CHAPTER 1
INTRODUCTION
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Plants have been used as a source of medicine science ancient time. The first written report of medicinal uses of plants was discovered at ‘Mesopotamia’ about 2600 BC (Cragg and Newman, 2005). According to the World Health Organization, approx. 80% of the world’s citizens rely for their primary health care on traditional medicines as most of the remedies use plants and plant products (Alves and Rosa, 2007; Bodeker et al., 2005; Bandaranayake, 2006). In the developing countries, remedies based on these plants have low cost with no side effects. Therefore, relatively high cost of synthetic medicines in developing countries often makes traditional herbal medicines an affordable choice for the people (Ekor, 2014). World trade of plant’s medicines and medicinal products is in billions of dollars. A current report suggests that global market for medicinal plants is around USD 60 billion growing at a vigorous pace of seven to eleven percent annually (Upadhyaya, 2017). There are at least 120 distinct chemical substances derived from plants, which are considered significant herbal drugs and represents a considerable proportion in the global market of drugs (Kong et al., 2003; Bandaranayake, 2006). In recent years, lot of efforts have been employed to identify novel molecules derived from the natural sources that exhibit a range of clinical and pharmacological activities. This has led to an extensive research on organic substances synthesized by various plants and microorganisms growing in diverse habitats and also displaying a varied range of habits.

Plant-endophyte interface provided insights into mutualism and metabolite fabrication by fungi to resist external biotic and abiotic stress, which in return benefits the host survival rate (Rajamanikyam et al., 2017). Endophytic fungi have been associated with plants for over 400 million years and considered as an important component of biodiversity (Krings et al., 2007; Rajamanikyam et al., 2017). The study of endophytes distribution, biodiversity and their biochemical characteristics has huge importance in plant sciences to comprehend and improve plant fitness (Sridhar and Raviraja, 1995; Selvanathan et al., 2011; Nair and Padmavathy, 2014; Verma et al., 2017). However, hidden potential of endophytic
fungi as promising sources of bioactive natural products continues to create a centre of attention, gives shed on the fungal biomass production to perk up the production of commercially important plant derived compounds with the participation of endophytic fungi (Strobel and Daisy, 2003; Wildman, 2003; Ashforth et al., 2010).

The term “endophyte”, originally introduced by de Bary (1866) refers to any organisms occurring within plant tissues, distinct from the epiphytes that live on plant surfaces. Since then, endophytes have been defined by various scientists (Wilson, 1995; Hallmann et al., 1997; Bacon and White, 2000; Pimentel et al., 2011; Subbulakshmi et al., 2012; Nair and Padmavathy, 2014; Singh and Dubey, 2015) as mutualists that colonise aerial parts of living plant tissues and do not cause symptoms of disease. Microorganisms associated with plants as compared to independent plants, have proved to offer material and products through high therapeutic potential. Carroll (1986), defined endophytes as microbes that colonize living, internal tissues of plants without causing any immediate harm, explicit negative effects”. Besides, extensively many researchers also defined endophyes as “Organisms that colonize internal plant tissues without causing any harm (Petrini et al., 1992; Petrini et al., 1993; Dreyfuss and Chapela, 1994; Bacon and White, 2000).

Historically, two endophytic groups clavicipitaceous (C) and non- clavicipitaceous (NC) have been reported with a large number of endophytic fungi. Clavicipitaceous or Balansiaceous endophytes are Class I endophytes represents a small number of phylogenetically related Clavicipitaceous species that are fastidious in culture and constrained to some grasses including repeatedly increase plant biomass, confer drought forbearance and produce chemicals that are toxic to animals and decrease herbivory (Stone et al., 2004; Bischoff et al., 2005; Mishra et al., 2014; Jalgaonwala et al., 2017). While, non- clavicipitaceous or non- balansiaceous endophytes are endophytes can be recovered from asymptomatic tissues of nonvascular plants, ferns, conifers, and angiosperms disseminated in plenty are extremely diversified relations with higher plants. Traditionally, these endophytes have been treated as a solitary group. However, on the basis of ecological interactions and difference in life history it has been further classified as class II endophytes, class III endophytes and class IV endophytes (Rodriguez et al., 2009). The first detailed description of a
Class II endophyte was a *Phoma* sp. in *Calluna vulgaris* (Rayner, 1915) and described as mycorrhizal, the fungus colonized all parts of the plant including the seed coat and did not form intracellular mycorrhizal structures. These endophytes in an individual host plant are quite limited and comprise a diversity of species, all of which are members of the Dikarya (Ascomycota or Basidiomycota) have ability to confer habitat specific stress tolerance to host plants (Rodriguez *et al*., 2008).

Class III endophytes are distinguished on the basis of their occurrence and horizontal transmission. These include vascular, non vascular plants, woody and herbaceous angiosperms in tropical forest and antarctic communities. Single plant may harbor hundreds of different endophytic fungi (Davis *et al*., 2003; Higgins *et al*., 2007; Murali *et al*., 2007). This also includes diverse endophytic fungi associated with leaves of tropical trees (Lodge *et al*., 1996; Frohlich and Hyde, 1999; Arnold *et al*., 2000; Gamboa & Bayman, 2001). Additionally, Melin (1922) reported a brown to blackish, pigmented fungus associated with terrestrial plant roots designated as ‘mycelium radicus astro virens’ (MRA). It was found to co-exist with mycorrhizal fungi and were referred to as ‘pseudomycorrhizal’ fungi. Further, the angiosperm’s roots tissues associated with dark pigmented fungi (Peyronel, 1924), dark septate endophytes often found in boreal and temperate forests associated with the fine roots of trees and shrubs, especially of conifers have been considered in the class (Richard and Fortin, 1974).

Class IV endophytes have darkly melanized septa and restricted to plant roots. They are generally ascomycetous fungi which are conidial or sterile and form melanized structures like inter and intracellular hyphae and micro sclerotia in the roots. This class of endophytes have been reported in host plants like non mycorrhizal from antarctic, arctic, alpine, sub-alpine, temperate zones and tropical ecosystems (Jumpponen, 2001; Jalgaonwala *et al*., 2011).

In addition, these endophytes are consistent source of unique natural amalgams with a high level of biodiversity and may also yield several compounds of pharmaceutical significance, which are currently attracting scientific community worldwide. Naturally, every plant in the world is reservoir of a number of endophytes. Hence, a
diversity of endophytic fungi has been reported from plants propagated in tropic
(Mohali et al., 2005; Ferreira et al., 2014), temperate (Ganley et al., 2004; Lugtenberg et al., 2016), xerophytic (Suryanarayanan et al., 2005) and aquatic
environmental conditions (Sraj-Krzcic et al., 2006).

There are twenty five hotspots of global biodiversity in the world and Rajasthan is
one of them, which offers suitable climate for the growth of phytodiversity with
diverse endophytic fungi. Consequently, interest on fungal endophytes has recently
surged, which has led to a substantial amount of research regarding an array of roles
by these fungi in host plants. The omnipresence of endophytic fungi, their extent in
collection in fungal biodiversity remains unclear (Hawksworth and Rossman,
1987; Gamboa et al., 2002; Jena and Tayung, 2013; Rajamanikyam et al., 2017).
Further, fungal endophytes are one of the largely untapped resources and poorly
investigated group of microorganisms realizing their importance. These endophytes
are having immense potential as they enhance the host resistance to herbivores
through the production of various natural bioactive compounds (Clay and Schardl,
2002), nutrient uptake (Malinowski et al., 2000) and also accelerate host tolerance
against various physical parameters such as heat (Redman et al., 2002), salinity
(Rodriguez et al., 2004) and plant biodiversity (Clay and Holah, 1999; Krings et al.,
2007; Arnold and Lutzoni 2007). Further, fungal symbionts have also profound
effects on plant ecology, fitness, and evolution (Brundrett, 2006). Besides, updated
estimate of worldwide fungal diversity estimated as 2.2 to 3.8 million (Hawksworth
and Lucking, 2017). Further, certain mycorrhizae, e.g. ectendomycorrhizae, ericoid
mycorrhizae and pseudomycorrhizae are indistinct (Bills, 1996) and some
mutualistic mycorrhizal fungi associated with plants of Ericaceae and Orchidaceae
family have been indicated as endophytes (Stoyke and Currah, 1991; Bayman et al.,
1997; Jiang et al., 2017; Guo, 2018). They are ubiquitous and occur within all
known plants including a broad range of host plant orders, families, genera and
species, mosses (Davey and Currah, 2006), ferns (Swatzell et al., 1996), grasses
(Muller and Krauss, 2005 ; Su et al., 2010), shrubs (Petriini et al., 1982), deciduous
and coniferous trees (Guo et al., 2008 ; Albrectsen et al., 2010 ; Mohamed et al.,
2010 ; Sun et al., 2011), lichens (Li et al., 2007), large trees (Oses et al., 2008 ;
Endophytic fungi represent an imperative and quantified constituent of fungal biodiversity, which have an effect and be affected by plant diversity and structure (Sanders, 2004; Krings et al., 2007). Approximately, all vascular plant species harbour endophytic bacteria and/or fungi (Sturz et al., 2000; Arnold et al., 2000). Moreover, the colonization of endophytes (Fungal/ Bacterial) in marine algae (Smith et al., 1989; Stanley, 1992), mosses and ferns (Petrini et al., 1992; Raviraja et al., 1996) have also been reported. The environmental conditions in which the host is growing also affect the endophyte population (Hata et al., 1998; Fuchs et al., 2017). Most fungal endophytes isolated to date belonging to ascomycetes and their anamorphs (Rungjindamai et al., 2008; Ilic et al., 2017; Shahzad et al., 2018), while some others to basidiomycetes (Rodriguez et al., 2009; Das et al., 2017; Katoch et al., 2017). However, Pan et al. (2016) documented obligatory role of fungal endophyte in biocontrol and on enhanced host disease resistance mechanism. Besides, the secondary metabolites produced by them being a continuous source of new lead compounds and chemical entities in the field of agriculture and medicine. The opportunity to discover new endophytes with promising properties from this unique ecosystem is alluring. Thus, every endophytic microbe contributes to their host defence with various applications in all sectors including agriculture, pharmaceutical, textile etc. Moreover, symbiotic association without fungal partner in plants could not withstand the extreme temperature, drought, salinity and pathogen attack (Saikkonen et al., 2010; Hartley et al., 2015; Potshangbam et al., 2017). In addition, endophytes commonly increase plant biomass under stressful conditions but the cellular mechanisms involved in stress tolerance and growth enhancement are poorly characterized. Endophytic fungi are considered as an important component of biodiversity as the distribution of endophytic mycoflora differs from host to host. Besides protective functions to host plant, endophytic fungi play important roles to initiate biological degradation of dead or dying host-plant, which is necessary for nutrient recycling. The “balanced antagonism” hypothesis was initially proposed to address how an endophyte controls host defences
mechanisms, ensures self-resistance before being incapacitated by the toxic metabolites of the host and manages to grow within its host without causing visible manifestations of diseases (Schulz and Boyle, 2005; Arnold and Lutzoni, 2007).

Endophytes are rich source of natural products displaying broad spectrum of biological activities. They produce diverse groups of metabolites such as alkaloid, flavanoids, steroids, xanthones, phenols, isocoumarines, perylene derivatives, quinones, furandiones, terpenoids, depsipeptides and cytochalasine, polyketides, peptides, proteins, lipids, shikimates, glycosides, isoprenoids (Mayer et al., 2011; Pavarini et al., 2012). These secondary metabolites of endophytic fungal origin associated with medicinal plants can be exploited for curing many diseases. In this respect, several attempts have been made to isolate and identify various bioactive metabolites from endophytic fungi (Anisha and Radhakrishnan, 2017; Kumar et al., 2017; Sharma et al., 2017). Moreover, endophytic fungi can grow in small to large fermenters to provide sufficient supply of bioactive compounds and thus can be exploited commercially. Presently, huge potential of endophytes are being used to produce biologically active natural products, which are useful not only in medical but also extensively in agriculture and industrial applications. Now a days, enzymes are being isolated from endophytic fungi, showing applications in energy, food, paper, textile, cosmetics, fine chemicals, biomaterials, leather, cellulose and detergent industries (Yadav et al., 2015; Upadhyay et al., 2016). Bioprospecting of these endophytes have been started for search of novel strains with promising bioactivities (Selvin et al., 2010; Paul et al., 2013; Rajamanikyam et al., 2017).

India is enormously being one of the megadiversity country in the world. Medicinal plants have been studied keeping in view their potential endophytic relations and have shown numerous benefits such as production of secondary metabolites of pharmacological concern, verve biomarkers and biological control agents against pests diseases (Bagchi and Banerjee, 2013; Bezerra et al., 2015). Henceforth, it is indispensable to recognize such relationships between endophytic fungi and their host medicinal plants. With respect, several efforts have been made by researchers for the study of endophytic fungi for host defence mechanisms i.e. *Pinus tabulaeformis* (Guo et al., 2008), *Atractylodes lancea* (Wang et al., 2009),
Calotropis procera (Nascimento et al., 2015), Monarda citriodora (Katock and Pull 2017). Hence, India offers vast scope for fungal diversity research. However, there are many challenges such as variations in geographical area, seasonal variations, soil texture, tendency of the researchers to select plants primarily based on easy availability and their vicinity. These hurdles need to be solved and scientists have to cross their boundary of close vicinity.

During the present research endeavour, Tinospora cordifolia (Willd.) Miers ex Hook F & Thoms, Adhatoda vasica Nees. and Murraya koenigii (L.) Spreng. were selected for the screening of the endophytic fungi and their potential roles in the synthesis of natural products and nanoparticles for biocidal activities. These are commercially important medicinal plant in Rajasthan. Cultivation of these medicinal plants was severely affected by a number of diseases. In T. cordifolia, secondary metabolites production has been affected by Phoma Leaf Spot Disease caused by Phoma putaminum, new bacterial leaf spot disease caused by Xanthomonas campestris, flat stem disease caused by phytoplasma (Shivanna et al., 2014; Achar et al., 2014; Achar et al., 2015). Besides, numerous fungal diseases of A. vasica have been reported such as a leaf spot disease caused by Rhizoctonia solani, alternaria blight disease caused by Alternaria alternata affects average yield loss (Verma et al., 2006; Singh and Verma, 2009). Further, mosaic disease in M. koenigii caused by the infection of potyvirus affected the growth of plant as stunting, mosaic patterns in colour of fruit and its malformation (Chandel et al., 2005).

The diseases in the above mentioned plants reported in different seasons and caused vast losses. Therefore, to protect plants from different severe diseases, the plant pathogenic fungi and the endophytic fungi in their respective host plant create close relationship and the host defence system (Xu et al., 2016). Hence, these endophytes improve host metabolism to secrete proteins and enzymes for adaptation as per the altered phyto-pathogenic fungal relations.

Keeping in view the diverse prospects of these endophytic fungi, following objectives were taken into account:
Objectives

• Histological localization and isolation of endophytic fungi from various explants of selected plant species
• Identification of isolated endophytic fungi using morphological, biochemical and molecular characterization
• Maintenance of pure culture and analysis of seasonal variation on diversity of fungal endophytes
• Biogenesis of nanoparticles using fungal extract and their characterization
• Analysis of antimicrobial activities using green synthesized nanoparticles against pathogenic bacteria and fungi