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Bioactive Compounds of Angiospermic Epiphytes For The Management of Fungal Pathogens In Crops - A Review

Hamzah Jumah Essa Barkah[#1] and Lokesh Siddalingegowda[*2]

[#1 *2]*Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysuru, 570 006, Karnataka, INDIA.*

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Abstract *- Plants on the earth are of great wealth gifted by Nature provide a variety of daily needs of our life. They are the chief ingredients of lively food, shelter, medicine, clothes, oxygen, fuel, rain water etc. Plants growing on higher plants are named epiphytes are also of great importance for human kind as food and medicine. Epiphytes have their innate bioactive contents in their components in a varied level. They are highly tolerant to the adverse conditions of the climate than their hosts. These resistant properties give us a clue to exploit their metabolites for the purpose of treating our crop seeds. Thus, polyphenols, tannins, flavonoids other antioxidants in them are promising as antifungal agents helpful to have eco friendly strategies in place of synthetic hazardous compounds being used in the agricultural system. The purified antifungal compounds will promise us to utilize them to for crop seed treatment in vitro at the time of sowing, which is highly helpful for boosting the crop irrespective of the species.*

Keywords - *Angiospermic Epiphytes, Bioactive compounds, Pathogenic fungi, Epiphytes Extracts, Antifungal Activity*

I. INTRODUCTION

Many of the higher angiospremic plants and their constituents have shown success in plant disease management and proved to be harmless and non-phytotoxic unlike synthetic fungicides (Dubey [1]). Some of the plants extract their component wise were also recommended to control fungal disease of several plants (Singh and Dwivedi [2]). Medicinal plants have been a major source of biologically active compounds for treating various diseases since many centuries. Plants are relatively cheap source of biological active material with a vast variety of primary and secondary metabolites. Plants have developed natural defense mechanisms themselves against microbial pathogen, before the man played an active role in protecting them. For example, the effect of polyphenols in microbes is attributed to interruption of various metabolic functions in them. Many secondary metabolites in angiospermic plants including their epiphytes are known to inhibit the microbial growth both in bacteria and fungi which readiliy causes the plant disease, mainly due to polyphenols, terpenen, alkaloids, flovoniods and antioxidants either in the crud or purified form (Kordali *et al* [3]; Castillo *et al* [4]). Nowadays, much importance is being given towards properties of endophytes for several ailments and even for plant disease management. In contract to this, the present study has emphasized to evaluate the efficacy of epiphytes in the management of fungal disease in crop plants. In this way, much information have been collected and compiled in different aspects such as to evaluate the antifungal efficacy of different epiphytes extracts, to check the species wise importance with respect to fungal growth and sporulation of pathogenic fungi *in vitro*. Apart from them, the enumerations of bioactive compounds have been considered as an initiative. Further, the efforts has been made in collect the information about the possible extent damage of cell membrane due to loss of ergosterol content and its relative with respect to electrolyte leakage due to epiphytes extracts. As an ultimate, this available information have been compiled with respect to structural analysis of the promising bioactive compounds were collected and compiled to find the lacunae among these aspects in the vise of facilitate the research activities with respect to the title of the current research. Accordingly the review of literature has been compiled under several aspects which includes importance of bioactive compounds of angiospemic epiphytes as antifungal agents, antifungal assay with respect to mycelial growth and spore germination inhibition, role in the management of fungal disease in crops, structural analysis of bioactive compounds in epiphytes, variability of ergosterol in fungal pathogens, their effect in the membrane damage and electrolyte leakage in the fungi, and finally, to evaluate their effect on the incidence of fungi in crop seeds as well as phytostimulation / toxicity.

II. Occurrence and distribution of epiphytes:

Epiphytes are the organisms grow upon living plants for support, may or may not parasites, usually independent of the host plants for water and nutrition. About 10% of vascular epiphytes are abundant and diverse in the wet tropics and are the part of the canopy community. Variety of morphological and physiological adaptations is necessary for them to conquer the epiphytes habit. Altitude, size and texture of the bark of host trees also influence their abundance and diversity. In relation to the habit, habitat, distribution and their vulnerability many reports are available (Hofstede *et al* [5]; Benmer and Vitousek [6]). Though epiphytes comprise 10% of the world's total vascular flora, their fate is tied to that of their hosts as they are threatened by the loss of tropical forests. In this respect their magnitude of disturbances and the type of vegetation that replaces the original forest in known to a little extent. As a part of epiphytic diversity in relation to survival and distribution in disturbed forests, the studies were conducted. Through, it has been claimed that the epiphytes are highly sensible to climate conditions. Tree size and age is also crucial in orchards and tree plantations. Large tree species were observed to be the best to carry the bulk of epiphytes diversity and biomass, because they have been available for enough time for slow growing epiphytes to complete their life cycles.

III. Relative occurrence of bioactive compounds in epiphytes

In 1971, Khanna and others [7] purified and characterized the two forms of enzymes glucan phosphorylase from the leaves of *Dendrophthe falcata*. They reported the variable sensitivity of these enzymes fractions to some phenolics present in the leaves of *D. falcata* such as phloridzin. Along with epiphytes lichen form species are also reported to contain/ produce biologically active metabolites with varied effects including antibiotic, antiviral, anti-inflammatory, analgesic, antiproliferative and cytotoxic activities (Boustie and Grube [8]). Mdee *et al* [9] have evaluated the antifungal property in several invasive plants species. In which they claimed the promising activity of acetone extracts of *Cestrum laevigatium* (flowers and leaves), *Nicotiana glauca* (flowers, leaves and seeds), *Solanum mouritianum* (fruits and leaves), *Lantara camara* (fruits, flowers and leaves) *Datura stramonium* (seeds), *Ricinus communis* (leaves) and *Compuloclinium macrocephalum* (leaves and flowers) in the inhibition of several plant pathogenic fungi like *Penicillium xanthium, P. expansum, Aspergillus niger, A. parasiticus, Colletotrichum gloeosporioides, Fusarium oxysporum, Trichoderma harzianum, Phytophthora nicotiana, Pythium ultimum* and *Rhizoctonia solani.* It was found that all the test fungal species there was growth inhibitor, in which minimum inhibitory concentration (MIC) was ranged between 0.08 to 2.5 mg/ml. In all the cases leaves extract was reported to be more effective than flowers/ seed extracts.

Certain species of epiphytes like *Cuscuta reflexa* and *Cassytha filiformis* exhibits close similarities superficially confuses in their identification. In order to have clarity in identification, Sharma *et al* [9] performed the morpho-anatomical studies. Considering there species, through which they could differentiate based on the color of the stem, stomata in epidermal layer, and numbers of vascular bundles, pith, and cortex and xylem vessels. As a part of priliminary phytochemical evaluation different solvent systems were tried for developing TLC with respect to comparative study to evident as crucial for differentiation. It is believed that nature has given cure of every disease among living organisms including plants in one or the other way. Researchers nowadays are emphasizing on evaluation and characterization of various plants and their constituents against a number of diseases based on the knowledge as claimed in ayurveda. However, the extraction of bioactive plant constituents has always been a challenging task for the scientists. Pertaining to this, Tiwari *et al* [11] have made an attempt to compile a review on extractants and extraction processes with their advantages and disadvantages. Hence, it has been concluded that the non standardized procedures of extraction lead to the degradation of the photochemical present in the plant and may lead to the variations results in a lack of reproducibility. Through which it has been suggested that the efforts should be made to produce batches with quality as consistent as possible, to develop and follow the elite extraction processes.

Plants irrespective of their habit may be herb, shrub, tree or epiphytes are known to contain an array of active compounds which varies with their surroundings. In parallel to this Stankovic, have estimated the high content of phenolics and flavonoids with their strong antioxidant activity in the methanolic extract of an herb, *Marrubium peregrinum* L., a member of Lamiaceae. They found the significant linear correlation between the values of phenolic content and antioxidant activity with the total phenolic content ranged from 27 to 89 mg/g of dry weight of extract, expressed as gallic acid equivalents. Sasidharan *et al* [12] have discussed the common problems and key challenges in the extraction, isolation and characterization of active ingredients found in botanicals. They also explained the strength and weaknesses of different extraction techniques, which are involved in the applications of common phytochemical screening assays, chromatographic techniques such as TLC, HPLC as well as immunoassay and Transform Infra Red (FTIR) spectroscopy.

El-Khateeb *et al* [13] have screened methanolic extracts of leaves of seedless grapes, *Zizyphus*, pomegranate and fig for their phytochemical constituents and also investigated for their antifungal activity *in vitro* with respect to several phytopathogenic fungi *viz., Alternaria solani, Botrytis cineria, B. fabae, Fusarium oxysporum* and *F. solani*. Their observations revealed an array of phytochemicals like terpenes, tannins, flavonoids, alkaloids, carbohydrates/ glycosides, phenolic glycosides and resins in all extracts except saponins. The HPLC analysis explored twelve polyphenolic compounds, includes pyrogallic acid, gallic acid, protocatechuic, catechin, p-hydroxy benzoic acid, pcoumaric acid, phenols, o-coumaric acid, salicylic acid, coumarin, quescetin and cinnamic acid with different concentrations. Among the extracts *Zizupus* and pomegranate leaves extract were found highly inhibitory to *B. fabae*. In the similar manner angiospermic epiphytes are also undoubtedly contain antifungal property in their foliages. Epiphytes are reported to be of ethno-medicinal importance. Shanavaskhan *et al* [14] have studied these aspects with respect to epiphytes and parasitic plants of Kerala. The tribes in those areas restored the epiphytes and parasitic medicinal plants occurring on all the trees for their use as drugs for the treatment of ailments. They claimed 13.4% of those plants present in Kerala, are valuable to be used to corrective measures for several diseases. However, the knowledge on the utilization of bioactive compounds present in these epiphytes for the treatment of plant diseases was found to be very limited.

Influence of different host species *Alstonia scholaris and Ficus virens* on the phytochemical constituents of *Cuscuta reflexa* was reported by Roy *et al* [15]. Preliminary investigations revealed the presence of flavonoids, glycosides and carbohydrates in the whole plant extracts using methanol, ethanol and water with their increasing order of polarity. Their study showed that all the mentioned parameters vary with the host on which *Cuscuta* parasitizes.

Among the angiospermic epiphytes, twenty five *Hoya* species belonging to Asclepiadaceae were facilitated for their systematic identification through compostion of latex triterpenes. The stable and species specific latex triterpenoids were considered as finger prints a very useful marker for identification (Baas *et al* [16]). Interestingly, Tambe and Bhambar [17] have estimated the total phenol, tannin, alkaloid and flavonoid content in the wood extracts of *Hibiscus tiliaceus* Linn. prepared using petroleum ether, ethyl acetate and methanol. Among this solvent ethyl acetate was found to be better in extracting more of phenol, tannin, alkaloid and flavonoids, comparatively. Hossain *et al* [18] have utilized the organic solvents (hexane, chloroform, ethyl acetate, butanol and methanol) with increasing polarities for their efficacy in extracting phenols and flavonoids content in the crude leaf extracts of *Thymus vulgaris.* Accordingly, they showed the highest content of phenols in butanol; lowest in methanol whereas highest content of flavonoids in methanol and lowest in hexane extract. Phytochemical analysis of leaf extracts of *Holoptelea integrifolia* and *Celestrus emarginata* revealed the presence of alkaloids, steroids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xantho proteiens, glycosides, steroids, phenols, resins, carboxylic acid group at varied concentrations. The studies are the evident of the occurrence of medicinally important bioactive compounds. Similar compounds may also be expected in angiospermic epiphytes which vary among the species and also environmental conditions where they grow. Similarly medicinally important bioactive compounds were extracted and identified in the leaf, bark and flower extracts of *Moringa pterygosperma*. Bargah, in [19] reported the reliable occurrence of large number of pharmacological values based compounds with antioxidants, antifungal, antibacterial, anti-inflammatory, diuretic activities. Out of these observations the ideas would be generated to use these antifungal from plants including epiphytes for the management of plant diseases too. Since the dawn of history, plants have been used for health and food needs by Monica *et al* [20] have determined the antiradical and antimicrobial activity of leaf extracts of *Hoya wightii* and *H. ovulifolia*, the epiphytic species growing in the rain forests, based on the agar well diffusion and poison food techniques. These plants are basically woody, twining or pendulous stem with adventitious roots in the internodes. These plants are known to contain a large number of bioactive metabolites in leaves, roots, flowers, fruits and seeds. The total phenolic content was estimated using FC reagent method. A positive correlation was observed between the phenolic content and antiradical activity of extracts. Considering the pathogenic impact of *Aspergillus niger* and *A. parasiticus* on crops, desirable strategies to prevent their growth and presence in food products, an array of natural phenolic compounds like isoeugenol, cresol, vanillis, euginol, carvacrol and thymol were evaluated for the inhibitory effect. Logarithm of the octanol water partition coefficient, refractive index and molar volume were demonstrated to be the descriptors that best explained the antifungal activity correlated to lipophilicity, reactivity of the components and steric aspect. These findings contributed a lot for the search of new components with antifungal activity (Pizzolitto *et al* [21]). *Cuscuta* is now accepted as belonging to the morning glory family, convolvulaceae on the basis of the work of the angiospremic phylogeny group. In *Cuscuta reflexa*, priliminary phytochemical assay was performed using different solvents system in TLC. Yield of phenol and flavonoids was reported to be 1.8, 3.6 and 4.5% respectively in petroleum ether; ethanol and aqueous extract (Teware [22]).

Plants including epiphytes are the reservoirs of natural chemical compounds with structural diversity. The exploration of new bioactive molecules to be used by pharmaceutical and agricultural industry, directly lead to synthesize more potent molecules. At this juncture, Ingle *et al* [23] reviewed the literature in respect of highlighting the analytical methodologies include extraction and analysis of novel compounds through various techniques like HPLC, TLC, HPTLC, GC, FTIR, NMR and Mass spectroscopy (MS).Parallel observations were made by Barkah and Lokesh (2018) with respect to phenol and tannins in the methanol and aqueous extracts of leaves and tender branches of *Cuscuta reflexa, Viscum orientale, Cymbidium bicolor, Bulbophyllum propinquum, Hoya ovulifolia* and *Dendrophthe falcata.* They found the variable content of phenol and tannins species wise, which have been used for seed treatment for the management of fungal diseases in some agricultural crops. Gul *et al* [24] have reported the occurrence of antioxidants in *Ephedra intermedia* an indigenous plant of Baluchistan which is being used as medicine for treatment of asthma and bronchitis. HLC was used for quantitative analysis of ephedrine alkaloids. They claimed that the methanolic extracts showed the antioxidant activity and proved powerful oxygen free radicals scavenging activities, which was near to the reference standard ascorbic acid.

IV. Antifungal activity of epiphytes

In general, studies on the biofungicidal properties of leaf extracts of some plants were made by many researchers (Srivastava and Lal [25]). Several plant species constituents are reported to shown success in plant disease control and proved harmless, non phytotoxic unlike synthetic fungicides. In parallel to this many plants extracts were found to have a detrimental effect on the fungal spore germination (Singh *et al* [26]) and many of the promising plants extracts were also recommended for the control of plant diseases (Singh and Dwivedi [27]). Spread of multidrug resistant strains of fungi both in animals and plants, makes it necessary to discover new classes of antifungal and allied compounds. This has lead to the search for new alternatives among medicinal plants used for their empirically antifungal properties. In this connection, it was found that a series of molecules with antifungal activity against different strains of fungi which are of great importance to mankind diseases and even plants. In this regard, they compiled a review about the main sources of molecules with anti-mycotic activity from natural environment irrespective of molecules and species. Fungal cell membrane has a basic role in the maintenance of cell, cell order and integrity. Some of the antifungal compounds are known to affect the synthesis of sterol, which is the specific membrane component. Considering this aspect Avis [28] compiled the literature in order to contain effective antifungal with high efficacy and increased longevity for combating the plant diseases. Antifungal activity of leaf extracts of different South African trees was examined against many phytopathogenic fungi. Among the extractants acetone was found suitable which extracted active principles in all the plant species chosen. *Bucida buceras* extract was reported to show highest antifungal activity against a range of phytopathogens. *B. salicina* extract was used as promising treatment to protect oranges against post harvest fungal pathogens (Mahlo [29; Mahlo *et al* [30]). This may also serve as useful information for extraction of bioactive antifungal molecules from epiphytes as an alternative to methanol. Routine usage of antifungal compounds leads to development of resistance through adoption or degradation. It makes necessary to discover new classes of antifungal agents for fungal infections both human and plants. Pertaining to these aspects Arif *et al* [31] summarized the information based on the literature on the utilization of natural products and their derivatives. Their observations accrued the information on a series of molecules with antifungal activity against several strains of fungi that colonize plant species. Antifungal activity of aqueous of *Cuscuta reflexa* was reported to be effective against many filed fungi such as *Fusarium solani, F. oxysporum and Macrophomina phaseolina*, which are common seed borne fungi in many crop plants. The effectiveness of aqueous extracts was recorded based on the well diffusion method, which indicated the growth inhibition directly proportional to the concentration of the extract. 30% concentration itself was reported to be highly inhibitory even up to 6 days of incubation in vitro. Dellavalle *et al* [32] have evaluated the antifungal efficacy of Uruguayan medicinal plants viz., *Salvia sclarea*, *S. officinalis* and *R. officinalis* against plant pathogenic *Alternaria* spp. Out of 29 species, the solvent extracts were assayed on different tissues of plants, 31% of the extracts inhibited the growth of the fungus, similar to the effects of a chemical fungicide, Captan.

Antimicrobial activity of crude extracts of leaves and fruits of *Viscum album* in ethyl acetate and methanol were reported to be highly active against both gram –ve and +ve bacteria in vitro including an isolate of yeast and a fungus. Both organic and aqueous extracts in disc diffusion method proved their efficacy against the test microbes in comparison with positive references Ciprofloxacin and Nystalin (Hussain *et al* [33]). The distinct group of epiphytic symbionts like lichens namely *Parmelia reticulata*, *Ramalna roesleri*, *Usnea lorgissima* and *Stereocaulon himalayense* were evaluated for their antifungal activity against nine soil borne plant pathogenic fungi like *Rhizoctonia bataticola, Sclerotium rolfsii, Altenaria alternata, Pythium debaryanum, Fusarium oxysporum, R. solani, Botrytis cinerea, Sclerotinia sclerotiarum and Pythium aphaniderantum* based on food poison technique. Hexane and dichloromethane extracts were found to be more effective compared to aqueous extracts against all the test species. The highest inhibitory activity was recorded with hexane extracts, comparatively (Goel *et al* [34]). *Cuscuta refelexa* though a parasitic plant belongs to family convolvulaceae has been to be antimicrobial. The ethanol extracts of stems of this plant was evaluated for its efficacy against few gram +ve, -ve bacteria as well as some fungi like *Penicillium citrinum* and *Aspergillus niger* using agar cup plate method. The results were analyzed using zone of inhibitions; it was found that the extract was much active against gram –ve bacteria and fungi, compared to gram +ve bacteria (Inamdar *et al* [35]).In the similar manner Sakshy *et al* [36] have compared the antioxidant activity of *Cuscuta reflexa* and *Cassytha filiformis*. In both the cases, alcoholic extracts there was neutralization of radical activity and inhibition of peroxidation reactions were observed. *Cuscuta reflexa* was proved to be more effective in the *in vitro* antioxidant activity than *Cassytha filiformis*, which were exposed as IC50 and the same was found rich in polyphenolic content. It is none other than an african mistletoe species which in being used as traditional and folkloric medicine was used to determine its antifungal property by Ukwueze and Osadebe [37]. The methanolic leaf extracts of the epiphyte growing on different host tree like *Kola acuminata, Irvingia gabonensis* and *Persea amaricana* were tested against clinical isolates of some pathogenic fungi. The effectiveness of these extracts were evaluated based on agar diffusion method using standard reference Ketoconazole as positive control, which exhibits better activities towards *Aspergillus* than *Candida.*

Studies on the evaluation of epiphytes for their ethno medicinal value were performed in Kerala. Shanavaskhan *et al* [38] have documented the ethno-medicinal importance of 28 epiphytes as well as parasitic species present in Kerala, which are of use for curing and corrective measures of several diseases in animals and plants. Common and locally available plants viz., *Azadirachta indica, Aloe vera, Ocimum sanctum, O. basilicum, Lantana camara* and *Asparagus* species were tested for their potential for growth inhibition in several pathogenic fungi like *Aspergillus* and *Rhizoctonia* species. The alcoholic extracts of all the plants were found comparatively potent to that of aqueous and acetone extracts. *Azadirachta indica* and *Aloe vera* extracts were observed to be more effective in reducing the radial growth of *R. solani, A. niger, A. flavus* and hence, were suggested for the management of infectious diseases caused by them in plants as well as in animals. In the similar manner epiphytes extracts can also be suggested for the diseases management in crop plants (Gujar and Talwanker [39). Monoterpenes in general are the main constituents of plant essential oils, gives unique odoriferous properties due to their lower boiling points. The natural pesticides properties of some monoterpenes make them useful as potential alternatives to synthetic pesticides. In connection with the common occurrence of monoterpenes in certain plant species, Marei *et al* [40] used 12 different monoterpenes against four plant pathogenic fungi *Rhizoctonia solani, Fusarium oxysporum, Penicillium digitatum and Aspergillus niger* to evaluate their inhibitory activity based on mycelial growth inhibitory technique. Apart from these, the pectin methyl esterase, cellulase and polyphenol oxidase inhibitory activity of monoterpenes in fungi was determined *in vitro*, in comparison with a standard reference, Canbedazim. Ukwueze *et al* [41] claimed that the *Loranthus* species contains phenols, tannins, alkaloids, flavoniods, terpenoids etc. which were isolated as different fractions in methanol, ethyl acetate, hexane, acetone and chloroform. The antimicrobial effects against *Bacillus subtilis and Pseudomonas aeruginosa, Staphylococcus aureus* and *E. coli* were confined and concluded to the mixed group of plant constituents rather than individual ones. Authors considered the bioassay guided evaluation based on clear zone formation as the criteria to decide the antimicrobial effect of photochemical, among which crude methanol extract showed preponderance of such constituents like alkaloids, flavonoids, terpenoids and tannins compared to the defatted methanol extract, ethyl acetate soluble fraction, hexane extract, acetone soluble fraction and chloroform soluble fraction. The chemical compounds extracted from natural sources with antifungal properties include essential oils, phenol compounds and peptides. Apart from higher flowering plants, there has been a growing interest in microalgae as a source of metabolites to obtain antifungal compounds to inhibit the fungal diseases in plants. Phenol compounds extracted from *Spirulina* sp. were reported to inhibitory to phytopathogenic fungi *Fasarium graminearum* (Pagnussatt *et al* [42]). These metabolites of *Spirulina* sp. were proved promising in inhibiting fungal multiplication; in terms of inactivation of enzymatic systems involve amylase and proteases of the fungus. Mistletoes are semi-parasites or epiphytes grow on various host trees and shrubs, depend on them for mineral nutrition and water although they produce their own carbohydrates through photosynthesis. These mistletoes was described as an all purpose herb due to rich traditional uses as ethnomedicine for several ailments including plant diseases. The leaves are known to contain an array of bioactive photochemicals. The uses of natural products such as phenols as an antifungal drug are of great importance both economically and for human health as well. Ajugol extracts from *Spathodea campanulata* and oleuropcin from olive have been proved to have antifungal property, which can be used efficiently as antifungal compounds for treating the seeds before sowing. Therefore exploiting the potent natural phenolics against fungal infections is of better antimicrobial strategies which could efficiently control plants infectious diseases in the field. It has been indicated that much research in still going on to explore their more benefits including epiphytes as natural gift (Ansari *et al* [43]). Plant metabolites are not only antifungal but expressed fungitoxic effect through the reduced production of spores and mycotoxins among fungi. Related to these aspects, Reports indicated the inhibition of mycelial growth, spores and aflatoxin production by *Aspergillus flavus* under their treatment with the methanolic extracts leaves of *Tamarix* spp.*, D. gridium, C. procera, H. scoparium, P. argentea* and *M. canescens*. These extracts showed inhibitory effect variedly on mycelial growth, sporulation and aflatoxin production by *A. flavus*. These findings revealed the importance of *D. gnidium* to protect grain spoilage against aflatoxigenic fungus. In this way the angiospermic epiphytes may be used against toxigenic fungi through seed treatment before sowing, to avoid afla root symptoms in dicots. It explained that the polarity level and species nature are playing a role in extracting the secondary metabolites using plants (Ghasemzadeh *et al* [44]). In parallel to this Senguttuvan *et al* [45] extracted more of photochemical using high polar solvent methanol from the leaves and roots of *Hypochaeris radiata*. It is clearly demonstrated the occurrence of powerful antioxidants including vitamin C in this plants and concluded to have high radical scavenging abilities, which is suggestible to save both animals and plants under stress conditions to avoid loss of membrane integrity due to oxidation of lipids in the cell membrane.

Colletotrichum musae is one of the postharvest pathogen in banana known to cause anthracnose severely. In order to reduce its incidence methanolic leaf extracts of *Prosopis juliflora* and *Acacia albida* were used *in vitro* to assess their antifungal property based on paper disc and spore germination assays in comparison with a recommended fungicide, Carberdazim. Aqueous extracts of *A. albida*, whose active principles were found to be heat stable and most effective among the test species (Bazie *et al* [46]). Similarly the heat stability of antifungal principle in epiphytes may also be checked due to their variable habitat. Plant leaf exudates contain many minerals and some bioactive compounds that may have antifungal property. Leaf exudates of *Jatropha curcas* L. has been examined for antifungal ability in vitro. The spore germination of phytopathogenic phylloplane fungus *Fusarium solani* was reported to be up to 97%, which indicates the strong antifungal property of the leaf exudates (Chandel and Pimpalgaonkar [47]). In the similar manner, the epiphytes which occupy the top canopy may also be assumed to possess antifungal properties in the leaf exudates. Plant extracts in general with various constituents were found to be effective against phytopathogenic fungi in vitro, in the greenhouse as well as in the field (Prithviraj *et al* [48]). Several alkaloids of plant species are known to affect biological functions of microbes both fungi and bacteria at very low concentrations (Singh *et al* [49]). Considering these aspects Singh *et al* [50] have used natural products viz., berberine, isolated from *Berberis aristata* and Santonin from *Artemisia meritima* to reduce spore germination of some fungi, individually and in combination. In this manner it may be assumed that epiphytes as a special group of plants may serve better as antifungal agents when their extracts are used in combination for treating the crop seeds. Fungi in general have chitinous rigid cell wall and an ergosterol containing cell membrane. In any antifungal laboratories, fungal cell membrane is one of the targets to assess the activity of antifungal agents due to their interference with the structural integrity of the lipid *bilayer*. Synthetic antifungal agents like polyenes, azoles, alkylamines, amino acids and peptides are being tested to their efficacy in the disruption of fungal cell membrane (Aderiye *et al* [51]). They have explained the binding of these compounds to sterols in the fungal cell membrane leads to electrolyte leakage. Similar antifungal activity of synthetic chemicals are also claimed to involve in the HMG-CoA reductase, which is involved in the ergosterol synthesis. Other enzymes of ergosterol synthesis like reductase, esterase is known to inhibit due to antifungal compounds. The lipopeptide in urn act in the increased membrane permeability of fungi leads to loss of electrolytes causes their reduced growth. Considering the epiphytes extract we may also expect the similar principles involved in the loss of cell membrane integrity make the fungi to become vulnerable to the surroundings. Bhattacharjee and Islam [52] have reported the broad spectrum antimicrobial properties of *Rhynchostylis retusa*, a monopodial, epiphytic, medicinal orchid. According to their observations, chloroform extract was superior over several bacteria and fungi tested. They exhibited maximum zone of inhibition in agar diffusion method, it was up to 15mm, comparatively. Based on the high degree of antimicrobial potential it has been suggested to use these extracts to cure the microbial infection. Considering the impact of *Aspergillus* species, Pizzolitto *et al* [53] have evaluated the efficacy of some natural phenol compounds like isoeugenol, carvacrol and thymol against the growth and establishment of toxigenic isolate of *Aspergillus parasiticus*, which also involves the determination of their physicochemical properties. They claimed the antifungal property of these phenolics is in correlation to lipophilicity and reactivity of the components. Several epiphytic orchids like *Luisia zeylanica, Phalidota pallida* of Western Ghats of Karnataka in India were evaluated for their antifungal effect against a few plant pathogenic fungi includes *Bipolaris sorokiniara, Colletotrichum capsici, Fusarium oxysporum* and *Curvularia* spp. based on poisoned food technique. Though there was a variable potential of epiphytes extracts on fungi *C. brevicapa* extract was proved as strong antifungal source which would help in safeguarding the crop plants (Shweta *et al* [54]). Majority of the research articles suggests the better usage of methanol for the extraction of photochemical from plants. Similar observations were made by Nidiry *et al* [55], where they extracted the potent antifungal compounds from aerial parts of *Andrographis panticulata* using methanol. In which their findings revealed the inhibition of mycelia growth in *Fusarium solani* and spore germination in *Alternaria solani*. Quantitative estimation also revealed 6.82% of andrographolide in methanol extract as active principles. It is possible to claim angiospermic epiphytes not legging behind in having similar antifungal in their aerial parts along with other compounds which vary depending upon the species and their habitat. In 2015, Sesan *et al* [56] have demonstrated the antifungal activity of six plants namely *Hyssopus officinalis*, *Satureja hortensis*, *Allium sativum, Tagetes patula* and *Menta* spp. against gray mold *Botrytis cinerea,* which causes deleterious effects among crop species. Both *in vivo* and *in vitro* studies indicated the promising effects at varied concentrations (10–100%). Thus, they recommend eco-friendly nature of these active compounds for the management of economically important diseases in crop species.

Chemical composition as well as antifungal potential of medicinal plants like *Margifera indica, Mentha spicata, Citrus limon, Eucalyptus camaldulensis* were tested against *Fusarium oxysporum, Aspergillus flavus, Rhizopus stolonifer* and *Penicillium digitatum* which causes severe diseases in plants. It was observed that *E. camaldulensis* rich in flavonoids reduced the incidence of diseases in egg plant in green house conditions due to seed treatment. The seed germination and the seedling growth were also increased over control. The incidence of *F.oxysporum*, wilt causing fungus was also found to be reduced due to *E. camaldulensis* at 80% concentration. The disease caused by this fungus was also reduced to a higher extent, comparatively (Ashiq *et al* [57]). These observations may remain in parallel to the activity of bioactive compounds in epiphytes also, which may be used for crop plant disease management caused by fungi.

V. Effect of plant extract on spore germination and enzyme activity in fungi

Maurya *et al* [58] determined the spore germination inhibition in both pathogenic and saprophytic fungi using an alkaloid-corypalmine. The compound was isolated from the leaves of *Corydalis caerophylla* (Family: Fumariaceae) occurring in himalyan region and was tested against the spore germination and germ tube elongation in *Ustilago cynodontis* picked from the diseased plants. Other test fungi like *Alternaria solani, A. brassicicola, A. melongena, Curvularia pallescens, C. lunata, Colletotrichum* sp., and *Helminthosporium* spp., also exhibited reduction in the spore germination percentage. In many cases 400ppm was found to be highly inhibitory with respect to spore germination and germ tube elongation. Bioactive compounds in plants also vary with respect to habitat, influenced by the various abiotic factors. Since, the epiphytes are growing at varied climate experiences the varied conditions of weather in turn possess the varied bioactive compounds, even correlation wise. Hence, there is a need to search for effective sources of biomolecules for the management of crop diseases caused by microbes. Chitosan, a biopolymer, non toxic to plants is known to enhance the resistance in plants and play a role in the reduction of fungal diseases in plants. In *Fusarium oxysporum, Radicis lycopersici* ultra structural alterations in the fungal spores due to chitosan, indicated their susceptibility. Thus, the related toxicity of chitosan to fungi due to rapid entry in to spores and high extracellular chitosanolytic activity leads to loss of spore germination and reduced hyphal growth in fungi, which was suggested to utilize the chitosan an abundant wastage of sea food industry could be made use with biological agents for the management of fungi in crops (Palma-Guerrero *et al* [59]). Based in this, it is possible to claim that the relative use of chitosan with epiphyte extracts may prove better for seed treatment in crops to get rid of microbial diseases.

Synthetic fungicides were compared with the plants extracts for their antifungal property against a few fruit not pathogenic fungi. Seed and leaf extracts of *Azadirachta indica* was reported have spore germination inhibitory effect of the test species used. *Datura metel, Tagetes patula* extracts were also found to inhibit the spore germination and mycelial growth of *Fusarium oxysoprum, Rhizopus artocarpi* and *Alternaria tenuis*. This gives an idea about the combitorial effect with epiphyte extracts seves still better antifungal agents either through seed treatmentor foliar spray among crop plant (Begum *et al* [60]). Chandel and Pimpalgaonkar [61] have isolated phylloplane and rhizosphere myalflora of *Jatropha curcas* to test the efficacy of host factors (leaf exudates) on spore germination inhibition. *Fusarium solani* showed high sensitivity to the leaf exudate than *Curvularia pallescens*. Variable response of fungi was observed in both species; may be due to their in built factors involved in the interaction with the leaf exudate constituents. Fungi are heterotrophs, get their nutrition from the substratum by producing suitable hydrolytic enzymes from their hyphal tips. With this context, Chandrasekaran *et al* [62] observed high protease activity in *Aspergillus flavus* and *A. niger* through plate assay method. The enzyme production form these fungi were observed to be varied with respect to temperature and pH of the medium. Similarly the fungi in response to the content of the medium also show variability in enzyme production, thus there may the change in the growth of the fungi also. Similar observation about the production protease enzyme by fungi was reported. Sandhya *et al* [63] compared the solid state and submerged fermentation using several agro-industrial residues like wheat bran, rice husk, rice bran, spent brewing grain, coconut oil cake, sesame oil cake etc., in the production of protease by several test fungi. Out of the substrate used wheat bran was found suitable for the many fungal species which favor them to produce more protease comparatively. In which 3.5 fold more enzyme production in solid state fermentation over submerged fermentation. These studies clearly indicate the influence of substrate on the enzyme production by fungi. Hence it is possible to claim the reduction of enzymes in poisoned food medium in vitro, by adding bioactive compounds of plant origin even may be from epiphytes. Concurrently, Thirunavukkarasu *et al* [64] developed and described a simple method to detect extracellular protease produced from fungi. This modified method enables the visualization of different types of proteases, also found sensitive than the conventional agar plate method which could be used for screening a large number of samples in a short time. This technique also can be used to screen several bioactive compounds from plants *in vitro* based on their ability to leave clear zone around the fungal colonies.

VI. TLC, HPTLC and HPLC analysis of plant extracts for bioactive compounds

Increase in phenolics content in the extracts can be correlated to the induction of resistance in treated plants against pathogenic fungi. HPLC analysis of medicinal plants crude extracts showed the occurrence of phenolic acid includes benzoic acid, cinnamic acid, caffeic acid, ferulic acid, gallic acid and tannic acid at varied amount. Thus, the study made by Singh *et al* [65] indicated the possible scientific basis for the use of plant extracts in the future development of antifungal and antioxidant agents for better treatment of diseased plants. Sahu *et al* [66] have extracted the bioactive compounds from medicinally important epiphyte

Dendrophthe falcata in ethanol, which showed the occurrence of carbohydrates, sterols, glycosides, flavonoids and phenols. For TLC analysis they have used toluene, ethyl acetate and formic acid in the ratio of 2.5: 1: 1 which facilitates the easy separation of the compounds in the crude extract of the whole plants. HPTLC reported of the extract in some solvent system showed the presence of eleven active chemical constituents. In the similar manner, Shailajan *et al* [67] reported the microwave assisted extraction of quercetin from hydroethanolic extract of *Cuscuta reflexa*. Chromatographic characterization of hydroethanolic extract of the plant was carried out in terms of quectetin content using HPTLC and RP-HPLC. The presence of quercetin in the samples was confirmed using Mass spectroscopy, which was found to be simple and rapid method. Corresponding observation were also made with respect to methanol extract of *Viscum orientale* leaves using ultra performance liquid chromatography indicated the occurrence of five polyphenol compounds like gallic acid, vanillic acid, caffeic acid, ellagic aicd and quercetin. Qunlitative antioxidant activity determined by TLC indicated the presence of antioxdants which justify the traditional medicinal value of the epiphyte (Khatun *et al* [68]).

Plants hemi-parasites are known to obtain a variety of primary compounds such as carbon, water and ions as well as secondary compounds from their hosts. Many hemi-parasites do not synthesis or modify the secondary compounds drawn from their hosts; hence the presence of certain secondary compounds varies within and among their populations, depending upon the host association of individual species. In this context Jadhav *et al* [69] have confirmed the transfer of c-glycosyl xanthone mangiferin and some flavoniods from *Mangifera indicia* (host) to that of epiphyte *Dendrophthe falcata* based on HPTLC. Many reports indicated the use of plant extracts as an effective way of controlling the plant diseases compared to synthetic chemicals. The plant extracts are known to elicit various defense enzymes in the host plants and thus incur resistance to the pathogens. Parallel information also made available by the work of Nisha *et al* [70] in rice plants suffering from bacterial blight in which the usage of *Azadirachta indica, Agle marmelos, Cassia auriculata and Vitex nigunda* leaf extracts in organic solvents elicited the activity of peroxidases, polyphenol oxidases and B1-3 gluconase in the hosts. This indicated that the defense enzymes are responsible for the control of diseases in host plants rather than antagonism by the extracts.

Peroxidases (E.C.1.11.1.7) are widely distributed in nature, found in plants, microbes and animals. They are known to catalyze the reduction of H2O2 (end product of oxidative metabolism) to water, rendering harmlessness to the living cells, thus serves as oxidizing agents. As this happens in the crop plants due to abiotic/ biotic stresses, the bioactive found in epiphytes are strong enough to perform this function as in any other medicinal plants. This knowledge has been utilized for the evaluation of peroxidases from many plant sources. Effect of bioactive compounds of plants on ergosterol content of fungi: The introduction of bioactive fungicidal compounds that inhibit ergosterol biosyntheses and membrane function in fungi of recent innovation in the uses of chemicals to control plant pathogens. Many fungicides having diverse chemical structure, biological activity, showed common mode of action revealed that ergosterol biosynthesis is rapidly inhibited. This inhibition eventually curtails the membrane synthesis, in turn the fungal growth. The details of toxic action, effect on sterol biosynthesis and site of action of antifungal compounds of varied origin are discussed by Siegel during 1981 itself (Siegel [71]). Seitz *et al* [72] assayed the ergosterol content in *Alternaria* and *Aspergillus* grown on milled rice based on HPLC method and suggests the feasibility of ergosterol as measure of fungal invasion and growth. It is known that some of antimycotics like tolnaftate and tolciclate block the biosynthesis of fungal cells and cell extracts, with accumulation of squalene. This was proved by the direct inhibition of microsomal squanlene epoxidase in *Candida albicans*. This may be true in case of many plant pathogenic fungi, in which plant based bioactive serves the similar function and play as antifungal agents. Hata *et al* [73] have determined the antifungal activity of Amino-piperidine derivatives against few strains of *Candida* spp. They claimed that these derivatives are known to inhibit the ergosterol synthesis, hence, inhibit the fungal growth. Similar compounds may also occur in the extracts of plants irrespective of their type. Stahl and Parkin [4 showed the relationship of ergosterol in the substrate and fungal biomass, in which ergosterol servers as an indicator of soil fungal biomass. These studies indicated the highly significant correlation between soil ergosterol content and measure of hyphal length. It also indicated the wide range in specific ergosterol content of living fungal biomass in soils is related to the total amount of fungal hyphae in soil. This idea may be considered to determine the antifungal activity of bioactive of plants including epiphytes. Synergistic antifungal activity was observed with respect to *S. cerevissiae* confirmed by the increased reduction in cellular level of ergosterol (Cabral *et al* [75]). Their work was aimed to detect the antifungal synergism between statins and azoles by means of an agar well diffusion bioassay. This synergistic effect may also be used to assess the antifungal effect of multiple bioactive of plants origin. Hata *et al* [76] showed the possibilities of determining the ergosterol based on LC/MS analysis in *Candida albicans* due to amino-piperidine derivatives. They also claimed that the inhibition of fungal growth is due to antifungal compounds which involved in inhabiting C-14 reductase in the ergosterol synthesis pathway. Though the ergosterol is the basic constituents of majority of eumycotic fungi, is not produced by all fungi. The occurrence of ergosterol in fungi also varied in their concentration with respect to different fungal species, due to their varied physiological states. The extraction and estimation of ergosterol also play an important role to assess the effectivity of antifungal compounds. At this juncture extraction protocols have been modified by Chiocchio and Matkovie [77], for the determination of ergosterol in cellular fungi by HPLC method. This method holds good in respect of standardization of ergosterol estimation in fungi treated with bioactive compounds of plants. Varied content of ergosterol in different wood decaying fungi was determined by Bhosle *et al* [78] based on semi-micro determination method. This would facilitate for the easy estimation of ergosterol in fungal biomass subjected to bioactive compounds of plants origin. Fungal establishment in grapes correlated with the determination of ergosterol content using HPLC method. This throws the light for the utilization of HPLC method for the estimation of ergosterol in fungi affected by phytochemicals (Porep *et al* [79]). GC-MS was found to be a quick, reliable method of determining ergosterol content in grains, which was found to superior over HPLC method (Hossain and Goto [80]). This method may holds good with respect to assessment of ergosterol in plants products and fungi.

VII. Effect of plants extracts on the electrolyte leakage in fungi

Electrolyte leakage from living cells, exposed to toxic compounds are found depended on concentration and duration of exposure. Higher the duration and concentration the cells succumbed to release of more electrolytes and thus, the calls losses their strength eventually leads to death. The electrolyte leakage studies can be a tool to assess the effect of plants bioactive compounds on the viability of target cells. Similarly, Kendra and Hadwiger [81] reported the inhibition of spore germination and growth of *F. solani* due to chitosan, based on electrolyte leakage from the cells which was dose and duration of exposure time. Relationship of electrolyte leakage and sterol in fungi was explained by Steel and Drysdale [82]. α–Tomatine from tomato which is an antifungal compound known to disrupt liposome membrane containing 3 β–hydroxy sterol leads to the more electrolyte leakage from pathogenic fungi. This concept may be quite allied to the plant secondary metabolites which play a role in the inhibition of fungal spore germination and growth. Influence of specific and nonspecific elicitors on electrolyte leakage, lipoxygenase activity was tested in *Cladosporium fulvum* infected tomato tissues. It is suggested that the elicitors involve in the electrolyte leakage in the plant tissues which leads to necrosis depending upon the light condition. The plant metabolites may also act as the elicitors induces the leakage of electrolytes from the pathogen and leads to death of the pathogenic propagules epiphytes are of distinct group of plants may be useful as antifungal in plant disease causing pathogenic fungi (Peever and Higgins [83]).

Few reports are available with respect to the induction of ethylene in plant tissues due to fungal protein (Bailey *et al* [84]). In the similar manner it is also possible for the ethylene induction in fungi by plant protein. The possible antifungal effects of plant proteins especially from epiphytes may also be claimed for the electrolyte leakage which eventually leads to the death of plant pathogenic fungi is constituent part of living cells of in response to stress. The reaction is mainly related to the effuse of K+, which is abundant in living cells. The stress induced electrolyte leakage is always accompanied by ROS generation and often leads to cell death. The fungi under the stress of plant metabolites are not left from these phenomena of Chromatographic and Spectrophotometric analysis of bioactive compounds from epiphytes. Chatterjee and Sahu [85] have isolated two new compounds from entire plant ethanol extract of *Cuscuta reflexa*. On the basis of IR, NMR and ES-MS analysis and by chemical transformation, two new compounds were elucidated as 3-methyl-3, 4, 5, 7-tetrahydroxy flavones and 3-methyl-4, 5, 7-trihydroxy flavones-3 glucoside. In which IR spectrum exhibited absorptions corresponding to hydroxyl and ketone groups. The chemical shift in 1H and 13C NMR spectral data supported the identification of the flavones on comparison with spectra of the compound consisting of glycoside ring. Reports indicated the antifungal activity of crude extracts of *Acorus calamus, Tinospora cordifolia and Celestrus paniculatus* at the dose of 500mg/ml. *T. cordifolia; C. paniculatus; A. calamus* extracts proved better in inhibiting the growth of *Helminthosporium* sp. ; *Alternaria solani*; respectively. In spite of all the test species the property was found concentration dependent. HPLC analysis performed with respect to all the extracts showed a varied amount of six different phenolic acids like Benzonic acid, Cinnamic acid, Caffeic acid, Ferulic acid, Gallic acid and Tannic acid. The HPLC fingerprint of the methanolic extract of all the plants showed major peaks at the retention times (min.) of 6.92, 5.73, 3.70, 3.40, 3.10 and 2.58 at a wavelength of 290 nm. Among the three extracts, Ferulic acid and Cinnamic acid were detected only in *T. cordifolia. C. paniculatus* revealed Benzoic acid in large amount. Thus, HPLC analysis of the plant extracts revealed wide variability in their phenolic content. The results of antifungal activity and chemical profiling indicated possible use of these extracts in seed treatment against seed-borne fungal pathogens (Singh *et al* [86]). In the similar manner it is possible to expect antifungal agents in the angiospermic epiphytes can be made use for seed treatment. Bioactive compounds in plants include alkaloids, phenolics, steroids; terpenoids etc. are of great potential value as antimicrobial, antioxidants, antifungal and are known for treatment of various ailments in plants, human beings and domestic live stocks. The adequate information of several plants extracts with respect to their bioactive compounds are lacking still. At this juncture, in order to identify such active compounds many efforts have been made using HPLC and NMR spectral analysis. In this context plants including different groups like climbers, twiners, epiphytes, trees, herbs and their components extracts were analyzed for their structural details based on HPLC and

NMR. Anand *et al* [87] elucidated the bioactive compounds of *Zehnerias cabra* of cucurbitaceae as Gypenoside using the root extract based on Nuclear Magnetic Resonance spectra analysis. In which the proton nuclear magnetic resonance spectral of the root sample were recorded and the chemical shift values of various signals were identified by the presence of gypenoside. The different chemical shifts of the proton according to their molecular environments with in the molecule were measured in the NMR apparatus relative to the standard. Based on the proton data, the structure of the compound confirmed the plant extract contains the Gypenoside group. These information prompted biologists to identify many more compounds in plants extracts based on structural analysis. Patra *et al* [88] used HPLC and 1H NMR spectroscopy for the detection of possible functional groups in the bioactive compounds of crude methanol extract of *Excoecaria agallocha.* They claimed that the combination of n-butanol : acetic acid : water in the ratio of 4 : 1 : 1 was the most appropriate for the separation of phenolic compounds in the crude extract. In HPLC chromatogram of the crude extract, 7 peaks were in leaf component 2 peaks stem components were observed. The 1H NMR spectroscopic analysis of crude extract revealed the presence of acylic aliphatic and α mono substituted aliphatic group of compounds. Thus, they concluded that the possible purification and characterization of bioactive compounds in methanol for pharmaceutical use.

Various plants parts are known to contain antioxidants, antibacterial and antifungal agents. Out of which saponins and polyphenols play a major role in inhibiting the fungal growth. Considering these valuable compounds in plants, Sharma and Paliwal [89] have characterized the saponins of *Moringa oleifera* pods based on TLC, HPLC and 1H NMR methods. These findings exhibited the presence of hydroxyl group (-OH), carboxylic acid, alkynes, esters and ethers. NMR spectrum of the isolated compounds revealed the presence of protons. Hence, it can be claimed that an array of plants species contain a variety of bioactive compounds involve in resisting the adverse effect of pathogenic microbes, indicated possible use in the formulation of seed /plants treating agents.

VIII. Summary of literature

Regarding the evaluation of phytoextracts for their bioactive compounds indicated the huge occurrence of saponins, flavonoids, flavones, tannins, polyphenols which play an important role as pharmaceutical products for various ailments among human being and even plant species. In present context, these plants invariably yield a variety of active compounds at varied concentration that depends upon the species habit and habitat which were operated by several abiotic and biotic factors. Thus, it is made possible to throw light upon search and utilization as natural, biodegradable, eco-friendly beneficial compounds for human benefits directly as well indirectly to crop and related plant species which are being severely affected by the fungal pathogens in the field. Apart from these, the literature indicated the feasible ways of extraction, purification and exploitation of the functional compounds/ molecules from the plants natural resources which is the hidden wealth of the nature. Information available in the review influences the possible usage of epiphyte extract and their active principles for seed treatment through feasible formulation. In the other hand the functional groups, which exhibits antifungal properties would be synthesized *in vitro* for further application to treat the seeds of agriculture crops. The literature survey indicated the lacunae left in the field for the identification and application of the elite compounds for the management of fungal diseases of crops. This has provided the platform to emphasize on the collection and extraction of many more common angiosperimc epiphytes *in vitro* and to estimate their total phenolics and tannin content. It has enlighten the researchers to assess their antioxidant and antifungal activities based on *in vitro* bioassay methods and to isolate the potential compound based on HPLC and to characterization based on NMR. These information's, thrown the light upon the scientists to pay much importance to quantify the ergosterol content in the target fungal pathogens as well as to determine the electrolyte leakage in the fungi for their effect on the membrane integrity. Hence, in this way there is a need to evaluate the efficacy of promising plant extract in the elicitation of the peroxidase activity in the crop seedlings challenging with pathogens and to evaluate the variation in chitinase activity in fungi. Ultimately, these informations provide the route for finding the new way to utilize the natural sources as potential antifungals as the eco-friendly management of fungal diseases of crops.

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X. References

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