii, Ranunculus ficaria*) in the Midwestern United States. *Allelopathy J*, *29*(1), 63-76.

Coblentz, B. E. (1990). Exotic organisms: A dilemma for conservation biology. *Conservation Biology*, 4(3), 261-265.

Collier, D. (2016). Uranium mine and mill remediation and reclamation. In *Uranium for Nuclear Power* (pp. 415-437). Woodhead Publishing.

Constancias, F., Terrat, S., Saby, N. P., Horrigue, W., Villerd, J., Guillemin, J. P., Biju-Duval, L., Nowak, V., Dequiedt, S., Ranjard, L. & Chemidlin Prévost-Bouré, N. (2015). Mapping and determinism of soil microbial community distribution across an agricultural landscape. *MicrobiologyOpen*, *4*(3), 505-517.

Corbin, J. D., & D'Antonio, C. M. (2012). Gone but not forgotten? invasive plants' legacies on community and ecosystem properties. *Invasive Plant Science and Management,* 5(1), 117-124.

Corbin, J. D., & D'Antonio, C. M. (2004). Effects of exotic species on soil nitrogen cycling: Implications for restoration. *Weed Technology*, 18(1), 1464-1467.

Cordero, J., de Freitas, J. R., & Germida, J. J. (2020). Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. *Canadian Journal of Microbiology*, *66*(1), 71-85.

Cronk, Q. C. and Fuller, J. L. (2014). *Plant invaders: the threat to natural ecosystems*. Routledge.

Crystal-Ornelas, R., Hudgins, E., Cuthbert, R., Haubrock, P., Fantle-Lepczyk, J., Angulo, E., Kramer, A. M., Ballesteros-Mejia, L., Leroy, B., Leung, B., López-López, E., Diagne, C., & Courchamp, F. (2021). Economic costs of biological invasions within North America. *NeoBiota*, 67, 485-510.

Čuda, J., Skálová, H., Janovský, Z., & Pyšek, P. (2015). Competition among native and invasive Impatiens species: the roles of environmental factors, population density and life stage. *AoB Plants*, *7*, plv033.

Cuddy WS, Neilan BA, Gehringer MM (2012) Comparative analysis of cyanobacteria in the rhizosphere and as endosymbionts of cycads in drought-affected soils. FEMS Microbiol Ecol 80(1):204–215

D'Antonio, C. M., & Meyerson, L. (2002). Exotic species and restoration: synthesis and research needs. *Restoration Ecology*, *10*(4), 703-713.

Daneshkhah, R., Grundler, F. M. W., & Wieczorek, K. (2018). The role of MPK6 as mediator of ethylene/jasmonic acid signaling in Serendipita indica-colonized Arabidopsis roots. *Plant molecular biology reporter*, *36*, 284-294.

D'antonio, C & Meyerson, L. (2002). Exotic Plant Species as Problems and Solutions in Ecological Restoration: A Synthesis. *Restoration Ecology*, 10(4), 703-713.

D'antonio, C. A. R. L. A., & Meyerson, L. A. (2002). Exotic plant species as problems and solutions in ecological restoration: a synthesis. *Restoration ecology*, *10*(4), 703-713.

Das, A. J., Kumar, M., & Kumar, R. (2013). Plant growth promoting rhizobacteria (PGPR): an alternative of chemical fertilizer for sustainable, environment friendly agriculture. *Research Journal of Agriculture and Forestry Sciences*, *1*(4), 21-23.

Davidson, A. M., Jennions, M., & Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology letters*, 14(4), 419-431.

Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of ecology*, *88*(3), 528-534.

Dawson, W., & Schrama, M. (2016). Identifying the role of soil microbes in plant invasions. *Journal of Ecology*, *104*(5), 1211-1218.

Dawson, W., & Schrama, M. (2016). Identifying the role of soil microbes in plant invasions. *Journal of Ecology*, *104*(5), 1211-1218.

de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, *56*(1), 87-94.

Deng, Y. C., Kong, Z. W., Luo, H. Y., Ning, L., & Qin, H. (2010). Allelopathic effect of invasive *Ipomoea cairica* on seed germination and seedling growth of five vegetables. *Seed*, *7*, 8-11.

Dhanalakshmi, R., & Manavalan, R. (2014). Bioactive compounds in leaves of Corchorus trilocularis L. by GC-MS analysis. *International Journal of PharmTech Research*, *6*(7), 1991-1998.

Dichio, B., Romano, M., Nuzzo, V., Xiloyannis, C., (2002). Soil water availability and relationship between canopy and roots in young olive trees (cv Coratina). Acta Hortic. 586, 255–258.

Divíšek, J., Chytrý, M., Beckage, B., Gotelli, N. J., Lososová, Z., Pyšek, P., Richardson, D.M. & Molofsky, J. (2018). Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nature communications*, 9(1), 4631.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The plant cell*, *7*(7), 1085.

Djanaguiraman, M., Vaidyanathan, R., Annie Sheeba, J., Durga Devi, D., Bangarusamy, U., (2005). Physiological responses of Eucalyptus globules leaf leachate on seedling physiology of rice, sorghum and blackgram. *Int. J. Agric. Biol*. 7, 35–38.

Dmitrović, S., Perišić, M., Stojić, A., Živković, S., Boljević, J., Nestorović Živković, J., Aničić, N., Ristić, M. & Mišić, D. (2015). Essential oils of two *Nepeta* species inhibit growth and induce oxidative stress in ragweed (*Ambrosia artemisiifolia* L.) shoots in vitro. Acta *Physiologiae Plantarum*, 37, 1-15.

Downey, P. O., & Richardson, D. M. (2016). Alien plant invasions and native plant extinctions: a six-threshold framework. AoB plants, 8, plw047.

Drake, J.A. (ed.). (1989). Biological Invasions: A Global Perspective. Wiley, Chichester pp. 85-110.

Drenovsky, R. E., Grewell, B. J., D'antonio, C. M., Funk, J. L., James, J. J., Molinari, N., Parker, I. M., and Richards, C. L. (2012). A functional trait perspective on plant invasion. *Annals of Botany*, 110, 141-153.

Duenas, M. A., Hemming, D. J., Roberts, A., & Diaz-Soltero, H. (2021). The threat of invasive species to IUCN-listed critically endangered species: A systematic review. *Global Ecology and Conservation*, 26, e01476.

Duggin, J. A., & Gentle, C. B. (1998). Experimental evidence on the importance of disturbance intensity for invasion of Lantana camara L. in dry rainforest–open forest ecotones in north-eastern NSW, Australia. Forest Ecology and Management, 109(1-3), 279-292.

Ehrenfeld, J. G. (2003). Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* (New York), 6(6), 503-523.

Ek M, Ljunquist PO and Stenström E .(1983). Indole-3-acetic acid production by mycorrhizal fungi determined by gas chromatography-mass spectrometry. *New Phytol* 94:583–588.

Ellstrand, N. C., & Schierenbeck, K. A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences*, 97(13), 7043-7050.

Elshamy, A.I.; Abd El-Gawad, A.M.; El Gendy, A.E.-N.G.; Assaeed, A.M. (2019). Chemical characterization of *Euphorbia heterophylla* L. essential oils and their antioxidant activity and allelopathic potential on *Cenchrus echinatus* L. *Chem. Biodivers*. 16, e1900051

Elsheikh, E. A., El-Keblawy, A., Mosa, K. A., Okoh, A. I., & Saadoun, I. (2021). Role of endophytes and rhizosphere microbes in promoting the invasion of exotic plants in arid and semi-arid areas: A review. *Sustainability*, *13*(23), 13081.

Elton, C. S. (1933). The ecology of invasions by animals and plants. Chicago, Illinois, USA: Springer Nature.

Erin, R. S. .(2012). Aseptic laboratory techniques: plating methods. *Journal of visualized experiments.*

Essl, F., Lenzner, B., Bacher, S., Bailey, S., Capinha, C., Daehler, C., ... & Roura-Pascual, N. (2020). Drivers of future alien species impacts: An expert-based assessment. *Global Change Biology,* 26(9), 4880-4893.

Falaki, F. (2019). Sample preparation techniques for gas chromatography. In *Gas Chromatography-Derivatization, Sample Preparation, Application*. IntechOpen.

Farhoudi, R., & Lee, D. J. (2013). Allelopathic effects of barley extract (*Hordeum vulgare*) on sucrose synthase activity, lipid peroxidation and antioxidant enzymatic activities of *Hordeum spontoneum* and *Avena ludoviciana*. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, 83, 447-452.

Farhoudi, R., Zangane, H.S., Saeedipour, S., (2012). Allelopathical effect of barley (*Hordeum vulgare* (L.) cv. Karon) on germination and lipidperoxidation of wild mustard seedling. Res. Crop. 13, 467–471.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579-590.

Flaishman, M. A., Eyal, Z., Zilberstein, A., Voisard, C., & Haas, D. (1996). Suppression of Septoria tritici blotch and leaf rust of wheat by recombinant cyanide-producing strains of Pseudomonas putida. *MPMI-Molecular Plant Microbe Interactions*, *9*(7), 642-645.

Flores-Palacios, A., Corona-López, A. M., Rios, M. Y., Aguilar-Guadarrama, B., Toledo-Hernández, V. H., Rodríguez-López, V., & Valencia-Díaz, S. (2015). Is allelopathic activity of *Ipomoea murucoides* induced by xylophage damage?. *PloS one*, 10(12), e0143529.

Flory, S. L., & Clay, K. (2010). Non-native grass invasion suppresses forest succession. *Oecologia*, 164(4), 1029-1038.

Foxcroft, L. C., Pyšek, P., Richardson, D. M., Genovesi, P., & MacFadyen, S. (2017). Plant invasion science in protected areas: progress and priorities. *Biological Invasions*, *19*(5), 1353-1378.

Frey, S. D., Knorr, M., Parrent, J. L., & Simpson, R. T. (2004). Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management*, *196*(1), 159-171.

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. *New Phytologist,* 176:22-36.

Funk, J. L., & Vitousek, P. M. (2007). Resource-use efficiency and plant invasion in low-resource systems. *Nature*, 446(7139), 1079-1081.

Gaggini, L., Rusterholz, H. P., & Baur, B. (2018). The invasive plant Impatiens glandulifera affects soil fungal diversity and the bacterial community in forests. Applied Soil Ecology, 124, 335-343.

Gallo, M. B., & Sarachine, M. J. (2009). Biological activities of lupeol. *Int. J. Biomed. Pharm. Sci*, *3*(1), 46-66.

Gao, X. X., Li, M., Xie, H., Gao, Z. J., Zhang, X. R., Zhang, Y. L., ... & Kong, J. H. (2012). Allelopathic effects of *Parthenium argentatum* on seed germination and seedling growth of three plant species. *Pratacul Tural Science*, *29*(6), 898-903.

Gatti, A. B., Ferreira, A. G., Arduin, M., & Perez, S. C. G. D. A. (2010). Allelopathic effects of aqueous extracts of Artistolochia esperanzae O. Kuntze on development of Sesamum indicum L. seedlings. *Acta Botanica Brasilica*, *24*, 454-461.

Geetha, K., Venkatesham, E., Hindumathi, A., & Bhadraiah, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on Vigna Radita (L.) R. Wilczek. *Int J Curr Microbiol Appl Sci*, *3*(6), 799-899.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., & Citterio, S. (2018). Effect of soil pH on the growth, reproductive investment and pollen allergenicity of Ambrosia artemisiifolia L. *Frontiers in plant science*, *9*, 1335.

Gfeller, A., Glauser, G., Etter, C., Signarbieux, C., & Wirth, J. (2018). Fagopyrum esculentum alters its root exudation after Amaranthus retroflexus recognition and suppresses weed growth. *Frontiers in Plant Science*, *9*, 50.

Ghimire, B. K., Hwang, M. H., Sacks, E. J., Yu, C. Y., Kim, S. H., & Chung, I. M. (2020). Screening of allelochemicals in *Miscanthus sacchariflorus* extracts and assessment of their effects on germination and seedling growth of common weeds. *Plants*, *9*(10), 1313.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

Gioria, M., & Osborne, B. A. (2014). Resource competition in plant invasions: emerging patterns and research needs. *Frontiers in Plant Science*, *5*, 501.

Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012.

Godoy, O. and Levine, J. M. (2014). Phenology effects on invasion success: insights from coupling field experiments to coexistence theory. *Ecology*, 95, 726-736.

Goel, A.,& Sheoran, L.S., (2003). Lipid peroxidation and peroxide scavenging enzymes in cotton seeds under natural ageing. *Biol. Plant.* 46, 429–434

Gómez-Aparicio, L., & Canham, C. D. (2008). Neighbourhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. *Journal of Ecology*, *96*(3), 447-458.

Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant physiology*, *26*(1), 192.

Grayston, S. J., Wang, S., Campbell, C. D., & Edwards, A. C. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, *30*(3), 369-378.

Grove, S., Haubensak, K. A., Gehring, C., & Parker, I. M. (2017). Mycorrhizae, invasions, and the temporal dynamics of mutualism disruption. *Journal of Ecology*, *105*(6), 1496-1508.

Guo W-Y, van Kleunen M, Pierce S, Dawson W, Essl F, Kreft H, Maurel N, Pergl J, Seebens H, Weigelt P, Pyšek P (2019) Domestic gardens play a dominant role in selecting alien species with adaptive strategies that facilitate naturalization. *Glob Ecol Biogeogr* 28:628–639.

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions?. *Trends in ecology & evolution,* 19(9), 470-474.

Hada, T., Shiraishi, A., & Furuse, S. (2003). Inhibitory effects of terpenes on the growth of Staphylococcus aureus. *Natural medicines 57*(2), 64-67.

Hadacek F, Bachmann G, Engelmeier D, Chobot V (2011) Hormesis and a chemical raison d'eˆtre for secondary plant metabolites. *Dose-Response* 9:79–116.

Haddadchi, G. R., &Gerivani, Z. (2009). Effects of phenolic extracts of canola (*Brassica napus* L.) on germination and physiological responses of soybean (*Glycine max* L.) seedlings. *International Journal of Plant Production* 3 (1), 63-73.

Hagan, D. L., Jose, S., & Lin, C. (2013b). Allelopathic exudates of cogongrass (*Imperata cylindrica*): Implications for the performance of native pine savanna plant species in the southeastern US. *Journal of Chemical Ecology*, 39(2), 312-322.

Hagan, D. L., Jose, S., Bohn, K., & Escobedo, F. (2013a). Cogongrass (*Imperata cylindrica*) invasion and eradication: Implications for soil nutrient dynamics in a longleaf pine sandhill ecosystem. *Invasive Plant Science and Management*, 6(3), 433-443. doi:10.1614/IPSM-D-12-00089.1

Hamelin, R. C. and Roe, A. D. (2020). Genomic biosurveillance of forest invasive alien enemies: A story written in code. *Evolutionary Applications*, 13, 95-115.

Hamilton, M. A., Murray, B. R., Cadotte, M. W., Hose, G. C., Baker, A. C., Harris, C. J., and Licari, D. (2005). Life-history correlates of plant invasiveness at regional and continental scales. *Ecology Letters*, 8, 1066-1074.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Matthias, D. & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews*, *79*(3), 293-320.

Hare, P.D., Cress,W.A., & van Staden, J., (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.* 21, 535–553.

Harikrishnan, H., Shanmugaiah, V., & Balasubramanian, N. (2014). Optimization for production of Indole acetic acid (IAA) by plant growth promoting Streptomyces sp VSMGT1014 isolated from rice rhizosphere. *Int J Curr Microbiol Appl Sci*, *3*(8), 158-171.

Harrison, M. J. (2005). Signaling in the arbuscular mycorrhizal symbiosis. *Annu. Rev. Microbiol.*, *59*, 19-42.

Hartmann, A., Rothballer, M., & Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and soil*, *312*, 7-14.

Hartmann, A., Schmid, M., van Tuinen, D., & Berg, G. (2009) Plant-driven selection of microbes. *Plant and Soil*, 321:235-257.

Harun, M.A.Y.A., Robinson, R.W., Johnson, J., & Uddin, M.N., (2014). Allelopathic potential of *Chrysanthemoides monilifera* subsp. monilifera (boneseed): a novel weapon in the invasion processes. South Afric. J. Bot. 93, 157–166.

Harvey, J. A., & Fortuna, T. M. (2012). Chemical and structural effects of invasive plants on herbivore–parasitoid/predator interactions in native communities. *Entomologia Experimentalis et Applicata*, *144*(1), 14-26.

Haubrock, P. J., Turbelin, A. J., Cuthbert, R. N., Novoa, A., Taylor, N. G., Angulo, E., Ballesteros-Mejia, L., Bodey, T.W., Capinha C., Diagne, C., & Essl, F. Courchamp, F. (2021). Economic costs of invasive alien species across Europe. *NeoBiota*, 67, 153-190.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics,* 125(1), 189-198.

Hejda, M., Chytrý, M., Pergl, J., & Pyšek, P. (2015). Native-range habitats of invasive plants: are they similar to invaded-range habitats and do they differ according to the geographical direction of invasion?. *Diversity and Distributions*, 21(3), 312-321.

Hess, K.M., Dudley, M.W., Lynn, D.G., Joerger, R.D. and Binns, A.N. (1991) Mechanism of phenolic activation of Agrobacterium virulence genes: development of a specific inhibitor of bacterial sensor/response systems. Proc. Natl Acad. Sci. USA, 88, 7854–7858.

Hierro, J. L., Maron, J. L., & Callaway, R. M. (2005). A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of ecology*, *93*(1), 5-15.

Hierro, J. L., Villarreal, D., Eren, Ö., Graham, J. M., & Callaway, R. M. (2006). Disturbance facilitates invasion: the effects are stronger abroad than at home. *The American Naturalist*, *168*(2), 144-156.

Hobbs, R. J., & Huenneke, L. F. (1992). Disturbance, diversity, and invasion: implications for conservation. *Conservation biology*, *6*(3), 324-337.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F., Ekblad, A., Högberg, M. N., ... & Read, D. J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, *411*(6839), 789-792.

Hollister, E. B., Engledow, A. S., Hammett, A. J. M., Provin, T. L., Wilkinson, H. H., & Gentry, T. J. (2010). Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. The ISME journal, 4(6), 829-838.

Hou, P. F., Chien, C. H., Chiang-Hsieh, Y. F., Tseng, K. C., Chow, C. N., Huang, H. J., & Chang, W. C. (2018). Paddy-upland rotation for sustainable agriculture with regards to diverse soil microbial community. *Scientific Reports*, *8*(1), 7966.

Hou, Y. P., Wei, W., & Zhai, W. (2016). Effects of litter from dominant tree species on seed germination and seedling growth of exotic plant Rhus typhina in hilly areas in Shandong peninsula. *Sci. Silvae Sin*, *52*(6), 28-34.

Hu X, Chen J, Guo J (2006), Two phosphate and potassium solubilising bacteria isolated from Tianmu Mountain, Zhejiang, China. *World Journal of Microbiology and Biotechnolology* 22: 983- 990.

Hu, C., & Kitts, D. D. (2005). Dandelion (Taraxacum officinale) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. *Phytomedicine*, *12*(8), 588-597.

Hu, X., Chen, J., & Guo, J. (2006). Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World journal of Microbiology and Biotechnology*, *22*(9), 983-990.

Huangfu, C., Chen, D., Wang, N., & Yang, D. (2010). The mutual allelopathic effect between invasive plant *Flaveria bidentis* and four forage species. *Acta Prataculturae Sinica*, *19*(4), 22-32.

Hubalek V, Wu X, Eiler A, Buck M, Heim C, Dopson M, Bertilsson S, Ionescu D (2016) Connectivity to the surface determines diversity patterns in subsurface aquifers of the Fennoscandian shield. ISME J 10(10):2447–2458

Hulme PE, Brundu G, Carboni M, Dehnen-Schmutz K, Dullinger S, Early R, Essl F, González-Moreno P, Groom QJ, Kueffer C, Kühn I, Maurel N, Novoa A, Pergl J, Pyšek P, Seebens S, Tanner R, Touza JM, van Kleunen M, Verbrugge LNH (2017) Integrating invasive species policies across ornamental horticulture supply chains to prevent plant invasions. *J Appl Ecol* 55:92–98.

Hulme, P. E. (2006). Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology,* 43(5), 835-847.

Hulme, P. E., & Weser, C. (2011). Mixed messages from multiple information sources on invasive species: a case of too much of a good thing?. *Diversity and Distributions*, *17*(6), 1152-1160.

Hulme, P. E., Pyšek, P., Nentwig, W., & Vilà, M. (2009). Will threat of biological invasions unite the European Union?. *Science*, *324*(5923), 40-41.

Huseynova, I. M., Sultanova, N. F., & Aliyev, J. A. (2014). Histochemical visualization of ROS and antioxidant response to viral infections of vegetable crops grown in Azerbaijan. *Plant physiology and biochemistry*, *81*, 26-35.

IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Inderjit, & Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta*, *217*, 529-539.

Inderjit, Callaway, R. M. (2003). Experimental designs for the study of allelopathy. *Plant and Soil*, 256(1), 1-11.

Inderjit, Evans, H., Crocoll, C., Bajpai, D., Kaur, R., Feng, Y. L., Silva, C., Carreón, J.T., Valiente-Banuet, A., Gershenzon, J. & Callaway, R. M. (2011). Volatile chemicals from leaf litter are associated with invasiveness of a neotropical weed in Asia. *Ecology*, *92*(2), 316-324.

Inderjit, Evans, H., Crocoll, C., Bajpai, D., Kaur, R., Feng, Y.L., Silva, C., Carreón, J.T., Valiente-Banuet, A., Gershenzon, J. and Callaway, R.M., 2011. Volatile chemicals

from leaf litter are associated with invasiveness of a neotropical weed in Asia. *Ecology*, *92*(2), pp.316-324.

Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H., & Shimada, J. (2004). The antibacterial effects of terpene alcohols on Staphylococcus aureus and their mode of action. *FEMS microbiology letters*, *237*(2), 325-331.

Irimia, R. E., Lopes, S. M., Sotes, G., Cavieres, L. A., Eren, Ö., Lortie, C. J., French, K., Hierro, J.L., Rosche, C., Callaway, R.M. & Pinho e Melo, T.M. (2019). Biogeographic differences in the allelopathy of leaf surface extracts of an invasive weed. *Biological Invasions*, *21*, 3151-3168.

Irwin, H.S. And Barneby C.B. (1982) The American Cassiinae. A synaptical revision of Leguminosae tribe Cassiieae subtribe Cassiinae in the New World. Memoirs of The New York Botanical Garden 35(2).

Islam, A. M., Hasan, M., Musha, M. M. H., Uddin, M. K., Juraimi, A. S., & Anwar, M. P. (2018). Exploring 55 tropical medicinal plant species available in Bangladesh for their possible allelopathic potentiality. *Annals of Agricultural Sciences*, *63*(1), 99-107.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in plant science*, *8*, 1617.

Jia, M., Chen, L., & Han, T. (2016). A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Frontiers in microbiology*, *7*, 179220.

Jiang, M., & Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of experimental botany*, *53*(379), 2401-2410.

Jiang, Y., Cao, Y. R., Wiese, J., Tang, S. K., Xu, L. H., Imhoff, J. F., & Jiang, C. L. (2011). Streptomyces sparsus sp. nov., isolated from a saline and alkaline soil. *International journal of systematic and evolutionary microbiology*, *61*(7), 1601-1605.

John, J., Bindu, G., Srimuruganandam, B., Wadhwa, A., & Rajan, P. (2020). Land use/land cover and land surface temperature analysis in Wayanad district, India, using satellite imagery. *Annals of GIS*, *26*(4), 343-360.

Joshi, J., & Vrieling, K. (2005). The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters*, *8*(7), 704-714.

Kalavati, P. , & Modi. H.A., (2012). Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. CIBTech Journal of Microbiology, 1, 8-14.

Kalayu, G. (2019). Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy*, *2019*, 1-7.

Kalisz, S., Kivlin, S. N., & Bialic-Murphy, L. (2021). Allelopathy is pervasive in invasive plants. *Biological Invasions*, 23, 367-371.

Kapoor, D., Rinzim, Tiwari, A., Sehgal, A., Landi, M., Brestic, M., & Sharma, A. (2019). Exploiting the allelopathic potential of aqueous leaf extracts of *Artemisia absinthium* and *Psidium guajava* against *Parthenium hysterophorus*, a widespread weed in India. *Plants*, *8*(12), 552.

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17(4), 164-170.

Khan, A. (2021). Biopesticides: Alternatives for management of *Callosobruchus maculatus*. *Journal of Biopesticides*, *14*(1), 59-78.

Kielak AM, Cipriano MA, Kuramae EE (2016) Acidobacteria strains from subdivision 1 act as plant growth-promoting bacteria. Arch Microbiol 198(10):987–993.

Kinlock NL, Dehnen-Schmutz K, Essl F, Pergl J, Pyšek P, Kreft H, Weigelt P, Yang Q, van Kleunen M (2022) Introduction history mediates naturalization and invasiveness of cultivated plants. *Glob Ecol* Biogeogr 31:1104–1119.

Kirk, J. L., Klirnomos, J. N., Lee, H., & Trevors, J. T. (2002). Phytotoxicity assay to assess plant species for phytoremediation of petroleum-contaminated soil. *Bioremediation Journal*, *6*(1), 57-63.

Kloepper, J. W. (1978). Plant growth-promoting rhizobacteria on radishes. In *Proc. of the 4th Internet. Conf. on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France, 1978* (Vol. 2, pp. 879-882).

Kong CH, Xuan TD, Khanh TD, Tran HD, Trung NT. (2019) Allelochemicals and Signalling Chemicals in Plants. *Molecules*. Jul 27;24(15):2737.

Kong, C. H., Chen, L. C., Xu, X. H., Wang, P., & Wang, S. L. (2008). Allelochemicals and activities in a replanted Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) tree ecosystem. *Journal of Agricultural and Food Chemistry*, *56*(24), 11734-11739.

Kong, C. H., Wang, P., & Xu, X. H. (2007). Allelopathic interference of *Ambrosia trifida* with wheat (Triticum aestivum). *Agriculture, ecosystems & environment*, *119*(3-4), 416-420.

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., ... & Aharoni, A. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proceedings of the National Academy of Sciences*, *117*(7), 3874-3883.

Kormas KA, Smith DC, Edgcomb V, Teske A (2003) Molecular analysis of deep subsurface microbial communities in Nankai Trough sediments (ODP Leg 190, Site 1176). FEMS Microbiol Ecol 45(2):115–125

Kornaś, J. (1990). Plant invasions in Central Europe: historical and ecological aspects. In Biological invasions in Europe and the Mediterranean Basin (pp. 19-36). Dordrecht: Springer Netherlands.

Koshila Ravi, R., Anusuya, S., Balachandar, M., & Muthukumar, T. (2019). Microbial interactions in soil formation and nutrient cycling. *Mycorrhizosphere and pedogenesis*, 363-382.

Koshila Ravi, R., Anusuya, S., Balachandar, M., & Muthukumar, T. (2019). Microbial interactions in soil formation and nutrient cycling. *Mycorrhizosphere and pedogenesis*, 363-382.

Kosola, K. R., Dickmann, D. I., Paul, E. A., & Parry, D. (2001). Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. *Oecologia*, *129*, 65-74.

Kourtev, P. S., Ehrenfeld, J. G., & Häggblom, M. (2002). Exotic plant species alter the microbial community structure and function in the soil. *Ecology*, *83*(11), 3152-3166.

Kourtev, P. S., Ehrenfeld, J. G., & Häggblom, M. (2003). Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology and Biochemistry*, 35(7), 895-905.

Kourtev, P. S., Ehrenfeld, J. G., & Huang, W. Z. (2002). Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. *Soil Biology and Biochemistry*, *34*(9), 1207-1218.

Kowalchuk, G. A., & Stephen, J. R. (2001). Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annual Reviews in Microbiology*, *55*(1), 485-529.

Kragelund C, Caterina L, Borger A, Thelen K, Eikelboom D, Tandoi V, Thomsen TR (2007) Identity, abundance and ecophysiology of flamentous Chlorofexi species present in activated sludge treatment plants. FEMS Microbiol Ecol 59(3):671–682

Kubešová, M., Moravcova, L., Suda, J., Jarošík, V., and Pyšek, P. (2010). Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. *Preslia*, 82, 81-96.

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant–soil feedbacks: a meta-analytical review. *Ecology letters*, *11*(9), 980-992.

Kumla, J., Suwannarach, N., Bussaban, B., Matsui, K., & Lumyong, S. (2014). Indole-3-acetic acid production, solubilization of insoluble metal minerals and metal tolerance of some sclerodermatoid fungi collected from northern Thailand. *Annals of microbiology*, *64*, 707-720.

Küster, E. C., Kühn, I., Bruelheide, H., and Klotz, S. (2008). Trait interactions help explain plant invasion success in the German flora. *Journal of Ecology*, 96, 860-868.

Ladha, J.K; Peoples M.B; Garrity, D.P: Capuno, V.T and Dart, P.T (1993) Estimating dinitrogen fixation of hedge growth vegetation using the nitrogen 15 natural abundance method. *Soil Sci Soc. Am.* J.57:732-737

Landi, M., Misra, B. B., Muto, A., Bruno, L., & Araniti, F. (2020). Phytotoxicity, morphological, and metabolic effects of the sesquiterpenoid nerolidol on *Arabidopsis thaliana* seedling roots. *Plants*, 9(10), 1347.

Langmaier, M., & Lapin, K. (2020). A systematic review of the impact of invasive alien plants on forest regeneration in European temperate forests. *Frontiers in Plant Science*, *11*, 524969.

Lankau, R. A. (2011). Resistance and recovery of soil microbial communities in the face of Alliaria petiolata invasions. *New Phytologist*, *189*(2), 536-548.

Lankau, R. A. (2012). Coevolution between invasive and native plants driven by chemical competition and soil biota. *Proceedings of the National Academy of Sciences*, *109*(28), 11240-11245.

Lankau, R. A., Nuzzo, V., Spyreas, G., & Davis, A. S. (2009). Evolutionary limits ameliorate the negative impact of an invasive plant. *Proceedings of the National Academy of Sciences*, 106(36), 15362-15367.

Latif, S., Chiapusio, G., & Weston, L. A. (2017). Allelopathy and the role of allelochemicals in plant defence. *In Advances in botanical research* (Vol. 82, pp. 19-54). Academic Press.

Lazzaro, L., Bolpagni, R., Buffa, G., Gentili, R., Lonati, M., Stinca, A., Acosta, A.T.R., Adorni, M., Aleffi, M., Allegrezza, M. & Angiolini, C. (2020). Impact of invasive alien plants on native plant communities and Natura 2000 habitats: State of the art, gap analysis and perspectives in Italy. *Journal of Environmental Management*, 274, 111140.

Lee, K., Lee, J. H., Kim, S. I., Cho, M. H., & Lee, J. (2014). Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against *Staphylococcus* aureus. *Applied microbiology and biotechnology*, *98*, 9447-9457.

Lee, S. J., Han, J. I., Lee, G. S., Park, M. J., Choi, I. G., Na, K. J., & Jeung, E. B. (2007). Antifungal effect of eugenol and nerolidol against *Microsporum gypseum* in a guinea pig model. *Biological and Pharmaceutical Bulletin*, *30*(1), 184-188.

Lee, T.M., & Lin, Y.H., (1995). Changes in soluble and cell-wall-bound peroxidase activities with growth in anoxia-treated rice (*Oryza sativa* L.) coleoptiles and roots. *Plant Sci*. 106, 1–7.

Leffler, A. J., James, J. J., Monaco, T. A., & Sheley, R. L. (2014). A new perspective on trait differences between native and invasive exotic plants. Ecology, 95(2), 298-305.

Li, J., & Jin, Z. (2010). Potential allelopathic effects of Mikania micrantha on the seed germination and seedling growth of Coix lacryma-jobi. *Weed biology and management*, *10*(3), 194-201.

Li, Y. P., Feng, Y. L., Chen, Y. J., & Tian, Y. H. (2015). Soil microbes alleviate allelopathy of invasive plants. *Science Bulletin*, *60*, 1083-1091.

Li, Z. H., Wang, Q., Ruan, X., Pan, C. D., & Jiang, D. A. (2010). Phenolics and plant allelopathy. *Molecules*, *15*(12), 8933-8952.

Liang, X., Zheng, H., He, C. Q., Xu, Q. Y., Zhan, Y. W., Lei, Y. R., Du, W. & Yang, J. N. (2012). Allelopathic effects of invasive *Spartina alterniflora* root exudates in soil on the offspring (seeds) of *Scirpus mariqueter. Allelopathy Journal*, *29*(2).

Lim, K. T., Amanah, A., Chear, N. J. Y., Zahari, Z., Zainuddin, Z., & Adenan, M. I. (2018). Inhibitory effects of (+)-spectaline and iso-6-spectaline from Senna spectabilis on the growth and ultrastructure of human-infective species Trypanosoma brucei rhodesiense bloodstream form. *Experimental parasitology*, *184*, 57-66.

Lin, W.X., 2010. Effect of self-allelopathy on AOS of *Casuarina equisetifolia* forst seedling. Fujian J. Agric. Sci. 25, 108–113.

Liu, D. L., & Lovett, J. V. (1993). Biologically active secondary metabolites of barley. I. Developing techniques and assessing allelopathy in barley. *Journal of Chemical Ecology*, *19*, 2217-2230.

Liu, F., Mo, X., Kong, W., & Song, Y. (2020). Soil bacterial diversity, structure, and function of *Suaeda salsa* in rhizosphere and non-rhizosphere soils in various habitats in the Yellow River Delta, China. *Science of The Total Environment*, *740*, 140144.

Liu, M.X., Li, B.W., Sun, R.D., Zhang, Y.Y., Song, J.Y., Zhang, G.J., Xu, L. & Mu, R.L. (2021**).** Characteristics of culturable microbial communities in rhizosphere /nonrhizosphere soil of *Ligularia virgaurea* in alpine meadow elevation gradient. *Acta Ecol. Sin.,* 41, 4853–4863. (In Chinese)

Lonsdale, W. M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, *80*(5), 1522-1536.

Lopes, N. P., Kato, M. J., Eloísa, H. D. A., Maia, J. G., Yoshida, M., Planchart, A. R., & Katzin, A. M. (1999). Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiapi Amazon Indians. *Journal of ethnopharmacology*, *67*(3), 313-319.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual review of microbiology*, *63*, 541-556.

Lukosi, N (1997) A brief note on possible control of *Senna spectabilis*, an invasive exotic tree at Mahale. *Pan Africa News* 4(2): .18

Luz, S. D. M., Souza Filho, A. P. D. S., Guilohn, G. M. S. P., & Vilhena, K. D. S. D. S. (2010). Atividade alelopática de substâncias químicas isoladas da Acacia mangium e suas variações em função do pH. *Planta Daninha*, *28*, 479-487.

Mack, R. N. (1996). Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biological conservation,* 78(1-2), 107-121.

Mahall, B. E., & Callaway, R. M. (1991). Root communication among desert shrubs. *Proceedings of the National Academy of Sciences,* 88(3), 874-876.

Mahall, B. E., & Callaway, R. M. (1992). Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology,* 73(6), 2145-2151.

Majumder, S., Acharyya, S., Ghosh, A., Chakraborty, S., Sarkar, S., Saha, S., & Bhattacharya, M. (2021). Insights into low biological activity of wax apple (*Syzygium samarangense*) juice by in vitro phytochemical investigation with special reference to metabolomics. *Asian Journal of Natural Product Biochemistry*, 19(1).

Mallon, C. A., Elsas, J. D., & Salles, J. F. (2015). Microbial invasions: the process, patterns, and mechanisms. *Trends in microbiology*, 23(11), 719-729.

Maqbool, N., Wahid, A., Farooq, M., Cheema, Z. A., & Siddique, K. H. M. (2013). Allelopathy and abiotic stress interaction in crop plants. *Allelopathy: current trends and future applications*, 451-468.

Marilley, L., & Aragno, M. (1999). Phylogenetic diversity of bacterial communities differing in degree of proximity of Lolium perenne and Trifolium repens roots. *Applied soil ecology*, *13*(2), 127-136.

Maron, J. L., & Vila, M. (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, 95(3), 361-373.

Masum, S. M., Hossain, M. A., Akamine, H., Sakagami, J. I., Ishii, T., Gima, S., Kensaku, T. and Bhowmik, P.C., & Bhowmik, P. C. (2018). Isolation and characterization of allelopathic compounds from the indigenous rice variety 'Boterswar'and their biological activity against *Echinochloa crus-galli* L. *Allelopath. J*, *43*(1), 31-42.

Masum, S.M., Hossain, M.A., Akamine, H., Sakagami, J.I., Ishii, T., Gima, S., Kensaku, T. and Bhowmik, P.C., 2018. Isolation and characterization of allelopathic compounds from the indigenous rice variety 'Boterswar'and their biological activity against Echinochloa crus-galli L. *Allelopath. J*, *43*(1), pp.31-42.

Matters, G. L., & Scandalios, J. G. (1987). Synthesis of isozymes of superoxide dismutase in maize leaves in response to O3, SO2 and elevated O2. *Journal of experimental botany*, *38*(5), 842-852.

McClean, R.H; Litsinger. J.A; Moody, K and Watson, AK (1992) The impact of ·alley cropping *Gliricidia sepium* and *Cassia spectabilis* on upland rice and maize production. *Agroforestry Systems* 20:213-218.

McGrath, D., & Binkley, M. A. (2009). *Microstegium vimineum* invasion changes soil chemistry and microarthropod communities in Cumberland Plateau forests. Southeastern Naturalist, 8(1), 141-156.

McKersie, B. D., Bowley, S. R., & Jones, K. S. (1999). Winter survival of transgenic alfalfa overexpressing superoxide dismutase. *Plant Physiology*, *119*(3), 839-848.

McLendon, T., & Redente, E. F. (1992). Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. *Oecologia*, 91(3), 312-317.

Meena, V. S., Maurya, B. R., & Verma, J. P. (2014). Does a rhizospheric microorganism enhance K+ availability in agricultural soils?. *Microbiological research*, *169*(5-6), 337-347.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews*, *37*(5), 634-663.

Mikiciński, A., Sobiczewski, P., Puławska, J., & Maciorowski, R. (2016). Control of fire blight (*Erwinia amylovora*) by a novel strain 49M of *Pseudomonas graminis* from

the phyllosphere of apple (Malus spp.). *European Journal of Plant Pathology*, *145*, 265-276.

Mikola, J., & Setälä, H. (1998). Productivity and trophic-level biomasses in a microbial-based soil food web. *Oikos*, 158-168.

Mikola, J., Yeates, G. W., Barker, G. M., Wardle, D. A., & Bonner, K. I. (2001). Effects of defoliation intensity on soil food-web properties in an experimental grassland community. *Oikos*, *92*(2), 333-343.

Miller, G., Shulaev, V., & Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. *Physiologia plantarum*, *133*(3), 481-489.

Mishra, S., Srivastava, S., Tripathi, R.D., Govindrajan, R., Kuriakose, S.V., Prasad, M.N.V., (2006). Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant Physiol. Biochem.* 44, 25–37.

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature*, 421(6923), 625-627.

Mitra, S. (Ed.). (2004). *Sample preparation techniques in analytical chemistry*. John Wiley & Sons.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, *7*(9), 405-410.

Mongkol, R., & Chavasiri, W. (2018). Antimicrobial and weed inhibitory activities of Senna spectabilis extracts against plant pathogens. *Int J Agric Technol*, *14*(7), 1445-1454.

Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, *98*(10), 5446-5451.

Moravcová, L., Pyšek, P., Jarošík, V., and Pergl, J. (2015). Getting the right traits: reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. *PLoS One*, 10, e0123634.

Morgan, L. M., Cory, C. C., Ylva, L., John, L. M., Laurent, P., David, B., Callaway, R. M. (2016). Exotic invasive plants increase productivity, abundance of ammonia-oxidizing bacteria and nitrogen availability in intermountain grasslands. *Journal of Ecology,* 104(4), 994-1002.

Morris, K. A., Stark, J. M., Bugbee, B., & Norton, J. M. (2016). The invasive annual cheatgrass releases more nitrogen than crested wheatgrass through root exudation and senescence. *Oecologia*, *181*, 971-983.

Murali, M., Amruthesh, K. N., Sudisha, J., Niranjana, S. R., & Shetty, H. S. (2012). Screening for plant growth promoting fungi and their ability for growth promotion

and induction of resistance in pearl millet against downy mildew disease. *Journal of Phytology*, *4*(5).

Muzell Trezzi, M., Vidal, R. A., Balbinot Junior, A. A., von Hertwig Bittencourt, H., & da Silva Souza Filho, A. P. (2016). Allelopathy: driving mechanisms governing its activity in agriculture. *Journal of Plant Interactions*, *11*(1), 53-60.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology*, *22*(5), 867-880.

NARO., (2004). Capacity building, training needs assessment, dissemination and replication strategy for invasive plant management in Uganda. Report submitted to CABI under the PDF-B phase of the UNEP/GEF-IAS Project; Removing Barriers to Invasive Plant Management in Africa. NARO, Entebbe, Uganda.

Nebo, L., Varela, R. M., Molinillo, J. M., Severino, V. G., Sarria, A. L., Cazal, C. M., ... & Macias, F. A. (2015). Phytotoxicity of triterpenes and limonoids from the Rutaceae and Meliaceae. 5α, 6β, 8α, 12α-tetrahydro-28-norisotoonafolin–a potent phytotoxin from *Toona ciliata*. *Natural Product Communications,* 10(1), 1934578X1501000107.

Neiva, I. P., Silva, A. A. D., Resende, J. F., Carvalho, R. D. C., Oliveira, A. M. S. D., & Maluf, W. R. (2019). Tomato genotype resistance to whitefly mediated by allelochemicals and Mi gene. *Chilean journal of agricultural research*, *79*(1), 124-130.

Ngwene, B., Boukail, S., Söllner, L., Franken, P., & Andrade-Linares, D. R. (2016). Phosphate utilization by the fungal root endophyte Piriformospora indica. *Plant and Soil*, *405*, 231-241.

Nic Lughadha, E., Bachman, S. P., Leão, T. C., Forest, F., Halley, J. M., Moat, J., ... & Walker, B. E. (2020). Extinction risk and threats to plants and fungi. Plants, People, Planet, 2(5), 389-408.

Nikolaeva, A. A., Golosova, E. V., & Shelepova, O. V. (2021). Allelopathic activity of *Acer negundo* L. leaf litter as a vector of invasion species into plant communities. In *BIO Web of Conferences* (Vol. 38, p. 00088). EDP Sciences.

Nilsson, M. C. (1994). Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. *Oecologia*, 98, 1-7.

Nishanthini, A., Mohan, V. R., & Jeeva, S. (2014). Phytochemical, ft-ir, and gc-ms analysis of stem and leaf of *Tiliacora acuminata* (lan.) Hook f & thomas

(menispermaceae). *International Journal of Pharmaceutical Sciences and Research*, *5*(9), 3977.

Nunes, O. C., Manaia, C. M., Kolvenbach, B. A., & Corvini, P. F. X. (2020). Living with sulfonamides: a diverse range of mechanisms observed in bacteria. *Applied microbiology and biotechnology*, *104*(24), 10389-10408.

Oliveira, M. T., Souza, G. M., Pereira, S., Oliveira, D. A., Figueiredo-Lima, K. V., Arruda, E., and Santos, M. G. (2017). Seasonal variability in physiological and anatomical traits contributes to invasion success of *Prosopis juliflora* in tropical dry forest. *Tree Physiology*, 37, 326-337.

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html.

Omezzine, F., Ladhari, A., & Haouala, R. (2014). Physiological and biochemical mechanisms of allelochemicals in aqueous extracts of diploid and mixoploid *Trigonella foenum-graecum* L. South *African journal of botany*, 93, 167-178.

Oracz, K., Bailly, C., Gniazdowska, A., Côme, D., Corbineau, F., & Bogatek, R. (2007). Induction of oxidative stress by sunflower phytotoxins in germinating mustard seeds. *Journal of chemical ecology*, *33*, 251-264.

Ozgur, R., Uzilday, B., Sekmen, A. H., & Turkan, I. (2013). Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology*, *40*(9), 832-847.

Pakeman, R. J., & Quested, H. M. (2007). Sampling plant functional traits: what proportion of the species need to be measured?. *Applied Vegetation Science*, *10*(1), 91-96.

Palazzini, J., Roncallo, P., Cantoro, R., Chiotta, M., Yerkovich, N., Palacios, S., Echenique, V., Torres, A., Ramirez, M., Karlovsky, P & Chulze, S. (2018). Biocontrol of *Fusarium graminearum* sensu stricto, reduction of deoxynivalenol accumulation and phytohormone induction by two selected antagonists. *Toxins*, *10*(2), 88.

Parida, A.K., Jha, B., 2010. Antioxidative defense potential to salinity in the euhalophyte *Salicornia brachiata*. *J. Plant Grow. Regul*. 29, 137–148.

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., ... & Von Holle, B. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, 1, 3-19.

Parker, J. D., Torchin, M. E., Hufbauer, R. A., Lemoine, N. P., Alba, C., Blumenthal, D. M., ... & Wolfe, L. M. (2013). Do invasive species perform better in their new ranges?. *Ecology*, 94(5), 985-994.

Patel, M. V., & Patel, R. K. (2014). Indole-3-acetic acid (IAA) production by endophytic bacteria isolated from saline dessert, the little Runn of Kutch. *CIBTech Journal of Microbiology*, *3*(2), 2319-3867.

Pawlak, S., Firych, A., Rymer, K., & Deckert, J. (2009). Cu, Zn-superoxide dismutase is differently regulated by cadmium and lead in roots of soybean seedlings. Acta Physiologiae Plantarum, 31, 741-747.

Pedro, A. C., Granato, D., & Rosso, N. D. (2016). Extraction of anthocyanins and polyphenols from black rice (Oryza sativa L.) by modeling and assessing their reversibility and stability. *Food Chemistry*, *191*, 12-20.

Pejchar, L., & Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. Trends in ecology & evolution, 24(9), 497-504.

Pélagie Michelin, K. T., Jean, A. N., Donatien, G., Paul Keilah, L., Stephen, L. T., & Jules-Roger, K. (2016). In vitro allelopathic effects of extracts and fractions of five plants on tomato seed germination and vigor index. *Cogent biology*, *2*(1), 1220661.

Pereira, J. S. (2007). Uma espécie altamente produtiva. *Pinhais e Eucaliptais, a Eloresta Cultivada. Público, FLAD, LPN, Lisbon*, 167-183.

Peter, A., Žlabur, J. Š., Šurić, J., Voća, S., Purgar, D. D., Pezo, L., & Voća, N. (2021). Invasive plant species biomass—Evaluation of functional value. *Molecules*, *26*(13), 3814.

Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. (2012). Hormonal modulation of plant immunity. *Annual review of cell and developmental biology*, *28*, 489-521.

Pikovaskya, R.T., (1948). Mobilization of herbicides in soil connection with vital activities of some microbial count. *Microbiologia* 17, 362–370.

Piršelová, B., Lengyelová, L., Galuščáková, Ľ., & Kuna, R. (2019). Impact of sugar beet shoot exudates on germination and root growth of wheat, barley and maize. *Listy Cukrovarnické a Reparské*, *135*(3), 112.

Prach, K., & Pyšek, P. (2001). Using spontaneous succession for restoration of human-disturbed habitats: experience from Central Europe. *Ecological Engineering*, *17*(1), 55-62.

Prajapati, K. B., & Modi, H. A. (2012). Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. *CIBTech J Microbiol*, *1*(2-3), 8-14.

Prajitha, T. and Sudhabai, R. (2022). Invasion of Alien *Senna spectabilis* (DC.) H.S. Irwin and Barneby. Causes loss of natural vegetation in the Wayanad Wildlife Sanctuary

of India and edaphic factors of the invaded areas assist its profuse sprouting. *Indian Forester*, 148(7): 724-732.

Prati, D., & Bossdorf, O. (2004). Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *American journal of Botany*, *91*(2), 285-288.

Pratley, J. E., Dowling, P., & Medd, R. (1996). Allelopathy in annual grasses. *Plant Protection Quarterly*, *11*, 213-214.

Prawoto, A. (2006). A Study of Allelopathy of Some Shade Trees to Coffea arabicaL. Seedlings. *Pelita Perkebunan (a Coffee and Cocoa Research Journal)*, *22*(1).

Preece, K. E., Hooshyar, N., Krijgsman, A. J., Fryer, P. J., & Zuidam, N. J. (2017). Pilot-scale ultrasound-assisted extraction of protein from soybean processing materials shows it is not recommended for industrial usage. *Journal of Food Engineering*, *206*, 1-12.

Puente-Sánchez F, Arce-Rodríguez A, Oggerin M, García-Villadangos M, Moreno-Paz M, Blanco Y, Rodríguez N, Bird L, Lincoln SA, Tornos F, Prieto-Ballesteros O (2018) Viable cyanobacteria in the deep continental subsurface. Proc Natl Acad Sci 115(42):10702–10707

Putten, W. V. D., Yeates, G. W., Duyts, H., Reis, C. S., & Karssen, G. (2005). Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges. *Biological invasions*, *7*, 733-746.

Pyšek, P. (2001). Past and future of predictions in plant invasions: a field test by time. *Diversity and Distributions*, *7*(3), 145-151.

Pyšek, P., & Richardson, D. M. (2010). Invasive species, environmental change and management, and health. *Annual review of environment and resources*, *35*(1), 25-55.

Pyšek, P., Jarošík, V., & Pergl, J. (2011). Alien plants introduced by different pathways differ in invasion success: unintentional introductions as a threat to natural areas. PloS one, 6(9), e24890.

Pyšek, P., Jarošík, V., Hulme, P. E., Pergl, J., Hejda, M., Schaffner, U., & Vilà, M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species traits and environment. *Global Change Biology*, 18(5), 1725-1737.

Pyšek, P., Jarošík, V., Hulme, P. E., Pergl, J., Hejda, M., Schaffner, U., & Vilà, M. (2012). A global assessment of invasive plant impacts on resident species, communities and

ecosystems: the interaction of impact measures, invading species' traits and environment. *Global change biology*, *18*(5), 1725-1737.

Pyšek, P., Jarošík, V., Pergl, J., Randall, R., Chytrý, M., Kühn, I., Tichý, L., Danihelka, J., Chrtek jun, J. Sádlo, J. (2009). The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. *Diversity and Distributions*, 15(5), 891-903.

Pyšek, P., Jarošík, V., Pergl, J., Randall, R., Chytrý, M., Kühn, I., Tichý, L., Danihelka, J., Chrtek jun, J. and Sádlo, J., 2009. The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. *Diversity and Distributions*, *15*(5), pp.891-903.

Pyšek, P., Křivánek, M., and Jarošík, V. (2009). Planting intensity, residence time, and species traits determine invasion success of alien woody species. *Ecology*, 90, 2734-2744.

Pyšek, P., Prach, K., and Smilauer, P. (1995). Relating invasion success to plant traits: an analysis of the Czech alien flora. *Plant Invasions: General Aspects and Special Problems*, 39-60.

Qamar N, Rehman Y, Hasnain S (2017) Arsenic-resistant and plant growth-promoting Firmicutes and γ-Proteobacteria species from industrially polluted irrigation water and corresponding cropland. J Appl Microbiol 123(3):748–758

Qi, M., Ma, X., Liang, B., Zhang, L., Kong, D., Li, Z., & Wang, A. (2022). Complete genome sequences of the antibiotic sulfamethoxazole-mineralizing bacteria Paenarthrobacter sp. P27 and Norcardiodes sp. N27. *Environmental Research*, *204*, 112013.

Qu, T., Du, X., Peng, Y., Guo, W., Zhao, C., & Losapio, G. (2021). Invasive species allelopathy decreases plant growth and soil microbial activity. *PloS one*, *16*(2), e0246685.

R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R version 4.3.1 using the Vegan Package

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed research international*, *2014*.

Rahi, P., Giram, P., Chaudhari, D., Kiran, S., Khullar, A., Chandel, M., ... & Mahajan, B. (2020). Rhizobium indicum sp. nov., isolated from root nodules of pea (Pisum

sativum) cultivated in the Indian trans-Himalayas. *Systematic and Applied Microbiology*, *43*(5), 126127.

Rahmonov, O. (2009). The chemical composition of plant litter of black locust (*Robinia pseudoacacia* L.) and its ecological role in sandy ecosystems. Acta Ecologica Sinica, 29(4), 237-243.

Rai, P. K. (2022). Environmental degradation by invasive alien plants in the anthropocene: challenges and prospects for sustainable restoration. *Anthropocene Science*, *1*(1), 5-28.

Rai, P. K., & Singh, J. S. (2020). Invasive alien plant species: Their impact on environment, ecosystem services and human health. *Ecological indicators*, *111*, 106020.

Raman, B. V., Samuel, L. A., Saradhi, M. P., Rao, B. N., Krishna, N. V., Sudhakar, M., & Radhakrishnan, T. M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian J Pharm Clin Res*, *5*(2), 99-106.

Rashid, M.H., Takashi, A., Uddin, M.N., 2010. The allelopathic potential of Kudzu (*Pueraria montana*). *Weed Sci*. 58, 47–55.

Rastogi G, Osman S, Kukkadapu R, Engelhard M, Vaishampayan PA, Andersen GL, Sani RK (2010) Microbial and mineralogical characterizations of soils collected from the deep biosphere of the former Homestake gold mine. South Dakota MicrobEcol 60(3):539–550 43.

Reinhart, K. O., Greene, E., & Callaway, R. M. (2005). Effects of Acer platanoides invasion on understory plant communities and tree regeneration in the northern Rocky Mountains. Ecography, 28(5), 573-582.

Reinhart, K. O., Packer, A., Van der Putten, W. H., & Clay, K. (2003). Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, *6*(12), 1046-1050.

Rejmánek, M. and Richardson, D. M. (1996). What attributes make some plant species more invasive?. *Ecology*, 77, 1655-1661.

Rejmanek, M., & Richardson, D. M. (1996). What attributes make some plant species more invasive?. *Ecology*, *77*(6), 1655-1661.

Rice, E. L. (1992). Allelopathic effects on nitrogen cycling. In *Allelopathy: basic and applied aspects* (pp. 31-58). Dordrecht: Springer Netherlands.

Rice, E. L. (2012). Allelopathy.

Rice, E.L., 1984. Allelopathy, Second edition. Academic Press, Inc., Orlando pp. 1–7.

Richardson, D. M., Pyšek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions,* 6(2), 93-107.

Ridenour, W. M., & Callaway, R. M. (2001). The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia*, *126*, 444-450.

Rietveld, W. J. (1983). Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. *Journal of Chemical Ecology*, *9*, 295-308.

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New phytologist*, *171*(1), 41-53.

Rodrigues, R. R., Pineda, R. P., Barney, J. N., Nilsen, E. T., Barrett, J. E., & Williams, M. A. (2015). Plant invasions associated with change in root-zone microbial community structure and diversity. PLoS ONE, 10(10), e0141424.

Roni, M. Z. K., Islam, M. S., & Shimasaki, K. (2018). In vitro seed germination and tracking the seedling growth of eustoma. *New Zealand journal of crop and horticultural science*, *46*(3), 224-242.

Rout, M. E., & Callaway, R. M. (2012). Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is not everywhere'. *Annals of Botany*, *110*(2), 213-222.

Rout, M. E., & Southworth, D. (2013). The root microbiome influences scales from molecules to ecosystems: the unseen majority1. *American journal of botany*, *100*(9), 1689-1691.

Roy, P., & Kumar, A. (2020). Arthrobacter. In *Beneficial Microbes in Agro-Ecology* (pp. 3-11). Academic Press.

Rusterholz, H. P., Schneuwly, J., & Baur, B. (2018). Invasion of the alien shrub Prunus laurocerasus in suburban deciduous forests: Effects on native vegetation and soil properties. Acta Oecologica, 92, 44-51.

Ryabchenko, B., Tulupova, E., Schmidt, E., Wlcek, K., Buchbauer, G., & Jirovetz, L. (2008). Investigation of anticancer and antiviral properties of selected aroma samples. *Natural Product Communications*, *3*(7), 1934578X0800300710.

Saadatnia H, Riahi H (2009) Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. Plant Soil Environ 55(5):207–212

Saeidian, S. (2013). Inhibitory effect of cysteine and glycine upon partial purified polyphenol oxidase of Pyrus communis. *Eur. J. Exp. Biol*, *3*, 476-483.

Sahu, A., & Devkota, A. (2013). Allelopathic effects of aqueous extract of leaves of *Mikania micrantha* HBK on seed germination and seedling growth of Oryza sativa L. and Raphanus sativus L. *Scientific World*, *11*(11), 90-93.

Sala, A., Smith, S. D., & Devitt, D. A. (1996). Water use by *Tamarix ramosissima* and associated phreatophytes in a Mojave Desert floodplain. *Ecological Applications*, *6*(3), 888-898.

Sampietro, D. A., Vattuone, M. A., & Isla, M. I. (2006). Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. *Journal of Plant Physiology*, 163(8), 837-846.

Sánchez, V. (2014). Characterization of Rhizobial Diversity and Relationship of Rhizobial Partner and Legume Performance in Four South Florida Pine Rockland Soils.

Sand, E. R. (2016). Government of Kerala District Survey Report.

Sankaran K.V., Sajeev T.V. and Suresh T.A. (2014). Invasive plant threats to forests in the humid tropics: a case study from Kerala state, Invasive Alien Species Management, India.

Sankaran, K. V., Suresh, T. A., & Sajeev, T. V. (2012). *Handbook on invasive plants of Kerala*. Kerala State Biodiversity Board.

Sanon, A., Beguiristain, T., Cebron, A., Berthelin, J., Ndoye, I., Leyval, C., Sylla, S. and Duponnois, R. (2009). Changes in soil diversity and global activities following invasions of the exotic invasive plant, *Amaranthus viridis* L., decrease the growth of native sahelian Acacia species. *FEMS Microbiology Ecology*, *70*(1), 118-131.

Santos, C. C. D. M. P., Salvadori, M. S., Mota, V. G., Costa, L. M., de Almeida, A. A. C., de Oliveira, G. A. L., Costa, J.P., de Sousa, D.P., de Freitas, R.M. and de Almeida, R. N. (2013). Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. *Neuroscience Journal*, *2013*.

Sathya A, Vijayabharathi R, Gopalakrishnan S (2017) Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech 7(2):102

Satyanarayana, P., & Gnanasekaran, G. (2013). An exotic tree Species Senna spectabilis (DC.) Irwin & Barneby (Caesalpiniaceae) naturalized in Tamil Nadu and Kerala. Indian Journal of Forestry, 36(2), 243-246.

Satyaprakash, M., Nikitha, T., Reddi, E. U. B., Sadhana, B., & Vani, S. S. (2017). Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 2133-2144.

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant physiology*, *101*(1), 7.

Scavo, A., Restuccia, A., & Mauromicale, G. (2018). Allelopathy: principles and basic aspects for agroecosystem control. *Sustainable agriculture reviews 28: ecology for agriculture*, 47-101.

Schmidt, J. P. and Drake, J. M. (2011). Time since introduction, seed mass, and genome size predict successful invaders among the cultivated vascular plants of Hawaii. *PLoS One*, 6, e17391.

Schnitzler, A. (1994). Conservation of biodiversity in alluvial hardwood forests of the temperate zone. The example of the Rhine valley. Forest ecology and management, 68(2-3), 385-398.

Schopfer, P., Plachy, C.& Frahry, G., (2001). Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide & hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol*. 125, 1591–1602.

Sekmen, A. H., Turkan, I., Tanyolac, Z. O., Ozfidan, C., & Dinc, A. (2012). Different antioxidant defence responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. *Environmental and Experimental Botany*, *77*, 63-76.

Selegato, D. M., Monteiro, A. F., Vieira, N. C., Cardoso, P., Pavani, V. D., Bolzani, V. S., & Castro-Gamboa, I. (2017). Update: Biological and chemical aspects of Senna spectabilis. *Journal of the Brazilian Chemical Society*, *28*, 415-426.

Shahab, S., Ahmed, N., & Khan, N. S. (2009). Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *African Journal of Agricultural Research*, *4*(11), 1312-1316.

Shahbaz, M., Mushtaq, Z., Andaz, F. & , Masood, A., (2013). Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L.)? *Sci. Horticult.* 164, 507–511.

Shea, K., & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, 17(4), 170-176.

Shi, Y., Lou, K., & Li, C. (2009). Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biology and Fertility of Soils*, 45, 645-653.

Shivanna, M. B., Meera, M. S., Kageyama, K., & Hyakumachi, M. (1996). Growth promotion ability of zoysiagrass rhizosphere fungi in consecutive plantings of wheat and soybean. *Mycoscience*, *37*, 163-168.

Shuvar, I., Korpita, H., Shuvar, A., Shuvar, B., & Kropyvnytskyi, R. (2021). Invasive plant species and the consequences of its prevalence in biodiversity. In *BIO Web of Conferences* (Vol. 31, p. 00024). EDP Sciences.

Siemann, E., & Rogers, W. E. (2001). Genetic differences in growth of an invasive tree species. *Ecology Letters*, *4*(6), 514-518.

Silva, F. D. O., Oliveira, Í. R. D., Silva, M. G. D. V., & Braz-Filho, R. (2010). Constituintes químicos das folhas de Senna spectabilis (DC) Irwin & Barneby var. excelsa (Schrad.) Irwin & Barneby. *Química Nova*, *33*, 1874-1876.

Simba, Y. R., Kamweya, A. M., Mwangi, P. N. & Ochora, J. M. (2013). Impact of the Exotic Weed, *Lantana camara* L. on Abundance of Native Plants in Nairobi National Park, Kenya: Implications for the Conservation of Wildlife. International Journal of Science and Research. 2: 294-300.

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81-102.

Simberloff, D. (2015). Non-native invasive species and novel ecosystems. F1000prime reports, 7.

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., ... & Vilà, M. (2013). Impacts of biological invasions: what's what and the way forward. Trends in ecology & evolution, 28(1), 58-66.

Singh, N.B., Sunaina., 2014. Allelopathic Stress produced by bitter gourd (*Momordica charantia*L.). J. Stress Physiol. Biochem. 10 (2), 5-14.

Singh, S., Mahajan, E., & Sohal, S. K. (2023). Immunomodulatory, cyto-genotoxic, and growth regulatory effects of nerolidol on melon fruit fly, *Zeugodacuscucurbitae* (Coquxillett)(Diptera: Tephritidae). Toxicon, 107248.

Singh, V. (2001). *Monograph on Indian subtribe Cassiinae (Caesalpiniaceae)* (pp. xviii+-279).

Sîrbu, C., Miu, I. V., Gavrilidis, A. A., Grădinaru, S., Niculae, M. I., Preda, C., ... & Anastasiu, P. (2022). Distribution and pathways of introduction of invasive alien plant species in Romania.

Sîrbu, C., Miu, I.V., Gavrilidis, A.A., Grădinaru, S., Niculae, M.I., Preda, C., Oprea, A., Urziceanu, M., Camen Comănescu, P., Nagoda, E. and Sîrbu, I.M. (2022). Distribution and pathways of introduction of invasive alien plant species in Romania.

Sivagurunathan, M., Devi, G. S., & Ramasamy, K. (1997). Allelopathic compounds in Eucalyptus spp. 313-320.

Skidmore, A. M., & Dickinson, C. H. (1976). Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. *Transactions of the British Mycological Society*, *66*(1), 57-64.

Smith, S. E., & Read, D. J. (1997). Mycorrhizal symbiosis. ix+-605.

Song, M., Li, X., Saikkonen, K., Li, C., & Nan, Z. (2015). An asexual Epichloë endophyte enhances waterlogging tolerance of *Hordeum brevisubulatum*. *Fungal Ecology*, *13*, 44-52.

Sprent, J. I., & Platzmann, J. (2001). *Nodulation in legumes* (p. 146p). Kew: Royal Botanic Gardens.

Stachel, S. E., Messens, E., Van Montagu, M., & Zambryski, P. (1985). Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature*, *318*(6047), 624-629.

Stamp, N., (2003). Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.* 78, 23–55.

Steenackers, W., Klíma, P., Quareshy, M., Cesarino, I., Kumpf, R.P., Corneillie, S., Araújo, P., Viaene, T., Goeminne, G., Nowack, M.K. and Ljung, K., 2017. cis-Cinnamic acid is a novel, natural auxin efflux inhibitor that promotes lateral root formation. *Plant Physiology*, *173*(1), 552-565.

Stefanowicz, A. M., Majewska, M. L., Stanek, M., Nobis, M. & Zubek, S. (2018). Differential influence of four invasive plant species on soil physicochemical properties in a pot experiment. Journal of Soils and Sediments. 18(4): 1409-1423.

Stinson, K., Kaufman, S., Durbin, L., & Lowenstein, F. (2007). Impacts of garlic mustard invasion on a forest understory community. Northeastern naturalist, 14(1), 73-88.

Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D. and Klironomos, J.N., 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS biology*, *4*(5), e140.

Stone, B. W., Weingarten, E. A., & Jackson, C. R. (2018). The role of the phyllosphere microbiome in plant health and function. *Annual plant reviews online*, 533-556.

Stres, B., Danevčič, T., Pal, L., Fuka, M. M., Resman, L., Leskovec, S., ... & Mandic-Mulec, I. (2008). Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. *FEMS microbiology ecology*, *66*(1), 110-122.

Sunmonu,T.O., & VanStaden, J., (2014). Phytotoxicity evaluation of six fast- growing tree species in SouthAfrica. *South Afric. J. Bot*. 90, 101–106.

Tahira, L. S., Tino, R. A., Bento, C. C., Tangerina, M. M. P., de Almeida, L. F. R., Franco, D. M., Sartori, A. L.B. and Sannomiya, M. (2021). The lupeol content in *Machaerium* species by HPLC-APCI-MS/MS and the allelopathic action. *Journal of Horticulture and Forestry,* 13(2), 44-50.

Talapatra, K., Das, A. R., Saha, A. K., & Das, P. (2017). In vitro antagonistic activity of a root endophytic fungus towards plant pathogenic fungi. *Journal of Applied Biology and Biotechnology*, *5*(2), 068-071.

Thang, P. T., Vien, N. V., Anh, L. H., Xuan, T. D., Duong, V. X., Nhung, N. T., ... & Ha, T. T. T. (2023). Assessment of allelopathic activity of *Arachis pintoi* Krapov. & WC Greg as a potential source of natural herbicide for paddy rice. *Applied Sciences*, 13(14), 8268.

Thapar, R., Singh, N.B., (2006). Effects of leaf— residues of *Croton bonplandianum*on growth and metabolism of *Parthenium hysterophorus*. Allelopathy J. 18, 255–266.

Thiébaut, G., Tarayre, M., & Rodríguez-Pérez, H. (2019). Allelopathic effects of native versus invasive plants on one major invader. *Frontiers in plant science*, *10*, 854.

Thompson, K., Hodgson, J. G., Grime, J. P., and Burke, M. J. (2001). Plant traits and temporal scale: evidence from a 5-year invasion experiment using native species. *Journal of Ecology*, 89, 1054-1060.

Thorpe, A. S., Thelen, G. C., Diaconu, A., & Callaway, R. M. (2009). Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *Journal of Ecology*, 641-645.

Tian, L., Shi, S., Ma, L., Tran, L. S. P., & Tian, C. (2020). Community structures of the rhizomicrobiomes of cultivated and wild soybeans in their continuous cropping. *Microbiological research*, *232*, 126390.

Tian, X. J., Shen, Y. M., & Tao, H. Z. (2015). Allelopathy of invasive *Tithonia diversifolia* on Bidens pilosa. *Ecol Environ Sci*, *24*(7), 1128-1131.

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction. P 179-244. *Biology of anaerobic organisms. John Wiley and Sons. New York, NY*.

Top, S. M., Preston, C. M., Dukes, J. S., & Tharayil, N. (2017). Climate influences the content and chemical composition of foliar tannins in green and senesced tissues of Quercus rubra. *Frontiers in plant science*, *8*, 423.

Torres, N., Herrera, I., Fajardo, L., & Bustamante, R. O. (2021). Meta-analysis of the impact of plant invasions on soil microbial communities. BMC Ecology and Evolution, 21, 1-8.

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current opinion in microbiology*, *5*(3), 240-245.

Tresch, S. (2013). Strategies and future trends to identify the mode of action of phytotoxic compounds. *Plant science*, *212*, 60-71.

Tsavkelova, E. A., Cherdyntseva, T. A., & Netrusov, A. I. (2005). Auxin production by bacteria associated with orchid roots. *Microbiology*, *74*, 46-53.

Turk, M. A., & Tawaha, A. M. (2003). Allelopathic effect of black mustard (Brassica nigra L.) on germination and growth of wild oat (Avena fatua L.). *Crop protection*, *22*(4), 673-677.

Valone, T. J., & Weyers, D. P. (2019). Invasion intensity influences scale-dependent effects of an exotic species on native plant diversity. *Scientific reports*, *9*(1), 18769.

Van der Putten, W. H. (2010). Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology & Evolution*, *25*(9), 512-519.

Van der Putten, W. H., Klironomos, J. N., & Wardle, D. A. (2007). Microbial ecology of biological invasions. *The ISME journal*, 1(1), 28-37.

van Kleunen M, Essl F, Pergl J, Brundu G, Carboni M, Dullinger S, Early R, González-Moreno P, Groom QJ, Hulme PE, Kueffer C, Kühn I, Máguas C, Maurel N, Novoa A, Parepa M, Pyšek P, Seebens H, Tanner R, Touza J, Verbrugge L, Weber E, Dawson W, Kreft H, Weigelt P, Winter M, Klonner G, Talluto MV, Dehnen-Schmutz K (2018) The changing role of ornamental horticulture in alien plant invasions. *Biol Rev* 93:1421–1437.

van Kleunen M, Xu X, Yang Q, Maurel N, Zhang Z, Dawson W, Essl F, Kreft H, Pergl J, Pyšek P, Weigelt P, Moser D, Lenzner B, Fristoe TS (2020) Economic use of plants is key to their naturalization success. *Nat Comm* 11:3201.

Van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J. M., & Fischer, M. (2010). Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology letters*, *13*(8), 947-958.

Van Kleunen, M., Weber, E., and Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, 13, 235-245.

Vanderhoeven, S., Dassonville, N., & Meerts, P. (2005). Increased topsoil mineral nutrient concentrations under exotic invasive plants in Belgium. *Plant and soil*, 275, 169-179.

Varsha-Narsian, J., Thakkar, J., & Patel, H. H. (1995). Inorganic phosphate solubilization by some yeast. *Indian J Microbiol*, *35*, 113-118.

Varughese, A. S., & Joseph, S. (2023). Influence of invasive plant species on soil properties in tropical riparian zones. Poll Res. 42 (1): 105-109.

Vieira, S., Sikorski, J., Dietz, S., Herz, K., Schrumpf, M., Bruelheide, H., Scheel, D., Friedrich, M. W. and Overmann, J. (2020). Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *The ISME journal*, *14*(2), 463-475.

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y. and Pyšek, P. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities, and ecosystems. *Ecology Letters*, 14(7), 702-708.

Vinayan, P. A., Anjankumar, B. N., Vishnu, N. M., Vaishnav, K., Unais, P., Ajayan, P. A., & Anoop, N. R. (2020). Mapping the distribution and abundance of the exotic invasive species, Senna spectabilis in the Wayanad Wildlife Sanctuary, Kerala. *Ferns-A society for nature conservation & Kerala Forests and Wildlife Department*.

Vinholes, J., Rudnitskaya, A., Gonçalves, P., Martel, F., Coimbra, M. A., & Rocha, S. M. (2014). Hepatoprotection of sesquiterpenoids: a quantitative structure–activity relationship (QSAR) approach. *Food Chemistry*, *146*, 78-84.

Vitousek, P. M., & Walker, L. R. (1989). Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological monographs*, 59(3), 247-265.

Vitousek, P. M., d'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American scientist*, *84*(5), 468-478.

Volga, V. R., Narayanan, M. R., & Kumar, N. A. (2013). Endemic trees of Western Ghats-A check list from Wayanad district, Kerala, India. International journal of plant, animal and environmental sciences, 3(2), 197-202.

Vranová, E., Inzé, D., & Van Breusegem, F. (2002). Signal transduction during oxidative stress. Journal of experimental botany, 53(372), 1227-1236.

Vujanović, D., Losapio, G., Milić, S., & Milić, D. (2022). The impact of multiple species invasion on soil and plant communities increases with invasive species co-occurrence. *Frontiers in Plant Science*, *13*, 875824.

Wagg, C., & Bender, S. F. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences,* 111(14), 5266-5270.

Wakibara, J. V. (1998). Observations on the pilot control of Senna spectabilis, an invasive exotic tree in the Mahale Mountains National park, Western Tanzania. *Pan Africa News*, *5*(1), 4-6.

Wakibara, J. V., & Mnaya, B. J. (2002). Possible control of *Senna spectabilis* (Caesalpiniaceae), an invasive tree in Mahale mountains National Park, Tanzania. *Oryx*, *36*(4), 357-363.

Walker, L. R., & Smith, S. D. (1997). Impacts of invasive plants on community and ecosystem properties. In Assessment and management of plant invasions (pp. 69-86). New York, NY: Springer New York.

Wang, C., Liu, Z., Wang, Z., Pang, W., Zhang, L., Wen, Z., ... & Yang, C. (2022). Effects of autotoxicity and allelopathy on seed germination and seedling growth in *Medicago truncatula*. *Frontiers in Plant Science*, *13*, 908426.

Wang, W. B., Kim, Y. H., Lee, H. S., Kim, K. Y., Deng, X. P., & Kwak, S. S. (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant physiology and Biochemistry*, *47*(7), 570-577.

Wang, W.Q., Sardans, J., Wang, C., Zeng, C. S., Tong, C., Asensio, D. & Penuelas, J. (2015). Ecological stoichiometry of C, N, and P of invasive Phragmites australis and native *Cyperus malaccensis* species in the Minjiang River tidal estuarine wetlands of China. Plant Ecology. 216(6): 809-822.

Wang, X., Radwan, M. M., Taráwneh, A. H., Gao, J., Wedge, D. E., Rosa, L. H., Cutler, H.G. and Cutler, S. J. (2013). Antifungal activity against plant pathogens of metabolites from the endophytic fungus *Cladosporium cladosporioides*. *Journal of agricultural and food chemistry*, *61*(19), 4551-4555.

Wang, X., Wang, J., Zhang, R., Huang, Y., Feng, S., Ma, X., ... & Roy, R. (2018). Allelopathic effects of aqueous leaf extracts from four shrub species on seed germination and initial growth of *Amygdalus pedunculata* Pall. *Forests*, *9*(11), 711.

Wang, Z. H., & Jiang, X. J. (2022). Contrasting responses of the microbial community structure and functional traits to soil pH in Purple Soils. *Huan Jing ke Xue= Huanjing Kexue*, *43*(7), 3876-3883.

Wardle, D. A., Bellingham, P. J., Fukami, T., & Bonner, K. I. (2012). Soil-mediated indirect impacts of an invasive predator on plant growth. *Biology Letters*, *8*(4), 574-577.

Wardle, D. A., Yeates, G. W., Nicholson, K. S., Bonner, K. I., & Watson, R. N. (1999). Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period. *Soil Biology and Biochemistry*, *31*(12), 1707-1720.

Weir TL, Park SW, Vivanco JM (2004) Biochemical and physiological mechanisms mediated by allelochemicals. *Curr Opin Plant Biol* 7:472–479

Whitelaw, M. A. (1999). Growth promotion of plants inoculated with phosphate-solubilizing fungi. *Advances in agronomy*, *69*, 99-151.

Whitelaw, M. A. (1999). Growth promotion of plants inoculated with phosphate-solubilizing fungi. *Advances in agronomy*, *69*, 99-151.

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences*, *95*(12), 6578-6583.

Willekens, H., Inzé, D., Van Montagu, M., & Van Camp, W. (1995). Catalases in plants. *Molecular Breeding*, *1*, 207-228.

Williams, A., & de Vries, F. T. (2020). Plant root exudation under drought: implications for ecosystem functioning. *New Phytologist*, *225*(5), 1899-1905.

Wilson LT (2006) Cyanobacteria: a potential nitrogen source in rice fields. Tex Rice 6(9):e10

Wilson, J. R., Dormontt, E. E., Prentis, P. J., Lowe, A. J., & Richardson, D. M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution*, 24(3), 136-144.

Wolfe, B. E., & Klironomos, J. N. (2005). Breaking new ground: soil communities and exotic plant invasion. *Bioscience*, *55*(6), 477-487.

Wu, H., Pratley, J., Ma, W., & Haig, T. (2003). Quantitative trait loci and molecular markers associated with wheat allelopathy. *Theoretical and Applied Genetics*, *107*, 1477-1481.

Wu, J., Chen, Z., & Peng, S. (2006). Allelopathic potential of invasive weeds: Alternanthera philoxeroide, Ipomoea cairica and Spartina alterniflora. *Allelopathy Journal*, *17*(2), 279-286.

Xia, Z. C., Kong, C. H., Chen, L. C., & Wang, S. L. (2015). Allelochemical-mediated soil microbial community in long-term monospecific Chinese fir forest plantations. *Applied Soil Ecology*, *96*, 52-59.

Xia, Z. C., Kong, C. H., Chen, L. C., Wang, P., & Wang, S. L. (2016). A broadleaf species enhances an autotoxic conifers growth through belowground chemical interactions. *Ecology*, *97*(9), 2283-2292.

Xia, Z., Bai, E., Wang, Q., Gao, D., Zhou, J., Jiang, P., & Wu, J. (2016). Biogeographic distribution patterns of bacteria in typical Chinese forest soils. *Frontiers in microbiology*, *7*, 1106.

Xiao, H. F., Feng, Y. L., Schaefer, D. A., & Yang, X. D. (2014). Soil fungi rather than bacteria were modified by invasive plants, and that benefited invasive plant growth. *Plant and soil*, *378*, 253-264.

Xuan, T. D., Toyama, T., Fukuta, M., Khanh, T. D., & Tawata, S. (2009). Chemical interaction in the invasiveness of cogongrass (*Imperata cylindrica* (L.) Beauv.). *Journal of Agricultural and Food Chemistry*, *57*(20), 9448-9453.

Xue, Y., Xia, H., Christie, P., Zhang, Z., Li, L., & Tang, C. (2016). Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review. *Annals of Botany*, *117*(3), 363-377.

Yahya, A. I., & Al-Azawi, S. K. (1989). Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. *Plant and Soil*, *117*, 135-141.

Yan, J., Zhang, X., Chen, X., Wang, Y., Zhang, F., & Wan, F. (2016). Effects of rhizosphere soil microorganisms and soil nutrients on competitiveness of Bidens pilosa with different native plants. *Biodiversity Science*, *24*(12), 1381.

Yan, X. F., Du, Q., Fang, S., & Zhou, L. B. (2010). Allelopathic effects of water extraction of Rhus typhina on Zea mays seeds germination. *Seed*, *29*(3), 15-18.

Yang, H.J., Wang, Q., Wan, Z.X., Zhang, Z.Y., Chen, D.D. & Tan, J. (2021). Structure and diversity of microbial communities in the rhizosphere and non-rhizosphere soil in areas with invasive *Solidago canadensis* L. *J. Biosaf*., 30, 235–243. (In Chinese)

Yang, K. X., Wu, X. H., Zheng, F. P., Fan, Z. W., Wu, R. D., Xu, G. F., Ynag, Y. H., Zhang, F. D., Yang, S. S. and Shen, S. C. (2023). Allelopathic effects of aqueous extracts of the invasive plant *Acmella radicans* on seed germination and seedling growth of four weeds. *Acta Agrestia Sinica*, *31*(12), 3759.

YANG, K.X., WU, X.H., ZHENG, F.P., FAN, Z.W., WU, R.D., XU, G.F., YNAG, Y.H., ZHANG, F.D., YANG, S.S. and SHEN, S.C., 2023. Allelopathic effects of aqueous extracts of the invasive plant Acmella radicans on seed germination and seedling growth of four weeds. *Acta Agrestia Sinica*, *31*(12), p.3759.

Yang, X., Deng, S., De Philippis, R., Chen, L., Hu, C., & Zhang, W. (2012). Chemical composition of volatile oil from *Artemisia ordosica* and its allelopathic effects on desert soil microalgae, *Palmellococcus miniatus*. *Plant Physiology and Biochemistry*, *51*, 153-158.

Yang, Y. A. N. G., & Liu, B. R. (2015). Distribution of soil nutrient and microbial biomass in rhizosphere versus non-rhizosphere area of different plant species in desertified steppe. *Acta Ecol. Sin*, *35*(22), 7562-7570.

Yannarell, A. C., Busby, R. R., Denight, M. L., Gebhart, D. L., & Taylor, S. J. (2011). Soil bacteria and fungi respond on different spatial scales to invasion by the legume *Lespedeza cuneata*. *Frontiers in Microbiology*, 2, 1-12.

Ye, S. F., Zhou, Y. H., Sun, Y., Zou, L. Y., & Yu, J. Q. (2006). Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of Fusarium wilt. *Environmental and Experimental Botany*, *56*(3), 255-262.

Yu, J.Q., & Matsui, Y., 1997. Effect of root exudates of cucumber (*Cucumis sativus*) and allelochemicals on uptake by cucumber seedlings. *J. Chem. Ecol.* 23, 817–827.

Yushu, W. A. N. G., Haiyan, S. H. E. N. G., Shasha, L. U. O., Yueming, H. U., & Lingling, Y. U. (2021). Characteristics of prokaryotic microbial community structure and molecular ecological network in four habitat soils around Lake Qinghai. *Ecology and Environment*, *30*(7), 1393.

Zeng, J., Wu, L., Zhang, X., Liu, Y., Deng, G., & Li, D. (2008). Oct-2-en-4-ynoyl-CoA as a specific inhibitor of acyl-CoA oxidase. *Organic letters*, 10(19), 4287-4290.

Zhang, A., Gao, Y., Xu, Y., Lei, F., & Zhang, L. (2011). Advances in studies on allelopathy of medicinal plants in China. *Zhongcaoyao= Chinese Traditional and Herbal Drugs*, *42*(10), 1885-1890.

Zhang, K., Bao, W.K.; Yang, B. & Hu, B. (2017). The effects of understory vegetation on soil microbial community composition and structure. *Chin. J. Appl. Environ. Biol.,* 23, 1178–1184. (In Chinese)

Zhang, P., Cui, Z., Guo, M., & Xi, R. (2020). Characteristics of the soil microbial community in the forestland of Camellia oleifera. *PeerJ*, *8*, e9117.

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine*, *13*, 1-26.

Zhang, X. H., Zhang, E. H., & Lang, D. Y. (2011). Autotoxic compounds from rhizosphere soil of Humulus lupulus L. extracts: identification and biological activity. *Agronomy Journal*, *103*(3), 695-701.

Zhang, Z., Fu, Q., Xiao, C., Ding, M., Liang, D., Li, H., & Liu, R. (2022). Impact of *Paenarthrobacter ureafaciens* ZF1 on the soil enzyme activity and microbial community during the bioremediation of atrazine-contaminated soils. *BMC microbiology*, *22*(1), 146.

Zhang, Z., Qiao, M., Li, D., Zhao, C., Li, Y., Yin, H., & Liu, Q. (2015). Effects of two root-secreted phenolic compounds from a subalpine coniferous species on soil enzyme activity and microbial biomass. *Chemistry and Ecology*, *31*(7), 636-649.

Zheng, L., & Feng, Y. L. (2005). Allelopathic effects of *Eupatorium adenophorum* Spreng. on seed germination and seedling growth in ten herbaceous species. *Acta Ecologica Sinica*, *25*(10), 2782-2787.

Zheng, Y., Feng, Y., Valiente-Banuet, A., Li, Y., Liao, Z., Zhang, J., & Chen, Y. (2015). Are invasive plants more competitive than native conspecifics? Patterns vary with competitors. *Scientific Reports*, *5*(1), 15622.

Zhiqun, T., Jian, Z., Junli, Y., Chunzi, W., & Danju, Z. (2017). Allelopathic effects of volatile organic compounds from Eucalyptus grandis rhizosphere soil on Eisenia fetida assessed using avoidance bioassays, enzyme activity, and comet assays. *Chemosphere*, *173*, 307-317.

Zhou, L., Wu, J., & Wang, S. (2003). Low-temperature stratification strategies and growth regulators for rapid induction of *Paris polyphylla var. yunnanensis* seed germination. *Plant growth regulation*, *41*, 179-183.