



Natural Products as Fungicide and Their Role in Crop Protection

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Abstract

Seeking solutions from nature for solving one and all problems is the age-old practice for mankind, and natural products are proved to be the most effective one for keeping up the balance of development as well as the “healthy, wealthy, and well” condition of mother nature. Fungal pathogens are proved to be a common and popular contaminant of agroecosystem that approximately causes 70–80% of total microbial crop loss. To meet the proper global increasing need of food products as a result of population explosion, managing agricultural system in an eco-friendly and profitable manner is the prime target; thus the word “sustainable agriculture” plays its part, and this package is highly effective when coupled with nature-derived fungicidal products that can minimize the event of fungal infections in agrarian ecosystem. Present study enlists the most common and effective natural products that might be of plant or microbial origin, their mode of action, day-by-day development of phytopathogenic resistance against the prevailing fungicides, and also their role in maintenance of sustainability of agricultural practices with special emphasis on their acceptance over the synthetic or chemical one. A large number of bioactive compounds ranging from direct plant (both cryptogams algae and moss and phanerogams)-derived natural extracts, essential oil of aromatic plants, and low-molecular-weight antimicrobial compounds known as phytoalexins to secondary metabolites that are both volatile and nonvolatile organic compounds of microbes (fungal and actinobacterial members) residing inside the host tissue, called endophyte, are widely used as agricultural bioweapons. The rhizospheric partners of plant, mycorrhizae, are also a prime agent of this chemical warfare and protect their green partners from fungal invaders and emphasize the concept of “sustainable agriculture.”

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J. Singh, A. N. Yadav (eds.), *Natural Bioactive Products in Sustainable Agriculture*, https://doi.org/10.1007/978-981-15-3024-1_9

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Keywords

Sustainable agriculture · Eco-friendly · Bioactive compounds · Algae · Moss · Phanerogams · Phytoalexins · Mycorrhizae · Chemical warfare

9.1 Introduction

Natural products are the best weapon for the survival of any type of problems regarding infection, pathogenesis, or protection from diseases. Due to their degradability in nature, they are the first options to be used by agriculturalists and plant biologist for combating fungal pathogenesis. The eukaryotic organism fungi have a separate kingdom in Whittaker's five kingdom classification and are prime member of this ecosystem as a potent decomposer. In spite of their heavy and multidimensional applications in agricultural, medicinal, and industrial field ranging from the production of life-saving medicines to food supplements, they are the cause of huge global crop loss each year and lead to economic exhaustion. Macro- and microscopic fungi-producing fruit bodies on different portions of a plant body (stem, leaf, fruit, root) lead to the death and decline of the crop species. Several methods have already been tried since the start of civilization for crop protection but because of plant and fungal coevolution, fungi have dominated on the green eukaryotes and caused significant reduction in the crop yield. Particularly in a country like India where the central GDP largely depends on agricultural output, the fungal pathogenesis has been a matter of grave concern for the agriculture department and policy makers. Huge amount of money and manpower is invested to fight against the fungal diseases for ensuring higher and qualitative yield, but still it has been a burning problem of today's conditions. The problem with chemical and synthetic tools for combating the parasitic infections includes their toxicity leading to quality deterioration and environmental pollution accompanied with side effects on human health. In a case study, it has been reported that the extreme use of antibiotics in agricultural field and their direct consumption by humans through their daily meal have led to resistance of those antibiotics in human fungal pathogens. So we are in search of bioactive agents that will be of biological origin, selective to their host, and produce no secondary symptoms with least negative impact. The problem with fungi is their secretion of various types of mycotoxins (aflatoxins, ochratoxins, patulin, fumonisin, zearalenone, deoxynivalenol, etc.) in the stored food products, causing postharvest loss of cereals, pulses, dry fruits, and spices. Mycotoxins are not only food spoilers but also potent disease-causing agents in humans leading to cancer, liver damage, kidney failure, and paralysis (Miller 1995). The severe effects of fungal crop loss are visible largely in tropical or subtropical regions where the temperature is moderately higher than the other parts of the world. Fungal devastation occurs in two phases: firstly, when the crops are growing on the field and, secondly, when they are stored for further transportation, postharvest loss. The third type of contamination occurs when the microscopic airborne pathogens like molds grow on cooked

foods and lead to food spoilage. At each and every level, scientists have developed techniques to minimize fungal food loss. On a gross annual estimate, almost 25% of agricultural food items are of no use due to fungal contamination (Pittet 1998). The major issues with fungi-related crop loss are deterioration as a result of increase of fatty acid conditions, change of color and texture of food items, poor nutritional conditions, and poor germinability of stored seeds (Dhingra et al. 2001). Reports from Asia and Africa include death of humans and animals due to consumption of mycotoxin-contaminated foods (Reddy and Raghavender 2007). Fungal pathogens are sometimes dependent on more than one host for their successful completion of life cycle and disease development (*Puccinia graminis* var. *tritici*, causal agent of black stem rust of wheat that requires *Berberis aristata* for successful infection other than their main target wheat plant). So physical controls like eradication of secondary or collateral host and burning of the old livestock and remnants of the field are the primary measures adopted by the farmers for disease-free crop production. So maintaining the sustainability along with less pathogenic infection is the deep ecological movement for crop maintenance. There are reports of resistance developed against the common and widely used antibiotics of agricultural importance. Blastocidin S, an antibiotic obtained from *Streptomyces* sp. (a type of actinobacteria predominantly present in soil samples), interacts with the protein synthesis and causes the death of the rice blast pathogens. Development of resistance of this antibiotic is reported to be present in some fungal pathogens that detoxify it by deamination (Dayan et al. 2009). Compounds of bacterial and fungal origin from both soil and endophytic sources are used as an alternative source over the chemical ones. Plant extracts especially essential oils from plant taxa of Lamiaceae family are of immense importance and are used as fungicidal or fungistatic. Most of the active ingredients act upon the fungal cell wall by either blocking the cellular processes like respiration, cell wall and cell membrane synthesis, ergosterol biosynthesis, protein synthesis, or DNA replication. Not only the secondary metabolites of plant and microbial origin but also the direct application of microorganisms in terms of biocontrol agent could be used as potent antifungals. Other than these, plants' own defense molecules, known as the phytoalexins, could provide a strong line of defense against mycorrhizae; the root symbionts of higher plants can physically, biologically, and biochemically protect the plant root from pathogenic invasion and provide an enhanced resistance conditions to their hosts. This study includes the role of these compounds as natural agents of antifungal property and their role in disease prevention.

9.2 Mycorrhizae as a Biocontrol Agent

9.2.1 Introduction

Mycorrhiza being the perfect example of symbiosis is known to be the oldest association between higher plant (both angiosperm and gymnosperm, monocot and dicot plants) and fungi and is an astonishing phenomenon of nature. The

mycorrhizal association is one of nature's privileges for maintaining the sustainability of agriculture. In present day's changing environment, haphazard use of pesticides (fungicides) and chemicals poses a great risk to the existence and survival of mycorrhizal species in its complete biologically active form. There is a need to increase awareness in order to save mycorrhizal fungi from extinction.

Plants form beneficial association with other variants of life forms (animals, bacteria, or fungi) to complete their life processes, to fight against pathogenic microorganisms, and most importantly to thrive in adverse environmental situations. The plant root and its associated living microbial flora are together called "rhizosphere," particularly the area of mycorrhizal occurrence. The term mycorrhiza is derived from two Greek words: *mycos* which means fungus and *rhiza* which means roots. In nature, more than 80% of angiosperms and almost all of gymnosperms are known to have mycorrhizal associations. The common two types of mycorrhizal associations that exist in nature are endomycorrhizae, also called arbuscular mycorrhizae (AM), for example, *Endogone* sp. and *Rhizophagus* sp., and ectomycorrhizae (EM), for instance *Amanita muscaria* and *Laccaria bicolor*. Mycorrhizal associations support its host plants to survive in untimely soil conditions and drought situations by increasing the surface area of root and efficiency of mineral uptake. Environmental threats including problems of temperature increase, climate changing, drought, and infertility of soil are some of the major challenges in agriculture and have to be mitigated to ensure global food security. In this context, mycorrhiza-based crop production is one of the key components of sustainable agriculture practices.

9.2.2 Interaction Between AM Fungi and Plant Pathogens

In most of the cases, AM fungi-mediated suppression of root pathogenic fungi is achieved by either morphological, physiological, or biochemical alterations of the host. Several experiments on fungistatic activity of the mycorrhizal species have been done, and fruitful results are found against pathogenic fungi such as *Aphanomyces* spp., *Botrytis fabae*, *Chalara (Thielaviopsis) basicola*, *Dothiorella gregaria*, *Fusarium oxysporum*, *Gaeumannomyces graminis* var. *tritici*, *Ganoderma pseudoferreum*, *Pythium ultimum*, *P. splendens*, *Phytophthora parasitica*, *P. cactorum*, *P. vignae*, *Rhizoctonia solani*, *R. bataticola*, and *Sclerotium rolfsii* (Lioussanne et al. 2009; Bagyaraj 2006; Bagyaraj and Chawla 2012). The most common outcome of AM fungal colonization is seen as an increase in number of branches, resulting in a relatively larger proportion of higher-order roots in the root system. Thickening of the cell walls due to lignification and production of polysaccharides in mycorrhizal plants are the common mode of prevention of penetration and growth of pathogens like *Fusarium oxysporum* and *Phoma terrestris*. A huge percentage of AM-root pathogen interaction studies have been conducted in crop plants of agricultural and horticultural importance. But the information available on forest tree species is scanty. Mycorrhizal technology can thus play an important role in production of low-cost quality seedlings and provide plant protection. Like other methods of biological control, AM fungi are not able to offer complete immunity against the

infection caused by plant pathogens. They could only impart a degree of resistance against soilborne plant pathogens. However, the possibility of biologically controlling soilborne plant pathogens looks promising.

9.2.3 Enhancement of Plant Defense Mechanism

AM fungi play a protective role for plants by activating the defense mechanisms for the better resistance of crop plants and thus may protect the host plant from further fungal pathogenic attack, thus working as a potent biocontrol agent. Researchers have proved that AM symbiosis triggers the activation of several defense-related genes and also expression of pathogenesis-related proteins. Evidences are drawn from modern techniques like molecular biology methods and immunological and histochemical analysis that strongly supports this concept. AM fungi first act as a biotrophic agent, and before entering the host plant's root cell, they cause a sharp change in endogenous salicylic acid that is reflected in quick accumulation of reactive oxygen species (ROS), a wide range of hydrolytic enzymes, and also the activation of phenylpropanoid biosynthetic pathway (Güimil et al. 2005, Paszkowski 2006, Roman et al. 2011). Research findings have proved that the amount of defense-related compounds (essential enzymes like PAL, phenylalanine ammonia-lyase, a product of phenylpropanoid pathway, enzymes needed for flavonoid or iso-flavonoid biosynthesis like chalcone isomerase) that act for the protection of plant from fungal and bacterial pathogen is higher in the case of mycorrhiza-inoculated plant than in the uninoculated ones (Volpin et al. 1994, 1995). Host's physiological and biochemical processes are greatly influenced by the mycorrhizal association in terms of decreased root exudation, higher concentration of phenylalanine and serine contents, ortho-dihydroxy phenols, increased membrane phospholipid content, etc. (Smith et al. 1994). When the phospholipid contents are high, it reduces the chances of root pathogenic attack, and higher concentrations of ortho-dihydroxy phenols show inhibitory activity against root rot pathogen *Sclerotium rolfsii* (causal agent of southern blight), whereas the non-mycorrhizal plants are affected by the southern blight disease. Tomato plants when inoculated with *G. fasciculatum* show inhibitory activity against root knot nematodes. Host-AM association leads to the formation of defense-related compounds like phytoalexins, chitinases (CHI), β -1,3-glucanase (GLU) (enzyme related to hydrolysis of fungal cell wall), peroxidases (POX), hydroxyproline-rich glycoproteins, and phenolics (St Arnaud and Vujanovic 2007). Synergistic effect of PGPRs along with AM fungi is proved to be a system of better protection than the use of AM fungi alone (Linderman 1994; Bagyaraj and Chawla 2012). Fungal wilt of common medicinal plant Indian coleus (*Coleus forskohlii*) caused by *Fusarium chlamydosporium* could be minimized by the joint action of AM fungus and *Trichoderma viride* and cause a sharp increase in root yield and root forskolin concentration and may also reduce the severe disease conditions (Singh et al. 2012).

9.2.4 Change in Rhizospheric Microbial Population

AM causes a drastic change in the rhizospheric microbiota and intentionally either removes directly the pathogenic microorganisms or stimulates the accumulation of potent microbial partners especially fungus that are heavily antagonistic to the plant pathogenic ones. Plants with mycorrhizal association harbor higher population of rhizospheric microorganisms, thus making it impossible for the pathogen to compete and invade the root. In the case of *Phytophthora cinnamomi*, the numbers of sporangia and zoospores are found to be reduced when rhizospheric soil extracts of AM plants are applied; it means the AM fungi are able to alter the microbial population and particular functional groups of rhizospheric microorganisms (Meyer and Linderman 1986; Larsen and Bodker 2003). They cause qualitative and quantitative changes in the fungal community by several factors like changed exudation patterns; altered root size and architecture; different physiological and biochemical parameters like sugar, organic acids, and amino acids; and also putative direct AM fungal effects (Toljander et al. 2007; Ahmed et al. 2013; Vigo et al. 2000). Fungistatic siderophore (low-molecular-weight chelating agents having higher affinity for ferric ion)-producing microorganisms are found to be crowded in mycorrhiza-infected roots and rhizospheric regions. Mycorrhizal plants are to be reported with more actinomycetes and bacterial (Gram-positive *Paenibacillus* sp. against *Phytophthora parasitica*) flora antagonistic to soilborne root pathogens (Azcon-Aguilar and Barea 1996; Budi et al. 1999).

9.2.5 Change in Root Anatomy

Apart from providing biochemical and physiological defense strategies, arbuscular mycorrhizal species also exhibit physical barrier of defense by changing the root anatomy, morphology, and even architecture in terms of increased nutrient uptake, meristematic and nuclear activities of root cell, higher rate of growths, and branching patterns (Atkinson et al. 1994; Gamalero et al. 2010; Gutjahr and Paszkowski 2013). Thus responses of root morphology as a result from AFM colonization seem to depend on plant characters, tap root system, etc. More benefits are seen in tap root system than fibrous root system in terms of gained biomass and nutrient acquisition. Though there is a gap of knowledge in how increased root branching caused by mycorrhizal infection help the plant to defend fungal pathogenesis, synergism is seen as something that can balance the suppressed root growth caused by several root pathogens and restore the root health.

9.2.6 Enhanced Nutrient Uptake by the Host and Competition Between the Symbiotic Partner and Pathogenic One

Mycorrhiza-mediated strengthening of the vascular system allows the higher rate of flow of nutrients, increased mechanical strength, and also inactivation of vascular

pathogens. In conditions of limited resource such as carbon requirement and space for inoculation, a competition between the symbiotic partner (mycorrhizae) and pathogenic fungi is very common and expected (Vos et al. 2014). In the direct warfare, mycorrhizae win over the pathogenic one and thus obtain higher amount of nutrients (almost 4–20% of total assimilated carbon by host plant) and occupy large areas of available root cortical cells (Jung et al. 2012; Vierheilig et al. 2008). Defeating the pathogenic fungi in terms of nutrient uptake and providing a little or no room for infection are probably the mightiest cause of biocontrol ability of AM fungus (Hammer et al. 2011). Output of AM and *Phytophthora* interaction indicates that the pathogen does not penetrate cortical arbuscular cells, suggesting that localized competition for infection site does occur between the pathogenic fungi and the AM fungus. Not only fungi but also plant-invading nematodes are in the queue for colonization and nutrient uptake (Smith 1988). The infection of southern root-knot nematode (*Meloidogyne incognita*, *M. exigua*) is reduced when the roots are priorly inoculated with symbiotic partners like in the case of coffee plants also (Alban et al. 2013; Dos et al. 2010). Reports have suggested that the number of infected sites is reduced within mycorrhizal root system than in the uninoculated one and thus strongly supports the mycorrhizal role as a biofungicide (Vigo et al. 2000). AM fungi can help the plant uptake of nutrients like phosphate, nitrogen, minerals, microelements (zinc), and water at a higher rate than the uninfected one (Baum et al. 2015; Parniske 2008), and as a result, they are provided with photosynthetic carbon (Smith and Smith 2011). The plants capable of absorbing higher amount of nutrients in terms of AM fungal association have the potential to tolerate pathogenic infections (Karagiannidis et al. 2002). Though the improved nutrition and increased tolerance are not involved in a cause–effect relationship, proofs are there that higher uptake of phosphate results in remarkable reduction in pathogenic infection in mycorrhizal plant but not in non-mycorrhizal plant (Bodker et al. 1998). Tomato plants already infected with *Rhizophagus irregularis* are not colonized by the pathogen *A. solani*, whereas non-mycorrhizal plant is affected by the pathogen (Fritz et al. 2006). Mixed action of arbuscular mycorrhizal fungi (AMF) *Glomus intraradices* and *Trichoderma harzianum* as a biocontrol agent significantly reduces the damping off disease caused by *Rhizoctonia solani* in the case of tomato seedlings (Amer and Seud 2008).

9.3 Phytoalexins as Plant Protectants

9.3.1 Introduction

In order to combat parasitic (fungal, bacterial, viral, nematoidal, and insectal) infection like mammalian cells, plant cells also develop defense systems that mediate the release of low-molecular-weight and short-lived (generally 72–96 h of existence) antimicrobial compounds or molecules known as the phytoalexins (Braga 1991; Echiverri et al. 2010; Paxton 1980). These secondary metabolites help the plant to withstand biotic and abiotic stress (Grayer and Kukubun 2001). Most of them being

Table 9.1 Mode of action of phytoalexin as an antifungal agent

Phytoalexin	Host plant	Mode of action	Target organism	References
Phaseolin or kievitone	<i>Phaseolus mungo</i>	Inhibition of glucose uptake of fungal cell	<i>Rhizoctonia solani</i>	VanEtten and Bateman (1971)
Rishitin, phytuberin, Anhydro- β -rotunol, solavetivone	<i>Solanum tuberosum</i>	Loss of motility and swelling of the zoospores, formation of cytoplasmic granules, and bursting of the cell membrane (leakage of electrolytes and metabolites)	<i>Phytophthora</i> sp.	Harris and Dennis (1977)
Stilbene, resveratrol, pterostilbene	<i>Vitis vinifera</i>	Disorganization of mitochondria Disruption of plasma membrane Uncoupler of ETS and blocker of photosynthesis	<i>Botrytis cinerea</i>	Pezet and Pont (1990), Adrian et al. (1997), Adrian and Jeandet (2012)
Camalexin	<i>Phaseolus vulgaris</i>	Induction of the fungal programmed cell death (PCD) by apoptotic mechanisms	<i>Botrytis cinerea</i>	Shlezinger et al. (2011)

lipophilic compounds can cross the plasma membrane and act inside the fungal cell causing cytoplasmic granulation of the infecting fungal cells, disorganization of the cellular components, rupture of the plasma membrane, and inhibition of the fungal enzymes and mycelial growth (Cavalcanti 2005). Mode of action of phytoalexins against fungal pathogenesis varies from species to species (Table 9.1). Metabolism of phytoalexin mediated by fungus involves the tendency for its increased polarity by addition of hydroxyl group (oxygenation), removal of methyl group (demethylation), etc. (Jeandet et al. 2014). Muller and Borger first enlightened the concept of phytoalexins almost 70 year ago (Muller and Borger 1940). The first reported case analyzed with the concept of phytoalexin was potato tuber infection by the different strains of causal organism of “late blight of potato,” *Phytophthora infestans*. This pathogenic fungus initiated the hypersensitive reactions that lead to the formation of some “plant secondary metabolite” that inhibited further infection of the same plant when infected with another strain of the same genus of *Phytophthora*. Muller and his coworker named this “principle” as “phytoalexins” that have protected the plant from secondary infection (Deverall 1982). Accumulation of phytoalexins in the green plant tissue clearly indicates the presence of remarkable amount of fungal and bacterial infections in the host tissue (Stoessl 1980). Phytoalexins are naturally occurring products secreted and accumulated temporarily by plants in response to pathogenic attack or abiotic stress and agents like heavy metal toxicity, UV radiation, and wounds on tissue (Naoumkina et al. 2007). The inducer agent may be of two types, elicitor and elicitin. The elicitors are commonly the oligosaccharides from fungal cell origin (like heptasaccharide from soja cell wall) (Sharp et al.

1984). The elicitor types of molecules are generally a type of glycoproteins secreted by the fungal cells (Cordelier et al. 2003). Reports on detailed investigations about phytoalexins have covered a very few families (Leguminosae and Solanaceae) of the green world (Ingham 1982; Kuc 1982). Though investigations on some selected number of species and genera are made from plant families including both monocotyledonous (Amaryllidaceae, Orchidaceae, Poaceae) and dicotyledonous plants (Apiaceae, Asteraceae, Convolvulaceae, Chenopodiaceae, Euphorbiaceae, Linaceae, Moraceae, Piperaceae, Rosaceae, Rutaceae) and even gymnospermic taxa (Ginkgoaceae) (Coxon 1982), cash crops like members of Poaceae (focusing on maize and rice), Vitaceae, and Malvaceae (cotton) have been studied for their phytoalexin production (Schmelz et al. 2014; Langcake and Pryce 1976; Jeandet et al. 2010; Sunilkumar et al. 2006). Though till date a lot of researches have already been performed regarding phytoalexins, a natural weapon against mycopathogens, but still to increase the fungitoxic effectivity of these stress metabolites, further advancement in design and genetic control is needed (Pont and Pezet 1990).

9.3.2 Biosynthetic Pathways and Regulatory Mechanism

Phytoalexin synthesis not only is dependent on pathogenic attack but also could be influenced by various abiotic factors such as temperature, humidity, and water availability (Fig. 9.1). There are evidences that different parts of the plant like leaves, flowers, stems, seeds, and root tubers are site of phytoalexin biosynthesis (Mikkelsen et al. 2003). Different biochemical pathways are used for producing various types of phytoalexins. The three most common pathways include (i) the

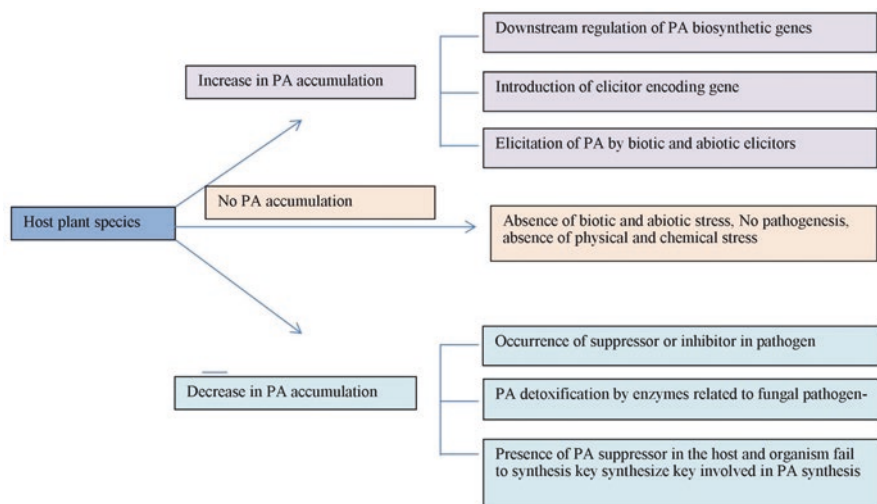


Fig. 9.1 PA production in host plant species is regulated by the interaction between the plant and pathogenic microbes

phenylpropanoic–polymalonic acid pathway, (ii) the methylerythritol phosphate (MEP) and geranylgeranyl diphosphate (GGDP) route, and (iii) the indole phytoalexin (IP) pathway (Jeandet et al. 2014). It is not always obvious that phytoalexins could be categorized not only by their chemical structure or biosynthetic pathway but also by their function and tissue specificity. Examples include the occurrence of momilactone A on different plant parts of rice plant (Lee et al. 1999; Cartwright 1981). Momilactone A is known to be residing in rice husks and rice stems constitutively, but they are also a phytoalexin of rice leaves. Further studies by Toyomasu and his coworkers conclude that momilactone A is constitutively synthesized and oozed out from root of rice plants. Still there is no sufficient data available to consider phytoalexins as ubiquitous throughout the whole plant kingdom. A lot of studies have revealed their complex biochemical synthetic machinery that involves their de novo synthesis, regulation, and mode of action (Jeandet et al. 2013 Ahuja et al. 2012). Regulatory mechanisms involve defense-related marker genes, calcium sensors, hormone signaling, phosphorylation cascades, and also their antipathogenic activity. There are reports on genetic engineering-mediated manipulation of phytoalexin production and increased disease resistance in the case of plants (Delaunais et al. 2009; Jeandet et al. 2012, 2013).

9.3.3 Fungal Pathogenesis: A Stimulus for Phytoalexins Production

Phytoalexins are secondary or stress metabolites that are produced when the host plant is infected with pathogenic fungus. Phytoalexin-mediated defense response includes the expression of lytic enzymes such as chitinases and glucanases and a number of pathogenesis-related (PR) proteins, oxidizing agents, and lignification of cell walls (Dixon and Lamb 1999). Mode of action of phytoalexin involves the coordinated synergism between several defense factors for the effective inhibition of the fungal pathogen (Purkayastha 2017; Mansfield 1999). In the case of *Sorghum* plant, significant infection caused by *Fusarium proliferatum* and *Fusarium thapsinum* stimulates the production of 3-deoxyanthocyanidin, apigeninidin, and luteolinidin and also the concentration of defense-related proteins like peroxidases, β -1,3 glucanases, and chitinases that help to fight the pathogenic infection (Huang and Backhouse 2004). Similarly, phytocassanes A, B, C, D, and E are produced as a result of *Magnaporthe oryzae*, *Rhizoctonia solani*, and *Phytophthora infestans* infections on rice plants. Remarkably phytocassane E, and momilactones A and B exert in vitro antifungal activity against *Magnaporthe oryzae*, *Botrytis cinerea*, *Fusarium solani*, *Fusarium oxysporum*, and *Colletotrichum gloeosporioides* (Koga et al. 1997; Fukuta et al. 2007). There are several ways of blocking the fungal infection in host plant tissues by phytoalexin-mediated response. That includes inhibition of fungal spore on the leaf surface and inhibition during and after penetration to host cell (Usman et al. 2018). The occurrence of fungal germ tube on the leaf surface and diffusion of fungal metabolites through the leaf cells cause the accumulation of phytoalexins by the underlying cells and provide the first line of induced chemical

defense (VanWees et al. 2003). Phytoalexins may be located on papillae or cell walls, thereby producing a localized, fungitoxic barrier to penetration (Friend 2016). Examples include occurrence of fungitoxic (against *Erysiphe graminis*) flavonoid (thought to be phytoalexin) on papillae of resistant barley leaves.

9.3.4 Fungal and Green Plant Extracts: In Vivo Inducers of Phytoalexins Production

Phytoalexins are known to be solely produced as a result of induction or stimulus by external agents. Fruitful evidences could be drawn regarding this fact. Induction of disease resistance in plants is developed through the direct and indirect involvement of elicitors. Extracts of fungal basidiocarp, essential oils of aromatic plants (Walters et al. 2013), and also synthetic chemicals like aminobutyric acid, salicylic acid, jasmonic acid, and acibenzolar-S-methyl (Garcia-Mier et al. 2013) are the inducers of phytoalexins production. Preparations of horse tail pteridophytic genus *Equisetum* sp. induce the production of glyceolin in soybean plant (*Glycine max*) cotyledons and significantly reduce the *Rhizoctonia solani* infection (Guimaraes et al. 2015). Further studies include the effect of aqueous extracts of basidiocarps of *Agaricus blazei*, *Lentinula edodes*, and *Pycnoporus sanguineus* (Arruda et al. 2012) on the production of glyceollins. Deoxyanthocyanidins and glyceolins are also synthesized by the tinctures of medicinal plants like *Ruta graveolens*, *Origanum majorana*, *Baccharis trimera* (Matiello and Bonaldo 2013), *Hymenolobium petraeum*, *Qualea albiflora*, and *Corymbia citriodora* (Matiello et al. 2016) that act as the elicitors of deoxyanthocyanidins and glyceolin production. Homeopathic preparations of species of *Calcarea* (*C. citriodora* and *Calcarea carbonica*), essential oils of *Eucalyptus globulus* (Telaxka et al. 2014; Oliveira et al. 2014), and mild concentrations of salicylic acid (Durango et al. 2013) are major elicitors of pistain production and accumulation in cotyledons of common bean (*Phaseolus vulgaris*). Silicon-mediated enhancement of disease resistance by peroxidase (POX), polyphenol oxidase (PPO), chitinases (CHI), β -1,3-glucanases (GLU), and phenylalanine ammonia-lyase (PAL) is found in the case of leaf spot of cotton plant caused by *Ramularia areola* (Curvêlo et al. 2013).

9.3.5 Modern Approaches Involving Amphibians' Extract as Defense Inducers of Plants

Southern Amazonian amphibian family Bufonidae represents the true toads, and their cutaneous secretions are of diverse source of bioactive compounds which can be fruitful as new chemical weapons for agrochemical development. Use of elicitors in the case of crop protection nowadays is becoming a very popular method of inducing response which are proved to be durable and broad-spectrum disease control mechanism where the plant's own resistance is used. A group of seven Brazilian scientists (Deice et al. 2019) evaluated the possibilities of methanolic extracts of

cutaneous secretions of two species of Bufonidae, *Rhaebo guttatus* and *Rhinella marina*, on synthesis of phytoalexins named glyceolin (soybean plant), deoxyanthocyanidins (*Sorghum* plants), and phaseolin (mung plant) in soybean cotyledons, sorghum mesocotyls, and bean hypocotyls, respectively. There is a direct relationship between the phytoalexins production and defense ability of the host plant against the fungal pathogenesis. Studies reveal that when the phytoalexin glyceolin is produced in higher amount in the soybean plant (cultivar TMG 132 RR) as a result of methanolic extracts of amphibian's (*R. guttatus*) cutaneous secretion (at a concentration of 0.2 mg/mL), stimulates the enzyme β -1,3-glucanase that can cause the hydrolysis of the fungal cell wall along with other defense-related enzymes (chitinase) is also produced in higher amount, but when suppression of glyceolin occurs, that particular enzyme is also not produced. There are evidences in the case of *Glycine max* that the effectivity of phytoalexins varies from cultivar to cultivar. Application of *R. marina* (amphibian) methanolic extracts induced glyceolin production in TMG 132 RR and Monsoy 8372 cultivars IPRO but did not induce TMG 132 RR cultivars to synthesize these defense-related compounds.

9.3.6 Phytoalexins Versus Phytopathogenic Fungi: A Direct Chemical Warfare

Less toxicity of phytoalexins than chemical fungicides is the reason for their universal acceptance. For over 75 years, phytoalexins have been a detailed area of study for its antimicrobial activity, especially antifungal properties. Several investigations include the in vivo bioeffectivity of the phytoalexins against serious plant pathogenic fungi (Table 9.2). Phytoalexin synthesizing genes have also been genetically modified to cope up with the pathogenic evolution. Still reports are there that include examples of cruciferous phytoalexins detoxification by fungal enzymes (Pedras and Abdoli 2017). Modification of pathogen to overcome phytoalexin-mediated damage includes curved germ tubes as a result of asymmetric growth of the germ tube. Phytoalexins are natural products of diverse chemical nature, for example, alkaloids, coumarins, isoflavonoid (coumestans, isoflavans, isoflavones, isoflavanones, pterocarpanes, pterocarpenes), lignans, polyacetylenes, pterocarpons (pisatin, phaseolin, glyceollin, medicarpin, and maackiain), terpenes, and non-isoflavonoid compounds (furanocetylenes and stilbenes) (Fig. 9.2) (Grayer and Kokubun 2001). Both in vitro and in vivo fungicidal activity are shown by sakuranetin (rice phytoalexins) against the blast fungus (Hasegawa et al. 2014). Reduction of green mold (caused by *Penicillium digitatum*) infections is achieved by the action of coumarin type of phytoalexin (scopoletin) of orange (Sanzani et al. 2014). The loss of apples production caused by *Penicillium expansum* and accumulation of patulin is minimized by the action of phenolic phytoalexins like resveratrol, scopoletin, scoparone, and umbelliferone (Sanzani et al. 2009). In the case of *Medicago sativa* (alfalfa), the isoflavonoid 7-O-methyltransferase provides increased resistance against *Phoma medicaginis* by synthesizing *maiackiain* (He and Dixon 2000). For soybean plants, transformation of resveratrol to pterostilbene

Table 9.2 Production of phytoalexin by host plant species in response to pathogenic fungal infection

Fungal pathogen	Name of phytoalexin	Host	References
<i>Phytophthora drechleri</i>	<i>Trans-trans</i> -3,11-tridecadiene-5,7,9-triene-1,2-diol	<i>Carthamus tinctorius</i> (safflower) (Asteraceae)	Allen and Thomas (1971)
<i>Helminthosporium turcicum</i>	Sativin, vesitol	Alfalfa (<i>Medicago sativa</i>), bird's-foot trefoil (<i>Lotus corniculatus</i>) (Nymphaeaceae)	Bonde et al. (1973)
<i>Ceratocystis fimbriata</i>	Ipomeamarone Xanthotoxin Polyacetylenes/ falcarinol Phenolics: xanthotoxin and 6-methoxymellein	Sweet potato (<i>Ipomoea batatas</i>) (Chenopodiaceae) <i>Pastinaca sativa</i> (parsnip root)	Johnson (1973)
<i>Cercospora beticola</i>	2',5-Dimethoxy-6,7-methylenedioxyflavanone, 2'-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone, betagarin, isoflavones, betavulgarin	<i>Beta vulgaris</i> (Chenopodiaceae)	Geigert (1973)
<i>Hendersonula</i> sp., <i>Phytophthora</i> sp.	Xanthoxylin	<i>Citrus limon</i> (Rutaceae)	Hartmann and Niehaus (1974)
<i>Rhizopus stolonifer</i> , <i>Aspergillus niger</i> , <i>Fusarium moniliforme</i>	Diterpenes, casbane	<i>Ricinus communis</i> (Euphorbiaceae)	Sitton and West (1975)
<i>Phytophthora infestans</i>	Dihydrophenanthrenes (loroglossol)	<i>Loroglossum hircinum</i> (Orchidaceae)	Ward (1975)
<i>Melampsora lini</i>	Coniferyl alcohol, coniferyl aldehyde	<i>Linum usitatissimum</i> (Linaceae)	Keen and Littlefield (1975)
<i>Botrytis cinerea</i>	Flavans, 7-hydroxyflavan, 7,4'-dihydroxyflavan, 7,4'-dihydroxy-8-methylflavan, trans-resveratrol (4,3',5'-trihydroxy stilbene), 6-methoxymellein, <i>p</i> -hydroxybenzoic acid, polyacetylene falcarinol	Daffodil (<i>Narcissus</i> sp.) (Amaryllidaceae) <i>Vitis vinifera</i> , grape vine (Vitaceae) Carrot root	Langcake and Pryce (1976), Coxon (1980), and Harding and Heale (1981)

(continued)

Table 9.2 (continued)

Fungal pathogen	Name of phytoalexin	Host	References
<i>Fusarium solani</i>	Furanopterocarpan, moracins E, F, G, and H	<i>Morus alba</i> (<i>Moraceae</i>)	Takasugi (1979)
<i>Bipolaris leersiae</i>	Brassicinal A Brassicinal C Brassinin Cyclobrassinin Dehydro-4- methoxycyclobrassinin, dioxibrassinin 1-Methoxybrassinin 4-Methoxybrassinin 1-Methoxyspirobrassinol Methyl ether	<i>Brassica oleracea</i> <i>B. rapa</i> <i>B. napus</i> <i>B. carinata</i> (<i>Brassicaceae</i>)	Takasugi et al. (1986), (1988); Monde et al. (1990a, b), (1991), (1994); and Gross et al. (1994)
<i>Phoma lingam</i>	Brassicinal A Brassilexin Camalexin Cyclobrassinin Methyl 1-methoxyindole-3-carboxylate Spirobrassinin	<i>B. rapa</i> , <i>B.</i> <i>campestris</i> <i>B. carinata</i> <i>Capsella</i> <i>bursa-pastoris</i> <i>B. napus</i> <i>R. sativus</i> (<i>Brassicaceae</i>)	Devys et al. (1988), Dahiya and Rimmer (1988), Browne et al. (1991), Conn et al. (1994), Pedras and Khan (1996), Pedras et al. (1997), and Pedras and Sorensen (1998)
<i>Sclerotinia sclerotiorum</i>	Cyclobrassinin	<i>B. napus</i> (<i>Brassicaceae</i>)	Dahiya and Rimmer (1988)
<i>Cladosporium</i> sp.	Camalexin, Cyclobrassininsulfoxide 1-Methoxycamalexin 6-Methoxycamalexin	<i>A. thaliana</i> <i>B. carinata</i> (<i>Brassicaceae</i>)	Devys and Barbier (1990)
<i>Alternaria brassicae</i>	Camalexin 1-Methoxybrassinin 6-Methoxycamalexin	<i>Arabidopsis thaliana</i> <i>B. napus</i> <i>B. oleracea</i> <i>C. bursa-pastoris</i> <i>Camelina sativa</i> (<i>Brassicaceae</i>)	Browne et al. (1991), Tsuji et al. (1992), and Gross et al. (1994)
<i>Rhizoctonia solani</i>	Camalexin Cyclobrassinin	<i>C. bursa-pastoris</i> <i>B. napus</i> (<i>Brassicaceae</i>)	Browne et al. (1991)
<i>Pythium ultimum</i>	Cyclobrassinin 1-Methoxybrassinin	<i>B. napus</i> <i>B. oleracea</i> (<i>Brassicaceae</i>)	Conn et al. (1994)

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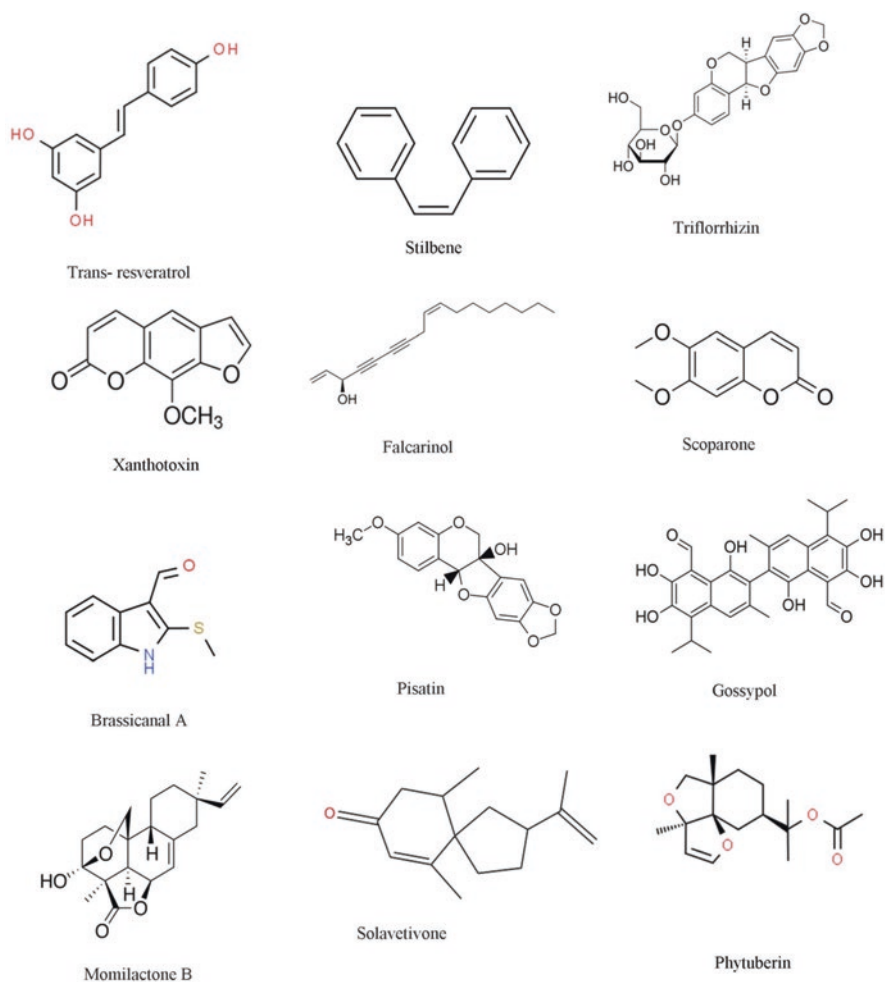
Table 9.2 (continued)

Fungal pathogen	Name of phytoalexin	Host	References
<i>Cladosporium cucumerinum</i>	1-methoxybrassinin, 1-methoxyspirobrassinin, cyclobrassinone, sinalexin, spiobrassinin	<i>B. oleracea</i> <i>B. carinata</i> <i>B. juncea</i> (Brassicaceae)	Gross et al. (1994)
<i>Phomopsis perniciosa</i>	Biphenyls, aunarperin, dibenzofurans, cotonefurans	<i>Aronia</i> sp., <i>Chaenomeles</i> sp., <i>Eriobotrya</i> sp., <i>Malus</i> sp. (Rosaceae)	Kokubun and Harborne (1995)
<i>Pyricularia oryzae</i>	Brassinin Spirobrassinin	<i>B. napus</i> <i>B. carinata</i> (Brassicaceae)	Storck and Sacristan (1995)
<i>Erwinia carotovora</i>	Camalexin	<i>C. bursa-pastoris</i> (Brassicaceae)	Jimenez et al. (1997)
<i>Fusarium oxysporum</i>	Camalexin	<i>Arabis lyrata</i> (Brassicaceae)	Zook et al. (1998)
<i>Bipolaris maydis</i>	3-Deoxyanthocyanidin, apigeninidin, luteolinidin, and apigeninidin 5-O-arabinoside	<i>Sorghum bicolor</i> (Poaceae)	Wharton and Nicholson (2000)
<i>Fusarium proliferatum</i> , <i>F. thapsinum</i>	Apigeninidin, luteolinidin	<i>Sorghum</i> sp. (Poaceae)	Huang and Backhouse (2004)
<i>Puccinia coronata</i>	Avenaluminins I, II, III	Barley (Poaceae)	Chaube and Pundhir (2005)
<i>Monilinia fructicola</i>	Trifolirhizin	Red clover (<i>Trifolium pretense</i>) (Fabaceae)	Chaube and Pundhir (2005)
<i>Penicillium expansum</i>	Resveratrol, scopoletin, scoparone, umbelliferone	Apple (<i>Malus</i> sp.) (Rosaceae)	Sanzani et al. (2009)
<i>Mycosphaerella fijiensis</i> (black Sigatoka disease)	Irenolone, musanolones	Banana (<i>Musa</i> sp.) (Musaceae)	Echeverri et al. (2012)
<i>Phytophthora megasperma</i>	Glyceollin	Soybean (<i>Glycine max</i>) (Fabaceae)	Ng et al. (2011)
<i>Fusarium graminearum</i> <i>Cochliobolus heterostrophus</i> , <i>Rhizopus microsporus</i> , <i>Colletotrichum sublineolum</i> , <i>Aspergillus flavus</i> , <i>Aspergillus sojae</i> , <i>Ustilago maydis</i>	Zealexins (sesquiterpinoid) Zealexins A1, A2, A3, B1	<i>Zea mays</i> (maize) (Poaceae)	Huffaker et al. (2011)

(continued)

Table 9.2 (continued)

Fungal pathogen	Name of phytoalexin	Host	References
<i>Cochliobolus victorise</i> <i>Fusarium</i> sp.	<i>Diterpenes (solenoids), momilactones A, B, phytocassane A–E, flavonoids (5,4-dihydroxy-7-methoxyflavanone)</i>	<i>Oryza sativa</i> (rice) (Poaceae)	Ahuja et al. (2012)
<i>Botrytis fabae</i>	Wyerone	Pea (<i>Pisum sativum</i>) (Fabaceae)	Slusarenko et al. (2012)
<i>Penicillium digitatum</i> (green mold symptoms)	Scopoletin	Orange (<i>Citrus sinensis</i>) (Rutaceae)	Sanzani et al. (2014)
<i>Rhizoctonia solani</i>	Pterostilbene	Soybean (<i>Glycine max</i>) (Fabaceae)	Zernova et al. (2014)

**Fig. 9.2** Different types of phytoalexins having major role in plant protection

includes protection against *Rhizoctonia solani* (Zernova et al. 2014). Scientists have proven that not only fungal infection acts as the stimulus for phytoalexin synthesis but also the hormone levels; phosphorylation cascades play a major role in this purpose. Cytokinin overexpression in *Nicotiana tabacum* is directly associated with its resistance against *P. syringe* by higher concentration of capsidiol and scopoletin (Grosskinsky et al. 2011.) The fungitoxicity of the phytoalexin could be enhanced by methylation or presence of electron-attracting groups on aromatic rings that is directly involved in affinity with membrane proteins being an uncoupler of ETS system.

9.4 Endophytes: An Untapped Source of Biofungicides

Endophytes are a type of hidden beneficial microorganisms that reside within the host plant causing no visible disease symptoms and syndrome and promote the plant to maintain its existence in typical harsh conditions. Sometimes they could be latent pathogens at a very distant path of the host's life cycle but are simply a unique area of research where plant science and their microbial association get new definition. Endophytes have been a constant and reliable source of exploration of bioactive compounds, but extensive search has not been performed till date, and that has given the endophyte biologists a great opportunity to search endophytic fungal and actinobacterial flora for the establishment of novel bioactive compounds. Selection of plant for endophytic isolation is the most vital part of this study. Exploitation of the proper isolates accelerates this search and opens up new angle of research. The search for uncommon products of agrochemical importance is a common demand of today's world. The safer the antifungal agents become, the more it is well accepted in the scientific community as well as agricultural market. In general, the screening of thousands of natural products ends up giving only one commercial product. So indeed it's a tough job to end the search of new antibiotics with a hopeful result. A total of 6 out of 20 of the popular prescribed medicines are of fungal origin, and it is a fact that 5% of the fungi have been described till date (Hawksworth 1991, 2001). So fungi serve as a continuous dependable source of new natural products. The intelligent screening procedure includes the selection of fungal flora of endophytic sources to open up the untapped potential of secondary metabolites synthesized by fungi.

9.4.1 Process of Screening of Antifungal Metabolites from Endophytic Origin

Microorganisms grown in the petri plates or culture broth constitute minimal growth medium needed for their survival. Any kind of stress or transfer of microorganisms on selective media acts as a stimulation for production of their secondary bioactive compounds. These secondary metabolites are produced for their survival in odd environments and strictly act as the selection force for the expression of their

antimicrobial-producing genes. These crude by-products of microbial cultures are filtered and purified for their industrial, medicinal, and agricultural exploitation. Soil microorganisms have been exploited for a long time for production of antibiotics, but microorganisms inhabiting plants are a new source in that respect.

Plants are selected usually with potent medicinal applications. Here the knowledge of ethnobotany and folk taxonomy contributes a lot in this selection procedure. A strong literature survey supports the plant selection. The plants are surface sterilized and plated in nutrient-less solid plates. The fungi emerge out from different explants using the decaying plant parts as their primary growth substance. The isolates are identified by microscopic structures focusing on their conidial morphology, spore sculpturing, and colony characters. Confirmatory identification includes 18s rRNA analysis. Endophytic fungi are tested for their antifungal activity against phytopathogenic fungi by one-to-one inhibition assay or antagonistic test (Fig. 9.3). Two agar portions containing fungal hypha of endophyte and pathogen are placed on opposite sides of the plate. If the growth of pathogenic one is arrested partially or completely, that endophytic isolate is marked as antifungal agent and selected for further studies. Another way of screening includes separating the agar plate into two equal halves, and two fungi are placed on two separate sides of the discontinuous plate. This test aims to screen the endophytes that produce volatile antifungals. If the isolate is potent enough to produce volatile organic compounds with fungi static or cidal activity, this will cease the growth of the pathogenic strain. Then that isolate would be qualitatively and quantitatively measured for their volatile emissions using GC-MS as the master equipment (Fig. 9.4). Liquid extracts of endophytic fungi are also tested for antifungal potentials by agar well-diffusion method. The fungal extract having antibiotic property shows clear zone of inhibition of growth of the pathogenic fungus surrounding the area of application of that fungal liquid. The potent isolate will be mass cultured, and the bioactive molecules will be extracted using organic solvents like ethyl acetate, ethyl ether, and n-hexane. Steps include purification of that fungal extract by column chromatography, detection of the compound by thin-layer chromatography, and analysis of compounds by HPLC and mass chromatography. Field experiment includes the synergistic effect of a pure compound with mixture of natural compounds, and the effectivity of a newly applied antifungal agent strictly depends on the host plant and pathogenic microorganism's interaction, environmental condition, and development of drug resistance by that organism. Application of pellets soaked in fungal extracts also is a method of determination of antifungal activity.

9.4.2 Diversity of Antifungal Metabolites

Secondary metabolites are itself diverse in nature. A variety of bioactive secondary metabolites are produced at significant concentrations by the endophytic microbial flora. The major components include quinones, phenols, phenolic esters, steroids, terpenoids, cytochalasins, benzopyranone, alkaloids, isocoumarins, and chromones.

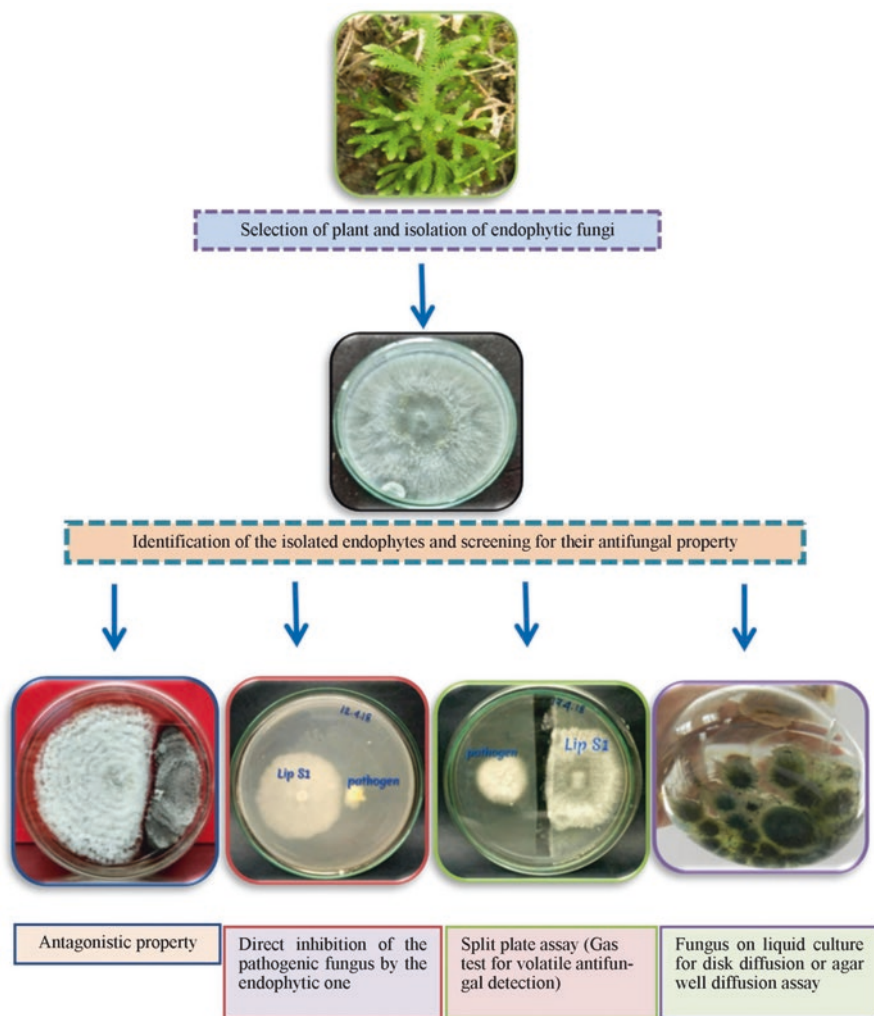


Fig. 9.3 Different approaches for the screening of endophytic fungi with antifungal activity

Till date, a large number of plants have been studied for their endophytic flora as antifungal agents (Table 9.3).

9.4.2.1 Alkaloids

Alkaloid was the first ever reported insecticidal bioactive product. Cryptocin was isolated from endophyte of *Tripterygium wilfordii*, a plant of Celastraceae family. The inner barks of the stem were used as explant, and *Cryptosporiopsis* cf. *quercina* was isolated as a potent endophyte active against *Pyricularia oryzae* and some other phytopathogenic fungi (Li et al. 2000). *Colletotrichum* sp. produces 6-isoprenylindole-3-carboxylic acid having inhibitory action against *Phytophthora*

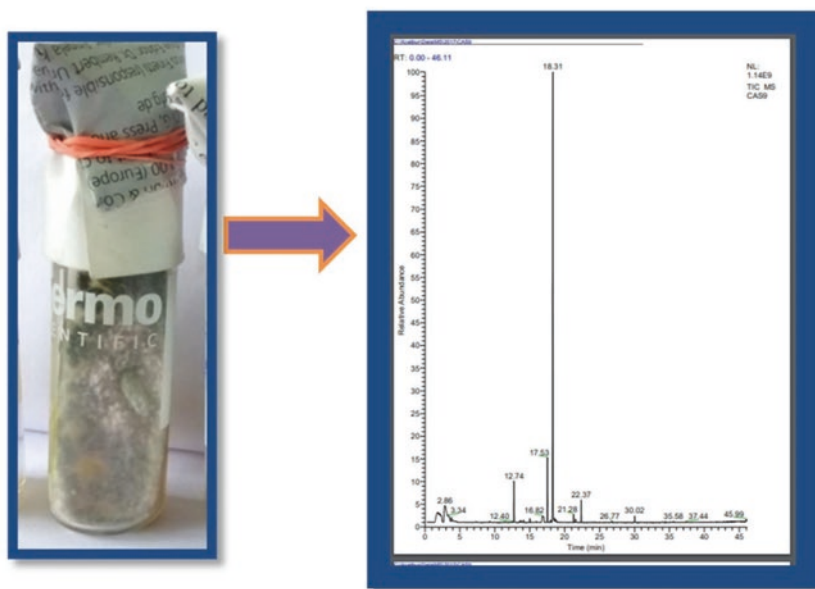
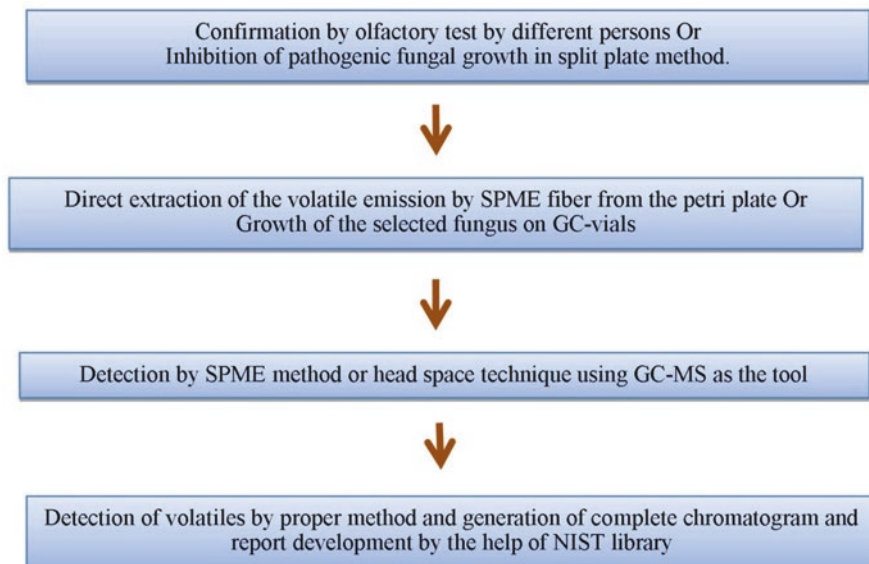


Fig. 9.4 Detection of volatile organic compounds of endophytic origin

capsici, a pathogen of Cucurbitaceae, Fabaceae, and Solanaceae, and also other phytopathogens *Rhizoctonia cerealis* and *Gaeumannomyces graminis* var. *tritici*, a common pathogen of Poaceae family (Lu et al. 2000). Epoxychochalsin H and chochalsins N and H were isolated as chloroform and methanolic extracts of

Table 9.3 Antifungal activity of endophytic fungi

Name of the endophytic isolate	Source plant	Antifungal against	References
<i>Ovulariopsis</i> sp. and <i>Alternaria</i> sp.	<i>Datura stramonium</i>	<i>Aspergillus niger</i> , <i>Colletotrichum gloeosporioides</i> , <i>Fusarium</i> sp., <i>Phytophthora nicotianae</i> , <i>Scopulariopsis</i> sp., <i>Trichoderma viride</i> , <i>Verticillium</i> sp.	Li et al. (2005a, b)
<i>Beauveria bassiana</i> , <i>Trichoderma koningii</i> , <i>Alternaria alternata</i> , <i>Phoma</i> sp., <i>Acremonium strictum</i>	<i>Zea mays</i> (maize) (roots)	<i>Fusarium oxysporum</i> , <i>Fusarium pallidoroseum</i> , <i>Fusarium verticillioides</i> , <i>Cladosporium herbarum</i>	Orole and Adejumo (2009)
<i>Alternaria</i> sp., <i>Chaetomium</i> sp., <i>Dothideomycetes</i> sp., <i>Thielavia subthermophila</i>	<i>Tylophora indica</i>	<i>Sclerotinia sclerotiorum</i> , <i>Fusarium oxysporum</i> , <i>Fusarium oxysporum</i>	Kumar et al. (2011)
<i>Chaetomium globosum</i>	<i>Withania somnifera</i>	<i>S. sclerotiorum</i>	Kumar et al. (2013)
<i>Nigrospora oryzae</i> , <i>Fusarium proliferatum</i> , <i>Guignardia cammillae</i> , <i>Alternaria destruens</i> , <i>Chaetomium</i> sp.	<i>Jatropha curcas</i>	<i>S. sclerotiorum</i>	Kumar and Kaushik(2013)
<i>Phytophthora infestans</i> , <i>Fusarium oxysporum</i>	<i>Triticum durum</i>	<i>Alternaria</i> sp., <i>Cladosporium</i> sp., <i>Penicillium</i> sp., <i>Aspergillus</i> sp., <i>Chaetomium</i> sp., <i>Phoma</i> sp.	Sadrati et al. (2013)
<i>Cladosporium</i> sp., <i>Curvularia</i> sp., <i>Penicillium</i> sp.	Moso bamboo (<i>Phyllostachys edulis</i>) (seeds)	<i>Curvularia eragrostidis</i> , <i>Pleospora herbarum</i> , <i>Arthrimum sacchari</i> , <i>Arthrimum phaeospermum</i>	Shen et al. (2014)
<i>Trichothecium</i> sp.	<i>Phyllanthus amarus</i>	<i>Penicillium expansum</i> (blue mold of apples)	Taware et al. (2014)
<i>Alternaria</i> sp., <i>Biscogniauxia mediterranea</i> , <i>Cladosporium funiculosum</i> , <i>Paraconiothyrium</i> sp.	<i>Opuntia humifusa</i>	<i>Colletotrichum fragariae</i> , <i>C. gloeosporioides</i> , <i>C. acutatum</i>	Silva-Hughesa et al. (2015)
<i>Rhexocercosporidium</i> sp., <i>F. solani</i>	<i>Sophora tonkinensis</i> Gapnep	<i>Alternaria panax</i> , <i>F. solani</i> , <i>C. gloeosporioides</i>	Yao et al. (2017)

(continued)

Table 9.3 (continued)

Name of the endophytic isolate	Source plant	Antifungal against	References
<i>Glomerella cingulate</i> , <i>Colletotrichum gloeosporioides</i> , <i>C. truncatum</i> , <i>Lasiodiplodia pseudotheobromae</i> , Dothideomycetes sp.	<i>Houttuynia cordata</i> Thunb.	<i>F. oxysporum</i> , <i>S. rolfsii</i> , <i>T. harzianum</i> , <i>Rhizoctonia</i> sp., <i>A. brassicicola</i> , <i>P. palmivora</i>	Aramsirirujwet et al. (2016)
<i>C. boninense</i> , <i>F. chlamydosporum</i> , <i>C. aerea</i> , <i>M. yucatanensis</i> , <i>Cladosporium</i> sp.	<i>Monarda citriodora</i> (leaf, roots, and flowers)	<i>F. solani</i> , <i>Sclerotinia</i> sp., <i>Colletotrichum capsici</i> , <i>A. flavus</i> , <i>A. fumigatus</i>	Katoch and Pull (2017)
<i>Trichoderma longibrachiatum</i> strain BHU-BOT-RYRL17, <i>Syncephalastrum racemosum</i> strain AQGSS 12, <i>Trichoderma longibrachiatum</i> voucher 50	<i>Markhamia tomentosa</i>	<i>Fusarium oxysporum</i> , <i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Botrytis cinerea</i>	Ibrahima et al. (2017)
<i>Penicillium simplicissimum</i> , <i>Leptosphaeria</i> sp., <i>Talaromyces flavus</i> , <i>Acremonium</i> sp.	Cotton roots (<i>Gossypium hirsutum</i>)	<i>V. dahlia</i> (Verticillium wilt disease)	Yuan et al. (2017)
<i>Aspergillus</i> sp., <i>Xylaria</i> sp., <i>Fusarium</i> sp., <i>Trichothecium</i> sp., <i>Oidium</i> sp.	<i>Camellia oleifera</i>	<i>Camellia oleifera</i> anthracnose pathogen	Yu et al. (2018)
<i>Penicillium</i> sp. (ARDS-2.3), <i>Aspergillus oryzae</i> (ARHS-1.1)	<i>Asparagus racemosus</i> Willd	<i>Botrytis cinerea</i> (gray mold), <i>Sclerotinia sclerotiorum</i> (stem rot), <i>Rhizoctonia solani</i> (root rot), <i>Fusarium oxysporum</i> (wilt)	Chowdhary and Kaushik (2018)
<i>Aspergillus</i> sp., <i>Curvularia</i> sp., <i>Fusarium oxysporum</i>	<i>Dendrobium lindleyi</i>	<i>Fusarium</i> sp., <i>Sclerotium</i> sp., <i>Colletotrichum</i> sp., <i>Curvularia</i> sp., <i>Phytophthora</i> sp.	Bungtongdee (2019)

Phomopsis sp., an endophyte of *Gossypium hirsutum*. It showed potent antifungal activity against species of *Bipolaris* (*B. sorokiniana*, *B. maydi*), *Botrytis* (*B. cinerea*), *Sclerotinia* (*Sclerotinia sclerotiorum*), *Rhizoctonia* (*R. cerealis*), and *Fusarium* (*Fusarium oxysporum*) (Fu et al. 2011). A lot of endophytes have been explored for their antifungal production, but only a few of them were positive for antifungal metabolites categorizing in alkaloids. The common alkaloids acting as the antifungal agents of endophytic fungal origin are gliotoxin, cryptocanadin, tyrocidine A, fumigaclavine C, fumitremorgin C, 1-N-methyl albonoursin, and phomapsichalasin.

9.4.2.2 Terpene Derivatives

The terpenoids, usually called isoprenoids, are large and diverse group of naturally occurring organic compounds derived from terpenes that are multicyclic. Sixty percent of all the known natural products are terpenoids in nature. Some endophytic fungicidal products are of terpenes by their native chemical structure. Endophytic isolates (*Hormonema* sp.) of gymnospermous plant *Juniperus communis* were reported to be antifungal producers of a triterpene glycoside enfumafungin (Pelaez et al. 2000). Known antifungal sterols of endophytic origin are 3 β -hydroxy-ergosta-5-ene, 3-oxoergosta-4,6,8,22-tetraene, etc. The sterols are strong inhibitors of *Helminthosporium sativum* (present name: *Bipolaris sorokiniana*), the asexual stage of *Cochliobolus sativus*, a common root rot pathogen of wheat and barley crops which also infects leaf and stems of Poaceae plants (Lu et al. 2000). Sesquiterpenes are reported to be the growth inhibitors of *Cladosporium phlei* (causal organism of leaf spot disease of timothy grass, *Phleum pratense*). This is a unique example where the host plant (*Phleum pratense*) itself harbors the endophyte (*Epichloe typhina*) that inhibits the growth of its leaf spot pathogen (*Cladosporium phlei*).

9.4.2.3 Isocoumarins

From the point of view of organic chemistry, isocoumarins are defined as the isomer of coumarin where the orientation of the lactone is reversely arranged. Zhang and his coworkers in the year 2008 isolated an endophytic fungus named *Microdochium bolleyi* from *Fagonia cretica* (also known as virgin's mantle of Zygothylaceae family), a herb of semiarid regions of Gomera. Isocoumarins were identified as the active compounds having antifungal activity against *Microbotryum violaceum* (previously known as *Ustilago violacea*), an obligate parasite of Basidiomycete group and a common infectant of members of Caryophyllaceae causing smut of anther. The four isolated and identified isocoumarins are monocerin, 12-oxo epimers of monocerin, and open-ring derivative compounds of monocerin. The compounds are obtained as mixtures by column chromatography followed by Sephadex LH-20 chromatography techniques. Preparative TLC further differentiated the four compounds. Monocerin and its analogues were previously reported as antifungal compounds from fungal sources of *Drechslera monoceras*, *Exserohilum monoceras*, *Helminthosporium monoceras*, *Exserohilum turcum*, and *Fusarium larvarum* (Aldridge and Turner 1970; Robeson and Strobel 1982; Grove and Pople 1979; Claydon et al. 1979). These secondary metabolites act on pathogens by interfering stages of divisional phases of cell cycle. The second isocoumarin was colorless oil. The third and fourth one are represented by the empirical formula of C₁₆H₂₀O₇ and C₁₆H₂₂O₇. The fourth one is structurally correlated to fusarentin 6,7-dimethyl ether. Both the compounds are of heptaketide in their origin, and it is revealed that fusarentins are the probable precursors of the active compounds monocerins (Scott et al. 1984; Axford et al. 2004). Dihydroisocoumarins, mellein (an isocoumarin derivative), (R)-7-hydroxymellein, and fonsecinone were reported from species of *Xylaria* (endophyte of *Piper aduncum*), *Pezicula*, *Penicillium* (*Alibertia macrophylla*), and

Aspergillus (Cynodon dactylon), respectively (Oliveira et al. 2011; Schulz et al. 1995; Song et al. 2004).

9.4.2.4 Phenolics as the Most Potent Antifungal from Endophytic Source

Phenols (popularly known as phenolics) represent a class of chemical compounds characterized with a hydroxyl group attached to an aromatic hydrocarbon group. Phenol (or carboic acid) is a colorless crystalline solid, aromatic compound having benzene rings. They are predominantly found in the plant kingdom as a response to stress and are of utmost importance. Endophytic culture extracts are also known to be rich sources of phenolics; usually they are directly proportional to the antioxidative property of any fungal isolate, but in some particular cases, they are characterized with their antifungal potentials against phytopathogenic fungus. Usually the liquid culture extracts of the endophytic isolates are subjected to solvent extraction using ethyl acetate, n-hexane, ethyl ether, etc. Those organic solvents are believed to extract the phenolics from the water-based culture broth. Those extracted compounds are further screened for their antifungal efficiency. Ethyl acetate extracts of endophytic *Phoma* sp. are reported to contain tetralone metabolites (derivatives of α -tetralone, 3,6,7-trihydroxy- α -tetralone) inhibiting the growth of two common broad phytopathogenic fungus *Fusarium oxysporum* and *Rhizoctonia solani*. Griseofulvin is known to be the first antifungal compound isolated from *Penicillium griseofulvum*. Later it is isolated from several species of fungi including endophytic *Penicillium canescens* and *Xylaria* sp. (member of Xylariaceae family). Griseofulvin from endophytic *P. canescens* of popular Chinese medicinal plant *Polygonatum cyrtoneuma* (Polygonaceae) showed strong inhibitory effectivity against phytopathogenic *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Colletotrichum orbiculare*, and *Didymella bryoniae* (Wang et al. 2010). Other than *Penicillium*, endophytic *Xylaria* sp. isolated as an endophyte of *Abies holophylla* yields griseofulvin and dechlorogriseofulvin for in vitro and in vivo effectivity against pathogenic *Magnaporthe grisea*, *Corticium sasakii*, *Blumeria graminis* (Park et al. 2005). The ascomycete fungus *Pestalotiopsis* is known to be a common plant pathogen but also has been reported many times because of their endophytic existence in the host plants. The two common species *Pestalotiopsis microspora* (host: tropical plant *Terminalia morobensis*) and *P. fici* are reported to be producing antifungal metabolites isopestacin and pestalofones D–E (Harper et al. 2003; Liu et al. 2009a, b). Chlorogenic acid and colletotric acids are antifungal phenolics of *Colletotrichum gloeosporioides* and *Sordariomycetes* sp., respectively (Chen et al. 2010; Zou et al. 2000). They were isolated from medicinal plants of China (*Artemisia mongolica* and *Eucommia ulmoides*) and effective against fungi imperfecti *Helminthosporium sativum*. Orcinol is used for the production of a dye called orcein used randomly for the staining of cells and chromosomes. Orcinol is popularly known for its antifungal activity too and has been isolated as a product of endophytic origin of *Penicillium* sp. from *Alibertia macrophylla* (a plant of Rubiaceae) showing bioactivity against *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* (Oliveira et al. 2014). Endophytic *Phomopsis* sp., *Dothiorella* sp., and *Diaporthe* sp. have also

been tested for their antifungal production and antifungal compounds that were detected (Brady et al. 2000; Xu et al. 2004; Huang et al. 2008).

9.4.3 Volatile Organic Compounds

Volatile organic compounds (VOCs) are said to be a type of **organic low-molecular-weight carbon-containing small compounds** (up to C₂₀) that have a high **vapor pressure** with low molecular mass (100–500 daltons) at **room temperature**. The high vapor pressure results from a low **boiling point** of that chemical compound, which causes a huge quantity of molecules to **evaporate** from the liquid, solid, or semisolid form of the compound and gets released into the surrounding environment. The endophytes are unique in their volatile emissions. The term mycofumigation that is very much popular with the treatment of agricultural phytopathogens is actually the output of VOCs that originated from endophytic isolates. The first ever reported volatile antibiotic producer was *Muscodor albus* (Xylariaceae family), an endophyte of *Guazuma ulmifolia* (a plant of Sterculiaceae family collected from tropical forest of SW Ecuador), isolated by Gary Strobel and his co-workers (Strobel et al. 2007). The major compounds isolated by GCMS are known to be involved in antifungal, antibacterial activity. Compounds include butanoic acid, 2-methyl-; butanoic acid, 3-methyl-; 2-butenal, 2-methyl-; butanoic acid, 3-methylbutyl ester; 3-buten-1-ol, 3-methyl; guaiol; 1-octene, 3-ethyl-; formamide, N-(1-methylpropyl); azulene and naphthalene derivatives; caryophyllene; phenylethyl alcohol; acetic acid, 2-phenylethyl ester; bulnesene; and various propanoic acid, 2-methyl- derivatives. These compounds were tested against a number of phytopathogenic fungi (*Botrytis cinerea*, *Mycosphaerella fijiensis*, *Pythium ultimum*, *Phytophthora cinnamomi*) showing partial or complete death or growth inhibition of those pathogens after 2 or 4 days of incubation. *Muscodor albus* was reported from a diverse type of host plants, i.e., *Myristica fragrans*, *Terminalia prostrata*, *Cinnamomum zeylanicum*, and *Ginkgo biloba*, by several workers (Worapong et al. 2001; Sopalun et al. 2003; Ezra and Strobel 2003; Mercier et al. 2004; Ezra et al. 2004a, b; Atmosukarto et al. 2005; Lacey and Naven 2006; Lacey et al. 2009; Strobel et al. 2007; Banerjee et al. 2010a, b; Corcuff et al. 2011; Alpha et al. 2015). The mycofumigants are effective against pathogen *Fusarium culmorum*, causal agent of seedling blight, foot rot, ear blight, stalk rot, and common rot of cereals. Sexual stage (teleomorph) of *Glomerella cingulata*, a fungus of Glomerellaceae, is a potent pathogen causing anthracnose-like symptoms of water-soaked, sunken spots and necrotic lesions on fruits of forest trees. This phytopathogen is strictly inhibited by the volatile emissions of this novel endophyte. Banerjee et al. (2010a, b) first reported *Muscodor albus* strain GBA from the USA as an isolate of *Ginkgo biloba* (first isolate of *M. albus* from *G. biloba*) and tested the biological efficacy of its volatile mixtures against agricultural pathogens and also evaluated its promises to be used as a commercial mycofumigant agent for controlling the fungal diseases in storage fruits and vegetables, that is, agricultural productions and during food transportation. The strain GBA in comparison to other strains of *Muscodor* E6 and CZ620 completely

inhibits and potentially kills the member of Phycmycetes, *Pythium ultimum* after 2 days of exposure of the mixture of volatiles. The organic compounds include alcohols, acids, esters, ketones, and lipids as their active components. 1-butanol, 3-methyl-, acetate was found in significant quantities. Vitrine, a terpenoid, was first isolated from *Muscodor albus* strain GAB. The volatile mixture is artificially produced by the mixture of the pure compounds, and that mixture is again checked for antifungal activity. A positive mycocidal or mycostatic effect similar to the effect of endophyte's volatile emission will confirm establishment of the endophyte and its mixture as the biocontrol or antifungal agent. *Myrothecium inundatum*, an endophyte of herbaceous *Acalypha indica* (Euphorbiaceae member collected from north-eastern part of India), produces unique mixture of volatile components having 3-octanone, 3-octanol, 7-octen-4-ol, sesquiterpenes, organic acids, methyl esters, naphthalene, 2-octanoic acid, heptanoic acid, etc. This endophyte produces foam in its liquid culture predominant with long-chain carbon compounds like octane, 1,4-cyclohexadiene, 1-methyl- and cyclohexane, and 1-ethylpropyl. The presence of this type of compounds emphasizes the concept of "mycodiesel" Several other species of *Muscodor*, for example, *Muscodor heveae*, *Muscodor ghoomensis*, *M. indica*, *M. camphora*, *M. suthepensis*, *Muscodor tigerii*, *Muscodor darjeelingensis*, *Muscodor strobilii*, *Muscodor kashayum*, *Muscodor musae*, *Muscodor sutura*, *Muscodor cinnamomi*, *Muscodor crispans*, *Muscodor vitigenus*, and *M. roseus*, were isolated from different parts of the world and from various types of hosts, for example, *Hevea brasiliensis*, *Cinnamomum camphora*, *Cinnamomum bejolghota*, *C. zeylanicum*, *Aegle marmelos*, *Musa acuminata*, *Prestonia trifidi*, *Grevillea pteridifolia*, *Erythrophleum chlorostachys*, *Paullinia paullinioides*, and *Hevea brasiliensis* (Meshram et al. 2013, 2015, 2017; Suwannarach et al. 2015; Saxena et al. 2015; Suwannarach et al. 2010, 2012, 2013; Kudalkar et al. 2012; Mitchell et al. 2010; Worapong et al. 2002; Daisy et al. 2002; Siri-udom et al. 2016), showing antifungal activity against a large number of phytopathogens including *A. fumigatus*, *Botrytis cinerea*, *Colletotrichum lagenarium*, *Ceratocystis ulmi*, *Cercospora beticola*, *Geotrichum candidum*, *Mycosphaerella fijiensis*, *Phytophthora cinnamomi*, *Phytophthora palmivora*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Verticillium dahlia*.

9.4.3.1 VOC Producers as the Postharvest Biocontrol Agents and Mycofumigants

Postharvest fungal disease is one of the prime causes of agricultural loss of crops. Use of biological agent to minimize this loss is one of the vital targets of agriculturalists, horticulturalists, and plant biologists. Several chemical agents have been already tested for practical applications, but endophytes are less explored organisms in this arena. Volatiles from endophytic source open up new scope of utilization of unique mixtures of chemicals to be used as mycofumigant agents. A large number of endophytes have already been screened for their postharvest disease management ability (Table 9.4). The volatiles of the endophyte could be considered as the natural fungicides. *Muscodor vitigenus*, an endophyte of *Hevea brasiliensis*, was analyzed in GC-MS for their volatile production. The isolates produce a unique mixture of

Table 9.4 VOCs produced by endophytic fungi with antifungal activity

Endophytic fungi	Source	VOCs emitted	Antifungal activity against	References
<i>Muscador albus</i>	<i>Guazuma ulmifolia</i> , <i>Myristica fragrans</i> , unidentified small vine, <i>Terminalia prostrata</i> , <i>Cinnamomum zeylanicum</i> , <i>Ginkgo biloba</i> ,	1-Butanol, 3-methyl-acetate, citrine (terpenoid), naphthalene, tetrahydrofuran, 2-methyl furan, 2-butanone, aciphyllene, azulene derivative. Germacrene B; acetic acid; methyl ester; 1-butanol; benzeneethanol; acetate; pyrrolidine; 2-heptanoic acid, 4-cyclopropyl-; bicyclo[3.1.1]heptane, 6-methyl-2-; propanoic acid, 2-methyl-	<i>Aphanomyces cochlioides</i> , <i>Aspergillus fumigatus</i> , <i>A. ochraceus</i> , <i>Fusarium culmorum</i> , <i>Glomerella cingulate</i>	Worapong et al. (2001), Sopalun et al. (2003), Ezra and Strobel (2003), Mercier and Jimenez (2004), Ezra et al. (2004a, b), Atmosukarto et al. (2005), Lacey and Naven (2006), Lacey et al. (2009), Strobel et al. (2007), Banerjee et al. (2010a, b), Corcuff et al. (2011), and Alpha et al. (2015)
<i>Glitocladium</i> sp.	<i>Eucryphia cordifolia</i>	1-Butanol; 3-methyl-octene, 1-propanol, 2-methyl-; 1-butanol, 2-methyl-; propanoic acid; octanone; 1,3,5,7-cyclooctatetraene (azulene); acetic acid; 2-phenylethyl ester; phenylethyl alcohol	<i>Pythium ultimum</i> , <i>Verticillium dahliae</i>	Stinson et al. (2003)
<i>Edenia gomezpompae</i>	<i>Callicarpa acuminata</i>	Naphthoquinone spiroketal, preussomerin EG 1-3	<i>Colletotrichum</i> sp., <i>Alternaria solani</i> , <i>Phytophthora capsici</i> , <i>Phytophthora parasitica</i> , <i>Fusarium oxysporum</i>	Macias et al. (2008)
<i>Glitocladium roseum</i> (NRRL 50072)	<i>Eucryphia cordifolia</i>	Undecane, 2,6-dimethyl; decane, 3,3,5-trimethyl; cyclohexene, 4-methyl; decane, 3,3,6-trimethyl; and undecane, 4,4-dimethyl	<i>Pythium ultimum</i>	Strobel et al. (2008a, b)

(continued)

Table 9.4 (continued)

Endophytic fungi	Source	VOCs emitted	Antifungal activity against	References
<i>Nodulisporium</i> sp.	<i>Lomatia fraseri</i> , <i>Olearia argophylla</i>	Isobutanol; origanene; myrcene; β -pinene; 1,3,8-p-menthatriene; 6-isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene; eucalyptol; benzene, 1-methyl-4-(1-methylethenyl)-; 2-cyclohexen-1-one, 2-(2-methyl-2-propenyl)-; β -elemene; caryophyllene; α -farnesene	<i>Rhizoctonia fragariae</i> , <i>Fusarium oxysporum</i> , <i>Sclerotium rolfii</i> , <i>Verticillium dahlia</i> , <i>Colletotrichum acutatum</i>	Mann et al. (2008)
<i>Oidium</i> sp.	<i>Terminalia catappa</i>	Esters of propanoic acid, 2-methyl-; butanoic acid, 2-methyl-; and butanoic acid, 3-methyl-5-Pentyl-2-furaldehyde	<i>Pythium ultimum</i>	Strobel et al. (2008a, b)
<i>Oxyporus latemarginatus</i>	<i>Capsicum annuum</i>		Controls postharvest apple decay and <i>Rhizoctonia</i> sp. root rot of moth orchid	Lee et al. (2009)
<i>Botrytis</i> sp. BTF 21,		Butane-2-methyl; β -butyrolactone; 2-butenedinitrile; 1-propanol, 2-methyl-; 1-butanol, 3-methyl-; 2-butenedinitrile	Biocontrol potential against phytopathogen <i>Fusarium oxysporum</i>	Ting et al. (2010)
<i>Cladosporium</i> sp. MIF01,				
<i>Penicillium</i> sp. BTF08				
<i>Hypoxylon</i> sp.	<i>Persea indica</i>	1,8-Cineole, 1-methyl-1,4-cyclohexadiene, (+)-alpha-methylene-alpha-fenchocamphoron (monoterpene)	<i>Botrytis cinerea</i> , <i>Phytophthora cinnamomi</i> , <i>Cercospora beticola</i> , <i>Sclerotinia sclerotiorum</i>	Tomscheck et al. (2010)
<i>Muscodora crispans</i>	<i>Ananas ananassoides</i>	Propanoic acid, 2-methyl-; methyl ester; propanoic acid, 2-methyl-; 1-butanol, 3-methyl-; 1-butanol, 3-methyl-, acetate; propanoic acid, 2-methyl-, 2-methylbutyl ester; and ethanol	<i>Pythium ultimum</i> , <i>Phytophthora cinnamomi</i> , <i>Sclerotinia sclerotiorum</i> , <i>Mycosphaerella fijiensis</i> (the black Sigatoka pathogen of bananas)	Mitchell et al. (2010)
<i>Muscodora cinnamomi</i>	<i>Cinnamomum bejolghota</i>	Azulene; butanoic acid, 2-methyl-, methyl ester; propanoic acid, 2-methyl-, methyl ester	<i>Rhizoctonia solani</i>	Suwannarach et al. (2010, 2012)

<i>Muscodor fengyangensis</i>	<i>Pseudotaxus chienii</i> , <i>Actinidia chinensis</i> , <i>Abies beshanzuensis</i>	Naphthalene derivatives; α -phellandrene; β -phellandrene; 2-cyclohexen; propionic acid, 2-methyl-; and methyl ester	<i>Botrytis cinerea</i> , <i>Aspergillus clavatus</i> , <i>Colletotrichum fragariae</i> , <i>Didymella bryoniae</i> , <i>Fusarium oxysporum</i> , <i>Magnaporthe oryzae</i> , <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i> , <i>Verticillium dahliae</i> , <i>Penicillium digitatum</i>	Zhang et al. (2010)
<i>Myrothecium inundatum</i>	<i>Acalypha indica</i>	1,4-Cyclohexadiene, 1-methyl- and cyclohexane, (1-ethylpropyl)3-octanone, 3-octanol, 7-octen-4-ol	<i>Pythium ultimum</i> , <i>Sclerotinia sclerotiorum</i>	Banerjee et al. (2010a, b)
<i>Nodulisporium</i> sp. CF016	<i>Cinnamomum loureirii</i>	β -Elemene, β -selinene, α -selinene, 1-methyl-1,4-cyclohexadiene	Postharvest biocontrol agent of apple and a potent biofumigant agent against <i>P. ultimum</i> , <i>R. solani</i> , <i>F. oxysporum</i> , <i>Phytophthora capsici</i> , <i>Sclerotinia sclerotiorum</i> , <i>Colletotrichum coccodes</i> , <i>Magnaporthe oryzae</i> , <i>Alternaria panax</i> , <i>Botrytis cinerea</i> , <i>Penicillium expansum</i>	Park et al. (2010)
<i>Phoma</i> sp.	<i>Larrea tridentata</i>	Alpha-humulene (sesquiterpene), alcohols, reduced naphthalene derivatives, trans-caryophyllene	<i>Verticillium</i> , <i>Ceratocystis</i> , <i>Cercospora</i> , <i>Sclerotinia Trichoderma</i> , <i>Colletotrichum</i> , <i>Aspergillus</i> sp.	Strobel et al. (2011)
<i>Phomopsis</i> sp.	<i>Odonitoglossum</i> sp.	1-Butanol, 3-methyl-; benzeneethanol; 1-propanol, 2-methyl-; and 2-propanone	<i>Pythium ultimum</i> , <i>Phytophthora palmivora</i> , <i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Fusarium solani</i> , <i>Botrytis cinerea</i> , <i>Verticillium dahliae</i> , and <i>Colletotrichum lagenarium</i>	Singh et al. (2011)

(continued)

Table 9.4 (continued)

Endophytic fungi	Source	VOCs emitted	Antifungal activity against	References
<i>Candida intermedia</i>	Strawberry	1,3,5,7-cyclooctatetraene; 3-methyl-1-butanol; 2-nonanone; pentanoic acid, 4-methyl-; ethyl ester; 3-methyl-1-butanol, acetate; acetic acid, pentyl ester	Postharvest control agent of <i>Botrytis</i> fruit rot in strawberry plants	Huang et al. (2011)
<i>Muscodor sutura</i>	<i>Prestonia trifidi</i>	Thujopsene, chamigrene, isocaryophyllene, butanoic acid, 2-methyl-	Antifungal activity against 12 pathogenic fungi, e.g., <i>A. fumigatus</i> , <i>Botrytis cinerea</i> , <i>Colletotrichum lagenarium</i> , <i>Ceratocystis ulmi</i> , <i>Cercospora beticola</i> , <i>Mycosphaerella fijiensis</i> , <i>Phytophthora cinnamomi</i> , <i>Phytophthora palmivora</i> , <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i> , <i>Verticillium dahlia</i>	Kudalkar et al. (2012)
<i>Nodulisporium</i> sp.	<i>Myroxylon balsamum</i>	1, 4-Cyclohexadiene, 1-methyl-; 1,4-pentadiene and cyclohexene, 1-methyl-4-(1-methylethenyl)-; alkyl alcohols starting with 1-butanol-3-methyl, 1-propanol-2-methyl, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol along with phenylethyl alcohol, secondary alkyl alcohols, esters, ketones, benzene derivatives, terpenoids	<i>Aspergillus fumigatus</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora cinnamomi</i> , <i>Sclerotinia sclerotiorum</i>	Mends et al. (2012)
<i>Muscodor musae</i>	<i>Musa acuminata</i>	Ethyl 2-methylpropanoate, methyl 2-methylbutanoate, 2-methylpropyl acetate, methyl 3-methylbutanoate, 2-methylpropyl propanoate, 2-methylpropan-1-ol, 3-methylbutanoyl acetate, 2-methylbutyl 2-methylpropanoate, 3-methylbutan-1-ol, 3-methyl-3-buten-1-ol, ethyl 2-hydroxy-2-methylpropanoate, 3-hydroxy-2-butanone	<i>Alternaria porri</i> , <i>Alternaria solani</i> , <i>Botrytis cinerea</i> , <i>Colletotrichum capsici</i> , <i>Colletotrichum gloeosporioides</i> , <i>Colletotrichum musae</i> , <i>Fusarium oxysporum</i> , <i>Fusarium solani</i> , <i>Nigrospora oryzae</i> , <i>Penicillium digitatum</i>	Suwanarach et al. (2013)

<i>Muscodor equiseti</i>	<i>Equisetum debile</i> , <i>Hevea brasiliensis</i>	Isobutyric acid, C ₄ H ₈ O ₂	Pathogens (filamentous fungi)	Suwarnarach et al. (2013) and Siri-udom et al. (2016)
<i>Muscodor kashayum</i>	<i>Aegle marmelos</i>	3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl; 1,6-dioxacyclododecane-7,12-dione; 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol; 2,4-di-tert-butylthiophenol and 4-octadecylmorpholine	Antifungal and mycofumigant agent	Meshram et al. (2013)
<i>Nodulisporium</i> sp.	<i>Thelypteris angustifolia</i>	Series of ketones, including acetone; 2-pentanone; 3-hexanone, 4-methyl-; 3-hexanone, 2,4-dimethyl; 2-hexanone, 4-methyl and 5-hepten-2-one; 1,8-cineole; 1-butanol, 2-methyl and phenethyl alcohol; cyclohexane, propyl	<i>Phytophthora palmivora</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i> , <i>Phytophthora cinnamomi</i>	UL-Hassan et al. (2013)
<i>Muscodor strobilii</i>	<i>Cinnamomum zeylanicum</i>	Viridiflorol, tetraoxapropellan, terpinolene, octadec-9-enoic acid, aspidofractinine-3-methanol	<i>Penicillium citreonigrum</i> , <i>Aspergillus japonicas</i>	Meshram et al. (2014)
<i>Muscodor darjeelingensis</i>	<i>Cinnamomum camphora</i>	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol, 1, 6-dioxacyclododecane-7, 12-dione and 4-octadecylmorpholine	Species of <i>Candida</i>	Saxena et al. (2014)
<i>Muscodor tigerii</i>	<i>Cinnamomum camphora</i>	4-Octadecylmorpholine, 1-tetradecanamine, N,N-dimethyl and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Inhibits the growth of fungal and bacterial pathogens like <i>Cercospora beticola</i> , <i>Penicillium marneffeii</i> , <i>Rhizoctonia solani</i>	Saxena et al. (2015)

(continued)

Table 9.4 (continued)

Endophytic fungi	Source	VOCs emitted	Antifungal activity against	References
<i>M. suthiensis</i>	<i>Cinnamomum bejolghota</i>	Ethyl 2-methylpropanoate, methyl 2-methylbutanoate, 2-methylpropylacetate, methyl 3-methylbutanoate, methylpropyl propanoate, methylpropan-1-ol, 3-methylbutanoylacetate, 2-methylbutyl 2-methylpropanoate, 3-methylbutan-1-ol, 3-hydroxy-2-butanone, 3-ethyl-2-methylpentane, 6-methyl-5-hepten-2-one, 3-methylhexane, 4-dimethyl-1-heptene, 2-ethylhexylacetate, 4,5-dimethyl-1,3-cyclopentanedione, 2-methylpropanoic acid	A potent biofumigant agent against <i>Penicillium digitatum</i> to control tangerine fruit rot	Suwanmarach et al. (2015)
<i>Muscodor ghoomensis</i> <i>M. indica</i> <i>M. camphora</i>	<i>Cinnamomum camphora</i>	Tetracontane; 4-octadecylmorpholine; N,Ndimethyl-1-pentadecanamine and cis-9-hexadecena; 4-octadecylmorpholine; 1,6-dioxacyclododecane-7, 12-dione; 1,4-dimethyl-7-prop-1-en-2-yl-2,3,3a,5,6,7,8,8a-octahydro-1H-azulen-4-ol	<i>Alternaria alternata</i> , <i>Arthrinium phaeospermum</i> , <i>Aspergillus flavus</i> , <i>Botrytis cinerea</i> , <i>Cercospora beticola</i> , <i>Colletotrichum gloeosporioides</i> , <i>Fusarium solani</i> , <i>Muscodor albus</i> , <i>Penicillium marnieffei</i> , <i>Rhizoctonia solani</i>	Meshram et al. (2015, 2017)
<i>Nodulisporium</i> sp. GS4d2llla	<i>Gliricidia sepium</i>	Mono- and sesquiterpenes, especially eucalyptol and limonene, amines, 2-pentylfuran, α -phellandrene, α -myrcene, 3-carene, butyl isocyanatoacetate, tetrahydro-3-methyl-furan, 1,8-nonadiene	<i>Phytophthora capsici</i> , <i>Pythium aphanidermatum</i> , <i>Phytophthora cinnamomi</i> , <i>Phytophthora parasitica</i>	Fernández et al. (2016)
<i>Xylaria</i> sp. PB3f3	<i>Haematoxylum brasiletto</i>	3-Methyl-1-butanol, thujopsene, 2-methyl-1-butanol, 2-methyl-1-propanol	<i>Pythium aphanidermatum</i> , <i>Phytophthora capsici</i> , <i>Alternaria solani</i> , <i>Fusarium oxysporum</i>	Sanchez et al. (2016)

<i>Daldinia</i> cf. <i>concentrica</i>	<i>Olea europaea</i>	Germaerene A, α -bulnesene, α -selinene, terpenes, β -elemene, phenylethyl alcohol, 4-heptyn-2-ol, isoamyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, 4-heptanone, isoamyl acetate and trans-2-octenal, 3-methoxy-2-naphthol	<i>Alternaria alternata</i> pathotype tangelo, <i>Alternaria alternata</i> , <i>Aspergillus niger</i> , <i>Botrytis cinerea</i> , <i>Colletotrichum</i> sp., <i>Coniella</i> sp., <i>Fusarium euwallaceae</i> , <i>Fusarium mangiferae</i> , <i>Fusarium oxysporum</i> , <i>Lasiodiplodia theobromae</i> , <i>Neoscytalidium dimidiatum</i> , <i>Penicillium digitatum</i> , <i>Phoma tracheiphila</i> , <i>Pythium aphanidermatum</i> , <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i>	Liarzi et al. (2016)
<i>Hypoxylon anthochroum</i> strain Blaci	<i>Bursera lancifolia</i>	Phenylethyl alcohol and eucalyptol	Anti phytopathogenic activity	Ulloa-Benitez et al. (2016)
<i>Muscodora vitigenus</i>	<i>Hevea brasiliensis</i>	Naphthalene, azulene, 3-methylbutan-1-ol, 3-methylbutyl acetate	Potent biocontrol agent	Siri-udom et al. (2016)
<i>Muscodora heveae</i>	<i>Hevea brasiliensis</i>	3-Methylbutan-1-ol, 3-methylbutyl acetate, 2-methylpropanoic acid, and azulene derivatives	<i>Phellinus noxius</i> , <i>Rigidoporus microporus</i> (causal organism of root rot disease in rubber)	Siri-udom et al. (2016)
<i>Nodulisporium</i> sp. strain GS4d211	<i>Solanum lycopersicum</i>	Caryophyllene, 4-methyl-2,6-di-tert-butylphenol, alcohols' mixture, phenylethyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, eucalyptol, ocimene, terpinolene	Potent postharvest biocontrol agent for tomato against <i>Fusarium oxysporum</i>	Romero et al. (2017)

naphthalene, azulene, 3-methylbutan-1-ol, and 3-methylbutyl acetate that partially or completely inhibits the growth of the phytopathogenic fungal species. *Colletotrichum gloeosporioides*, a commercially significant plant pathogen, usually acts as a secondary invader of injured tissue or saprophyte and causes bitter root in variety of crops; tropical fruits like yams, papaya, avocado, coffee, sweet pepper, tomato; and also perennial grasses. *Rhizoctonia solani*, a commercially significant plant pathogen of Basidiomycotina, causes symptoms of brown patch on turf grass, damping off of soybean seedlings, black scurf of potatoes, root rot of sugar beet, belly rot of cucumber, sheath blight of rice, etc. Another phytopathogen named *Fusarium oxysporum*, the causal agent of *Fusarium* wilt or koa wilt, and *Rigidoporus microporus*, the causal agent of white root rot disease on tropical crops like cacao, were suppressed in terms of their growth upon exposure to the volatiles of *M. vitigenus*. *Phytophthora parasitica* (oomycetous fungi) causes destructive diseases of a wide range of crop plants (*Arabidopsis thaliana* and *Medicago truncatula*, the two guinea pigs of plant science), nursery and ornamental plants, and forest ecosystems. Another phytopathogenic species of *Ganoderma australe* that forms white heart rot in *Tilia* trees, *Quercus* sp. (oaks), *Fagus* sp. (beech), and *Betula* sp. (birch) is found to be inactive when exposed to the volatiles. *Phellinus noxius* is reported to be a serious threat to almost 200 plants covering 59 families of tropical forests of Asia, Africa, Japan, Taiwan, and the Pacific Islands, which is inhibited significantly by *M. vitigenus* volatiles. So *M. vitigenus* opens up scope for the biocontrol of all these seven harmful phytopathogens and ensures the complete protection of host from fungal attack. This could be concluded as natural fungicides. So mycofumigation by these volatiles could minimize the loss caused by these pathogens. Stinson and his co-workers (2003) isolated endophytic *Gliocladium* sp. (Hypocreaceae) from *Eucryphia cordifolia* and reported multiple number of volatile compounds, for example, 1-butanol, 3 methyl; octene; 1-propanol, 2-methyl-; 1-butanol, 2-methyl-; propanoic acid; octanone; 1,3,5,7-cyclooctatetraene (azulene); acetic acid; 2-phenylethyl ester; and phenylethyl alcohol. These volatiles are lethal to major phytopathogens, for example, *Pythium ultimum* and *Verticillium dahliae*. *Pythium ultimum* is known to infect plants causing damping off and root rot disease of corn, soybean, potato, and wheat. *Verticillium dahlia* causes *Verticillium* wilt resulting in curled and discolored appearance of leaves of almost 350 species of eudicots of temperate regions. So using *Gliocladium* sp. as a biocontrol agent in tropics may reduce the agricultural loss to some extent. *Edenia gomezpompae*, a member of Pleosporaceae isolated as an endophyte of *Callicarpa acuminata*, was reported to produce naphthoquinone spiroketal showing antifungal activity against *Colletotrichum* sp., *Alternaria solani*, and *Phytophthora capsici* (Macias et al. 2008). Lee et al. (2009) performed mycofumigation with *Oxyporus latemarginatus* EF069 volatiles for control of postharvest apple (*Malus pumila*) decay and *Rhizoctonia* root rot infection on moth orchid (*Phalaenopsis* sp.). Apple is an important economic fruit, and biocontrol of apple fungal pathogens by volatile emissions of endophytic fungi is completely an innovative way of treatment. Suwannarach and his coworkers in the year 2010 isolated a new species of Muscodor, *Muscodor cinnamomi* CMU-Cib 461, from a member of Lauraceae named

Cinnamomum bejolghota. This isolate was known to produce azulene, a new compound detected first from any *Muscodor* species. This species was tested in vitro and in vivo for antifungal activity against a common worldwide devastating pathogen *Rhizoctonia solani* (causal agent of damping off). The VOCs produced by this fungi include (S)-(+)-5-methyl-1-heptanol; ethyl acetate; propanoic acid, 2-methyl-, methyl ester; cis-2,4-dimethylthiane; S,S-dioxide; cyclopentane; butanoic acid, 2-methyl-, methyl ester; 1-butanol, 3-methyl-, acetate; β -humulene; azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-; 1S-(1. α ., 7. α ., 8a. β); and eudosma-4(14),11-diene 1,1,1,5,7,7,7-heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane. *Rhizoctonia solani*-infected seedlings were treated with volatile mixtures to assess the mycofumigation property. In vivo experiment was conducted on four seedlings of bird pepper, bush bean, garden pea, and tomato. It was concluded that 30 gm of *Muscodor cinnamomi* prepared on rye grain solid media is the minimum dose required for inhibition of *Rhizoctonia* infection and total control and elimination of damping off symptoms. *Muscodor cinnamomi*-infected soil does not show any seed germination inhibition in comparison to *Rhizoctonia solani*-infected soil. So it is a type of pioneer study of using endophytic species as potent agents of fumigation and biocontrol. *Candida intermedia* strain C410 (Saccharomycetaceae) was isolated as an endophyte of strawberry (*Fragaria ananassa*), and the volatile emission was known to be a mixture of 49 organic compounds including esters, alcohols, alkenes, alkanes, alkynes, organic acids, ketones, and aldehydes of which 1, 3, 5, 7-cyclooctatetraene and 3-methyl-1-butanol were the most dominant (Huang et al. 2011). Volatiles of strawberry endophyte were itself useful as postharvest control agent for the host plant against *Botrytis* fruit rot. Other compounds include 1,3,5,7-cyclooctatetraene; 3-methyl-1-butanol; 2-nonanone; pentanoic acid, 4-methyl-, ethyl ester; 3-methyl-1-butanol, acetate; acetic acid, pentyl ester; and hexanoic acid, ethyl ester that were found to be extremely inhibitory to conidial germination (reproductive growth) and also vegetative (mycelial) proliferation of *B. cinerea*. When the fruits are exposed to *C. intermedia* synthetic volatiles or itself to the fungus, the incidence of *Botrytis* fruit rot reduces significantly. Strawberry fruits inoculated directly with the endophyte also remain disease-free. So mixtures of *Candida intermedia* C410, the unique natural products, are useful as mycofumigation technique or for postharvest disease management by biological control policies.

Tangerine fruit (*Citrus tangerine*), the commercial citrus crop of Northern Thailand, faces huge postharvest losses due to pathogenesis of green mold (*Penicillium digitatum*). The pathogen is the prime cause of worldwide deterioration of tangerine fruits by mycopathogenesis. Out of 32 detected compounds, the most predominant were 2-methylpropanoic acid and 3-methylbutan-1-ol. Other compounds include carbitol, octanoyl chloride, azulene, 3-methylhexane, 2-methylpropan-1-ol, 2,3-butanediol, caryophyllene, 2-methylbutyric acid, ethyl 2-hydroxypropanoate, etc. The pathogen was treated both in vitro and in vivo for their inhibition by endophytic volatile components. In both cases, pathogen growth was restricted. During transportation of the fruits, fungus causes huge crop loss by infecting the fruits; when fruits were inoculated with 30 gm of rye grain culture of

M. suthepensis (1 month old), the disease development is ceased. So it is a classic example of mycofumigation by the biocontrol agent of tangerine fruit for the control of rot lesions caused by *P. digitatum* infection. The in vivo application requires the proper surface sterilization (using sodium hypochlorite) of the targeted parts where the inoculation is going to be done, for example, fruit, stem, root, and leaf. Usually infection on fruits for assessing the biocontrol potential is the most common and popular method. The seeds will be washed in distilled water, and using sterile needle, uniformly the whole area would be done, and the whole area would be infected or inoculated with the endophytic liquid extracts containing spore suspensions. *Muscodor albus* VOCs are potent enough to cause a significant reduction of in vitro spore germination of the *Tilletia* species *T. horrida*, *T. indica*, and *T. tritici*. Endophytic *Nodulisporium* spp., *Trichoderma* spp., *Phomopsis* spp., and *Oxyporus latemarginatus* are reported to produce VOCs that inhibit mycelial growth of phytopathogenic fungi (Lee et al. 2009; Park et al. 2010; Ajith and Lakshmidevi 2010; Amin et al. 2010). Black sigatoka disease (also known as leaf spot or black leaf streak disease) of banana (*Musa paradisiaca*) is caused by *Mycosphaerella fijiensis* (ascomycete fungus). This phytopathogen is inhibited by the volatile emissions of *Muscodor sutura*, an endophytic isolate of *Prestonia trifidi*. The volatiles are effective also against *Ceratocystis ulmi*, the causal agent of Dutch elm disease of American elm (*Ulmus americana*). The volatile mixtures include thujopsene, chamigrene, isocaryophyllene, and butanoic acid, 2-methyl- that are potent inhibitors of the common anthracnose pathogen of cucumber, muskmelon, and watermelon (members of cucurbits), *Colletotrichum lagenarium*. So this unique endophyte and its chemical mixtures are potent mycofumigants and ensure crop protections against destructive pathogens like *C. ulmi* and *C. lagenarium*, *Sclerotinia sclerotiorum* (causing white mold, cottony rot, water soft rot, stem rot, drop, crown rot, and blossom blight diseases of the host), and also *Phytophthora palmivora* (oomycete fungi), the causal agent of bud root of palms and areca nut predominantly occurring in regions of South India (Kudalkar et al. 2012). Liarzi and his coworkers tested the biological control efficacy of the endophytic *Daldinia* cf. *concentrica*, isolated from olive tree (*Olea europaea* L.) of Israel against 18 phytopathogens, and the unique mixtures of 27 volatile were effective against the phytopathogenic mycelial growths. The mixtures include a variety of organic compounds: 3-methyl-1-butanol, 2-methyl-1-butanol, 1-methyl-1,3-cyclohexadiene, 1-methyl-1,4-cyclohexadiene, 4-heptanone, isoamyl acetate, 4-heptyn-2-ol, 2-octenal, octanal, β -elemene, α -guaiene, β -selinene, α -selinene, α -bulnesene, germacrene A, etc. The unique mixtures having broad-spectrum antifungal property could be used for fumigation for eliminating the pathogenic infections of *Aspergillus niger* (mold-causing organism on fruits of economic importance). So the endophytic *D. cf. concentrica* opens up opportunities for fungal disease control in food and agricultural industries (Liarzi et al. 2016). *Nodulisporium* sp. strain GS4d2III1 (*Hypoxylon anthochroum*) and *Hypoxylon anthochroum* strain Blaci are potent enough to be used as biopesticide against *Fusarium oxysporum*, a common contaminant of *Solanum lycopersicum* var. *cerasiforme* (cherry tomato) causing a great percentage of crop loss globally. Six VOCs of alcohols' mixture, phenylethyl

alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, eucalyptol, ocimene, and terpinolene, were detected and applied together with synergistic effect and individually both in vitro and in vivo. Inoculation of pathogen on the cherry tomato fruits yields significant reduction in *Fusarium* contamination. Both agar dilution techniques and gas test were done to assess the in vitro antifungal activity, and the endophytic volatile mixtures were effective in both the cases. Volatiles kill the pathogens probably by interfering cell membrane permeability, hyphal morphology, and respiratory activity of the pathogenic *Fusarium oxysporum*. So it is a great opportunity to use the unique mixture of volatile organic compounds of the endophytic isolate to reduce the crop loss caused by the pathogenic infection on the commercially valuable plant of cherry tomato worldwide. Endophytic *Phoma* sp. (Didymellaceae) and *Phomopsis* sp. (Valsaceae) were isolated from *Larrea tridentata* and *Odontoglossum* sp. (Strobel et al. 2011; Singh et al. 2011). The volatiles detected are effective against phytopathogens *Verticillium* sp., *Ceratocystis* sp., *Cercospora* sp., *Sclerotinia* sp. *Sclerotinia* sp., and *Botrytis* sp.

9.5 Seaweeds as Natural Fungicides

Algae are diverse group of autotrophs and the leading producers of O₂ in the ecosystem. They range from prokaryotic unicellular to eukaryotic complex multicellular forms involved in the marine and terrestrial food chain. Antifungal activity of the seaweed (members of Phaeophyceae and Rhodophyceae) is a major weapon for natural fungicides along with their antibacterial, anti-protozoan, and antiviral activities. Algal seaweeds are potent holders of large number of secondary metabolites including phenolics, terpenes, alkaloids, and lectins which are not directly involved in photosynthesis and reproduction and thus fall under the category of secondary metabolites. They are common antimicrobial of algal origin that act on the target organisms by altering the microbial cell permeability accompanied with the loss of internal macromolecules or sometimes interfere with the membrane function causing cellular disintegrity ultimately leading to cell death (Abu-Ghannam and Rajauria 2013). Several studies include antifungal activity of algal members against human pathogens; a very few studies include their efficacy against plant pathogens (Cheung et al. 2014; Singh et al. 2007; Stirk et al. 2007; Padmakumar and Ayyakkannu 1997; Ismail et al. 2014; Genovese et al. 2013; Lopes et al. 2015). Padmakumar and Ayyakkannu tested 80 species of algae against a variety of bacterial and fungal pathogens. Out of the all screened organisms, 70% exhibited antibacterial efficiency, and only 27.5% inhibited fungal growth. Polysaccharides found in the cell wall and deposited in terms of storage food from red and brown algal sources include ulvans (obtained from *Ulva* sp.), alginates and fucans (from *Fucus* sp.), laminarin (*Laminaria* sp.), and carrageenans that can induce defense responses in plants against phytopathogens by pathogen-associated molecular patterns (MAPs) and are capable of inducing plant resistance (Vera et al. 2011). Polysaccharides stimulate regular cellular changes associated with pathogen perception and defense activation by change in Ca²⁺ concentration and burst due to oxidative stress

activation of salicylate, ethylene, and jasmonate biosynthetic pathways and by activating pathogenesis-related proteins (PRPs) (Jaulneau et al. 2010; Zhao et al. 2012). As a result of the depolymerization of the polysaccharides, the obtained oligosaccharides induce protection against a variety of fungal, viral, and bacterial diseases by accumulation of the antimicrobial compounds in the cell. Algal polysaccharides as an alternative weapon over the synthetic agricultural drugs for controlling plant disease have been widely studied (Stadnik and Freitas 2014; Hahn et al. 2008).

9.5.1 Laminarin: Defense-Inducing Polysaccharide from Brown Algae

Brown algae *Laminaria digitata*, a genus of Phaeophyceae, is commonly called seaweeds and known to be the potent producers of kelp, an iodine-rich substance needed for the normal functioning of thyroid gland. *Laminaria* produces laminarin, glucan polysaccharide-containing 1,3-linked β -d-glucose moiety, a reserve food material found on the vacuoles of the vegetative cells of this genus. β -Glucans are involved as a major part of daily diet and obtained from the brands of common cereals. They are involved in the defense responses of agricultural crops like tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), pepper (*Piper nigrum*), watermelon (*Citrullus lanatus*), grape (*Vitis vinifera*), apple (*Malus* sp.), and pear (*Pyrus communis*). Elicitation of defense response by laminarin against causal agents of gray mold (*Botrytis cinerea*) and downy mildew (*Plasmopara viticola*) in grapevine plants remarkably suppresses their infection up to 55% and 75%, respectively (Copping et al. 2004). So, natural product from brown algae *Laminaria* sp. known as laminarin or laminaran can act as the biofungicide or biocontrol compounds. Use of laminarin significantly reduces the mycelial growth and aflatoxin production in *Aspergillus flavus* and ensures its use as a fungicide (Liangbin et al. 2012). The advantage of using laminarin over other products is that as it breaks down finally to glucose molecules, it has no maximum residue limit (MRL) on the plant treated with this product. So, there is no need of preharvest interval constraint. This has been a prime cause why laminarin has substituted five popular fungicides involved in the treatment of apple scab (*Venturia inaequalis*) in France (Mery et al. 2013). This phyto-pharmaceutical is used widely in France and some countries of Europe in the name of Vacciplant (major active constituent is laminarin). Laminarin has broad-spectrum applicability on fire blight of apples and pears in Greece, France, Belgium, Switzerland, Portugal, and also Morocco. It is effective for apple scab disease in France and Belgium and for curing storage diseases of apples caused by *Gloeosporium* sp. in Belgium. Laminarin comes out as a fungicide of natural origin after being eligible in 33 tests between 2001 and 2011 in several parts of Europe, for example, France, Belgium, Italy, and Poland, on natural contamination of orchards on several sensitive strains of scab fungus including Golden Delicious, Golden Smoothie, Read Cheaf, Galaxy, Gala, and Pink Lady. Laminarin is applied widely against secondary scab (to minimize secondary scab during summer and up to harvest) as a result of its uniqueness in its mode of action. It does not involve cell

death of the host plant or hypersensitivity induction in the host organism but rather stimulates plants' natural resistance (Klarzynski et al. 2000). Aziz et al. (2003) reported its effectiveness in tobacco plants, wheat, strawberries, apples, and vines. The application of laminarin and alginate reduced the development of wilt symptoms caused by *Verticillium dahliae* on olive twigs, stimulating its phenolic metabolism (Salah et al. 2018). Moreover, alginates reduced pathogen growth in vitro. Laminarin induces the release of H₂O₂ in cells of tobacco plants and leads to the increase in PAL activity (phenylalanine ammonia-lyase) and causes the accumulation of PR-1, PR-2 (glucanase), PR-3 (chitinase), and PR-5. Concerning red algae polysaccharides, carrageenans induced protection against a broad range of pathogens such as tobacco mosaic virus (TMV), *B. cinerea*, and *E. carotovora* on tobacco (Vera et al. 2011). Again on tobacco, Mercier et al. (2001) showed that carrageenan infiltrated the leaves and increased the expression of genes coding for a sesquiterpene cyclase involved in the synthesis of the antimicrobial terpenoid capsidiol, PR-3 proteins (basic chitinases), and proteinase inhibitor with antipathogenic activity. An adequate percentage of growth and spore germination inhibition of *Botrytis cinerea* was mediated by the hexane extracts of *Laminaria digitata* and *Undaria pinnatifida*. *Porphyra umbilicalis*, laverbread, is an edible seaweed (Corato et al. 2017). Other than B-glucan polysaccharides (laminarin) of *Laminaria*, other secondary metabolites (phenols, terpenes) of phaeophyceean algae (*Sargassum* sp.) showed effectivity against common pathogens *Fusarium solani*, *Rhizoctonia solani*, *Aspergillus* spp., *Fusarium oxysporum*, *Penicillium* spp., and *Botrytis cinerea* (Khallil et al. 2015; Ibraheem et al. 2017; Mabrouk et al. 1985; Liu et al. 2014).

9.5.2 Cyanobacterial Polysaccharides Versus Fungal Infection

Cyanophycean blue-green algae are abundant all over the world and ranging from pond ecosystem to oceanic system. Though they have been reported to produce a large number of toxins and involved in death and disease of cattle and human being, they are of serious interest from the point of view of natural fungicidal products. Drawing the similarities with bacteria, they are characterized with a mucilaginous or gelatinous sheath composed of polysaccharides which are the weapon against fungal pathogenesis. Cyanobacterial polysaccharides (POL) show higher disease resistance against *B. cinerea* when they are applied on the intact fruit (preharvest conditions when fruit is attached to the plant) rather than the fruit detached (postharvest conditions) from the plant (Zheng et al. 2011; Feliziani et al. 2015; Yao and Tian 2005). Polysaccharides are involved in elicitation as elicitors for development of local and systemic disease resistance and expression of defense enzyme synthesis, for example, chitinases and glucanases that are involved directly in antifungal responses (Paulert et al. 2009; Reymond and Farmer 1998; Sharma et al. 2014). Water extracts of common BGA *Anabaena* sp., *Ecklonia* sp. (common edible marine algae of Japan and Korea), and *Corallina* sp. (hard seaweed of Corallinaceae family) exhibit antifungal activity against *Podosphaera xanthii* (causal agent of powdery mildew of cucurbits) on zucchini plant, *Cucurbita pepo*, of Cucurbitaceae

(Roberti et al. 2015, 2016). In the recent past, fungi inhibitory ability of algal members has been reported by several workers (Righini et al. 2018; Corato et al. 2017; Khallil et al. 2015; Ibraheem et al. 2017). In vitro growth inhibition of *Aspergillus oryzae* and *Penicillium notatum* has been seen by cyanophycean *Anabaena laxa* (Frankmölle et al. 1992). Devastating plant pathogens *Pythium* sp., *Fusarium* sp., and *Rhizoctonia* sp. were restricted by extracts of *Anabaena* sp. (Moon et al. 1992; Manjunath et al. 2010). The use of BGA extract as the growth inhibitor of pathogenic *Chaetomium globosum*, *Cunninghamella blakesleeana*, *Aspergillus oryzae*, *Rhizoctonia solani*, *Fusarium* sp., *Pythium* sp., and *Sclerotinia sclerotiorum* is reported. The extracts of *Phormidium fragile* and *Nostoc muscorum* (Rizk 2006) are effective control agents of sugar beet pathogens (*Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium verticillioides*). The common root rot pathogen *Rhizoctonia solani* is inhibited by the extracts of cyanophycean algae *Nostoc entophyllum* and *N. muscorum* (Osman et al. 2011). The recent investigations of Dukare et al. (2011) included the inhibitory activity of three strains of BGA (C4, C8, C12) against *F. solani*, *F. oxysporum*, *F. oxysporum* f. sp. *lycopersici*, *F. moniliforme*, *P. debaryanum*, and *R. solani*. Righini et al. (2018) reported for the first time about the resistance-inducing ability of the polysaccharides of the brown (*Ecklonia* sp.), red (*Jania* sp.), and cyanophycean algae (*Anabaena* sp.) and their possible role in disease control ability. Study on zucchini plant revealed the enhancement of defense-related enzyme on the plant mediated by the treatment of *Anabaena* extract (Roberti et al. 2015). Water extracts of *Ecklonia* sp., *Anabaena* sp., and *Corallina* sp. are potent antifungals against *Podospora xanthii* on zucchini plant (Roberti et al. 2016).

9.6 Plant Amphibians as an Alternative Source

Bryophytes, the simplest member of the broad umbrella of Embryophyta, are situated between algae and pteridophytes, are known to be plant amphibians growing in the marshy or shady habitat, and require water for their fertilization and for the perfect swimming motility of their sperms. They have been evaluated for their antimicrobial activity for a long time. It has been proved that these cryptograms are rich source of bioactive secondary metabolites and can easily be exploited as an alternative source of fungicidal compounds. As they grow in marshy habitats and can protect themselves from biotic (ultraviolet rays, heat stress, and predation) and abiotic stress (fungal or bacterial attack), they are store house of diverse bioactive chemicals (Xie and Lou 2008). Members of Hepaticopsida and mosses (the evolved members of bryophytes) are known to possess antifungal activity and are rich source of flavonoids, terpenoids, bibenzyls, and fatty acids of therapeutic importance (Krzaczkowski et al. 2008). Bryophytes are known to possess antibiotic property (Banerjee and Sen 1979; Banerjee 2000; Singh et al. 2007; Shirzadian et al. 2009; Savaroglu et al. 2011). Their antibiosis has been evaluated against a large number of plant and human pathogenic fungus (Mekuria et al. 2005). Antimicrobial compounds from bryophyte can cure the problems of conventional antibiotic resistance (Vanden Bossche et al. 1998). The antifungal efficacy is tested by disc diffusion

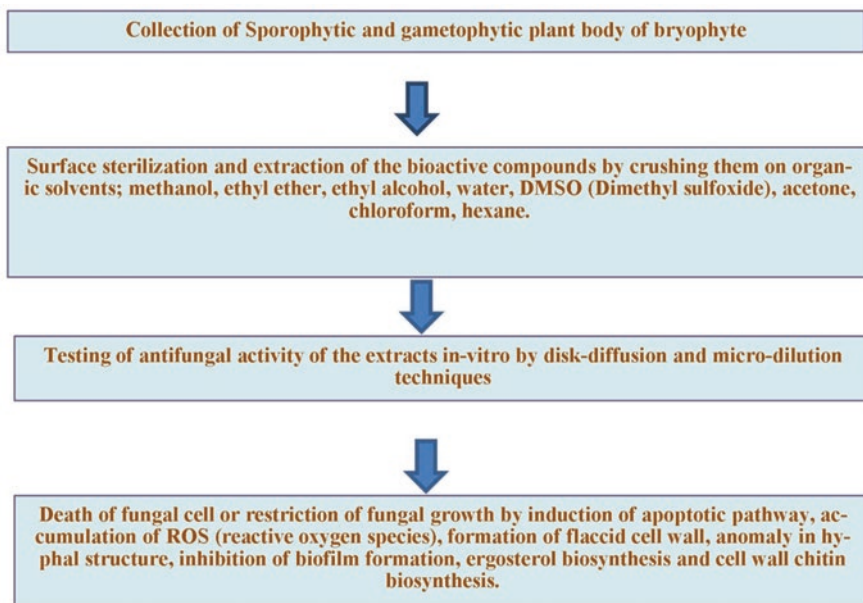


Fig. 9.5 Pharmacological extraction of bioactive compounds from bryophytes

assay and microdilution method (Fig. 9.5). Different concentrations of the extracts are prepared and checked for their antifungal efficacy against phytopathogenic fungi. They may be fungicidal or fungistatic in nature, interfering at cellular, genetic level and creating blockage at metabolic pathways. Extracts are made on several organic solvents or water extractions and also mixture of one or two organic solvents. The solvents popularly used are ethanol, methanol, chloroform, ether, dimethyl sulfoxide (DMSO), acetone, chloroform, and hexane (Table 9.5). Sporophytes and gametophytes of different bryophytes at different stages of growth and at a different amount are first surface sterilized and then crushed on the organic solvents and used as antifungals in vitro against the fungal pathogens (Wolters 1964). A large number of phytopathogenic fungi (*A. niger*, *R. bataticola*, *F. moniliforme*, *Penicillium funiculosum*, *T. viride*, *P. ochrochloron*, *A. versicolor*, *A. fumigatus*, *Trichoderma viride*, *Aspergillus niger*, *A. flavus*, *P. funiculosum*, *Tilletia indica*, *Sclerotium rolfsii*, *R. solani*, *Penicillium ochrochloron*, *Alternaria alternate*, *Botrytis cinerea*, *Botryodiplodia theobromae*, *F. oxysporum* f. sp. *gladioli*, *Penicillium expansum*, *P. chrysogenum*, *Trichoderma viride*) are reported to be partially or completely inhibited by the bryophyte extracts of *Marchantia polymorpha*, *Atrichum undulatum*, *Physcomitrella patens*, *Rhodobryum ontariense*, *Ctenidium molluscum*, *Ptilidium pulcherrimum*, *Hypnum cupressiforme*, *Fontinalis antipyretica* var. *pyretica*, *Plagiochasma appendiculatum*, and *Dumortiera hirsuta* (Sabovljevic et al. 2011; Pejin et al. 2012; Veljic et al. 2009; Gahotri and Chaturvedi 2011; Alam et al. 2011; Deora and Jain 2008; Dey and De 2011; Deora and Suhalka 2017).

Table 9.5 Antifungal activity of extracts of bryophytes

Extractions	Bryophyte genus	Antifungal against	References
Organic solvent extracts	<i>Scleropodium purum</i> , <i>Sphagnum fimbriatum</i> , <i>S. nemoreum</i> , <i>S.</i> <i>subsecundum</i> , <i>Pogonatum aloides</i> , <i>P.</i> <i>urnigerum</i> , <i>Polytrichum</i> <i>commune</i> , <i>P. formosum</i> , <i>Plagiothecium</i> <i>denticulatum</i> , <i>Mnium</i> <i>hornum</i> , <i>Oligotrichum</i> <i>hercynicum</i> , <i>Atrichum</i> <i>undulatum</i> , <i>F.</i> <i>antipyretica</i>	<i>C. cerebella</i> , <i>B. alli</i> , <i>P. oryzae</i> , <i>P.</i> <i>versicolor</i> , <i>F. bulbigenum</i> , <i>Rhizoctonia solani</i> ,	Wolters et al. (1964) and Savaroglu et al. (2011)
A-Herbertenol, β-herbertenol, α-formylherbertenol, β-bromoherbertenol	<i>Herbertus aduncus</i>	<i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i>	Matuso et al. (1986)
5- and 7-Hydroxycalamenenes, drimenol, drimenal, viridiflorol, gymnomitrol, bisbenzyls	<i>Bazzania trilobata</i>	<i>Botrytis cinerea</i> , <i>Cladosporium</i> <i>cucumerinum</i> , <i>P.</i> <i>infestans</i> , <i>Pyricularia oryzae</i> , <i>Septoria tritici</i>	Scher et al. (2004)
Trans-β- methylthioacrylate	<i>Balantiopsis cancellata</i>	<i>Cladosporium</i> <i>herbarum</i>	Labbe et al. (2005)
Ether, alcohol, and hexane extract	<i>Pallavicinia lyellii</i> , <i>Scapania verrucosa</i>	<i>Aspergillus niger</i> , <i>F.</i> <i>oxysporum</i> , <i>P.</i> <i>oryzae</i>	Subhisha and Subramoniam (2005), Guo et al. (2008)
Methanol and ethanol extracts	<i>Pleurozium schreberi</i> , <i>Palustriella commutata</i> , <i>Homalothecium</i> <i>philippeanum</i> , <i>Anomodon attenuatus</i> , <i>Rhytidium rugosum</i> , <i>Hylocomium splendens</i> , <i>Dicranum scoparium</i> , <i>Leucobryum glaucum</i>	Variety of phytopathogens <i>A. niger</i> , <i>P. ochrochloron</i>	Sabovljevic et al. (2006), Veljic et al. (2009)
Aqueous extracts	<i>Plagiochasma</i> <i>appendiculatum</i> , <i>Dumortiera hirsute</i>	<i>Alternaria alternate</i> , <i>A. niger</i> , <i>Botrytis</i> <i>cinerea</i> , <i>Botryodiplodia</i> <i>theobromae</i> , <i>F.</i> <i>oxysporum</i> f. sp. <i>gladioli</i> , <i>Penicillium</i> <i>expansum</i> , <i>P.</i> <i>chrysogenum</i> , <i>Trichoderma viride</i>	Deora and Jain (2008) and Alam et al. (2011)

(continued)

Table 9.5 (continued)

Extractions	Bryophyte genus	Antifungal against	References
Acetone, ethanol, chloroform, and distilled water extracts	<i>Thuidium delicatulum</i> , <i>Plagiochasma appendiculatum</i> , <i>Bryum argenteum</i> , <i>B. cellulare</i>	<i>A. niger</i> , <i>R. bataticola</i> , <i>F. moniliforme</i>	Bodade et al. (2008)
Methanolic and chloroform extracts	<i>Ctenidium molluscum</i> , <i>Ptilidium pulcherrimum</i> , <i>Marchantia polymorpha</i> , <i>Hypnum cupressiforme</i> , <i>Fontinalis antipyretica</i> var. <i>pyretica</i>	<i>Trichoderma viride</i> , <i>Aspergillus niger</i> , <i>A. flavus</i> , <i>P. funiculosus</i> , <i>Tilletia indica</i> , <i>Sclerotium rolfsii</i> , <i>R. solani</i> , <i>Penicillium ochrochloron</i>	Veljic et al. (2009), Gahotri and Chaturvedi (2011)
DMSO extracts	<i>Marchantia polymorpha</i> , <i>Atrichum undulatum</i> , <i>Physcomitrella patens</i> , <i>Rhodobryum ontariense</i>	<i>Penicillium funiculosus</i> , <i>T. viride</i> , <i>P. ochrochloron</i> , <i>A. versicolor</i> , <i>A. fumigatus</i>	Sabovljevic et al. (2011), Pejtin et al. (2012)
Acetone and methanol extracts	<i>Riccia gangetica</i>	<i>Curvularia lunata</i>	Deora and Suhalka (2017), Deora and Guhil (2015, 2016)

9.7 Ray Fungi–Based Antifungal Activities

Actinobacteria are a group of gram-positive filamentous bacteria that are called as the branched bacteria or ray fungi (from Greek *actis*, ray beam, and *mykes*, fungus) and are characterized with the high G + C content occurring in mostly aerobic conditions but occasionally being anaerobes (Ludwig and Klenk 2005; Olanrewaju and Babalola 2019). Their morphology varies from forming branching filaments or mycelial growth to external spores. They are ubiquitous in nature ranging their distribution from soil and human microbiota to plant and even animal kingdom. They are predominant in aquatic as well as terrestrial ecosystem playing a major part in mineralization and recycling of organic matters leading to soil formation (Sharma et al. 2014). They are not only free-living members of the ecosystem but also a plant symbiont or endophyte, contributing to the plants' survival in extreme conditions and pursuing several bioactivities in vivo and in vitro. Actinomycetes produce a diverse range of secondary metabolites, for example, antibiotics, antitumor, insect-repellent, and immunosuppressive agents, and plant growth-promoting regulators (PGPRs) that are of immense pharmaceutical and agricultural importance. They are the prime producers of diverse antibiotics after the landmark discovery of penicillin in the year 1928. The single genus of *Streptomyces* sp. itself produces 76% of the total known bioactive (10,000 are produced by actinobacteria out of 23,000 produced by microorganisms, almost 45%) compounds from actinobacterial and

bacterial source (Berdy 2012) and is known to be the prime organism in the pharmaceutical world. They are equally profitable when isolated from plant source and designated as endophytic actinomycetes. So exploitation of the actinobacterial novel bioactive compounds both from endophytic and non-endophytic source is the ultimate way to fight against human and plant diseases. Here we focus only on actinobacterial compounds' antifungal activity and role in plant protection from deadly diseases caused by severe phytopathogens leading to irreparable crop loss and economic breakdown of agricultural sectors.

9.7.1 Isolation, Identification, and Detection of Bioactive Compounds with Anti-phytopathogenic Activity

Actinomycetes from soil source are selected based on the enrichment culture technique and are plated on selective media for isolation. Antifungal agents, for example, nystatin and cycloheximide, are supplemented for the inhibition of fungal contamination. For isolation of endophytic actinobacteria from plant source, plants are first selected and surface sterilized for the elimination of the epiphytic contaminants and finally plated on the selective growth media like starch casein nitrate agar (SCNA), chitin-vitamin B, tap water yeast extract agar (TWYA), soybean, humic acid-vitamin B (HV), yeast extract casamino acid (YECA), modified Gause, and glycine-glycerol (Ivantiskaya et al. 1978; Küster 1959; Küster and Williams 1964; Williams and Davies 1965; Hayakawa and Nonomura 1987; Crawford et al. 1993). International Streptomyces Project (ISP) medium is also popular media used for isolation, and they are supplemented with amino acids (L-asparagine for ISP 5, tryptone for ISP 1), inorganic trace salts, starch or carbohydrate sources (malt extract for ISP 4), and agar as solidifying agent. pH set at near to optimum or slightly basic is mandatory for proper isolation techniques using ISP medium. The actinomycete isolates are grown in solid or liquid medium for their antifungal bioactivity detection. Antagonistic activities of the potent isolates are tested by growing them on both sides of the fungal hyphae, and isolate having anti-phytopathogenic activity will inhibit the growth of the pathogens. Actinobacterial aqueous- or solvent-based extracts will be evaluated for either fungistatic or fungicidal activity by agar well-diffusion techniques. Soluble bioactive compounds of antifungal importance will be extracted using wide range of organic solvents followed by purification by column and thin-layer chromatographic techniques. HPLC analysis will be the most useful method for the detection of the purity of the compound, and further NMR studies are needed for the proper identification of the bioactive compound. Cell line studies are made with the coupling of bioinformatics tools for the proper knowledge about their mode of action.

9.7.2 Role in Plant Protection

9.7.2.1 Actinobacterial Flora Producing Antibiotics of Agricultural Importance

Actinobacteria can be a part of plant as endophyte, rhizospheric soil as symbiont for plant growth-promoting substance producer, and organisms' normal microbial flora as gut microorganism. So they are ubiquitous in their distribution. Out of several biologically potent compound produced from the actinobacterial source, antibiotics are the major contribution of these microorganisms toward human civilization. All the known antibiotics (blasticidin, mildiomycin, natamycin, validamycin, kasugamycin) are of actinobacterial (most of them are the *Streptomyces* sp.) source showing protective activity for the plants against agricultural fungal pathogens (Tables 9.6 and 9.7).

9.7.2.2 Endophytic Actinomycetes

As human are dependent completely on nature and more particularly natural components of agricultural origin and importance, dependence on agricultural crops is of a known fact. But the problem arises when the crops are affected most by the fungal pathogens leading to huge crop loss, and thus the search for novel antibiotics is on, and the search has shifted to actinobacterial source, and endophyte plays an important role in this respect. There are significant reports of antifungal compounds from bacterial origin, but now the focus has shifted to microbes of endophytic origin (Table 9.8). Till date, a huge number of antibiotics are already reported and have minimized the crop loss to a notable amount (Fig. 9.6). Antibiotics and other antifungal compounds include munumbicins A, B, C, D, E-4, and E-5, vanillin, saadamycin, 5,7-dimethoxy-4-p-methoxyphenyl coumarin, coronamycin, and fistupyron isolated from different strains of *Streptomyces* sp. (*Streptomyces aureofaciens* CMU Ac 130, *Streptomyces* sp. NRRL 30562, *Streptomyces* sp. Hedaya48, *Streptomyces* sp. MSU-2110, *Streptomyces* sp. TP-A0569) and from different parts (stem, leaf, inflorescence, root, fruit, internal healthy tissues) of diverse medicinal plants, for example, *Kennedia nigricans* (Fabaceae), *Aplysina fistularis* (yellow-green candle sponge or yellow tube sponge), *Monstera* sp. (Monsteraceae), *Allium fistulosum* (Liliaceae), and *Zingiber officinale* (Zingiberaceae) of different regions all over the world. The bioactive compounds are effective against a wide range of phytopathogens including *Magnaporthe oryzae*, *Fusarium graminearum*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *cubense*, *F. verticillioides*, *Colletotrichum* sp., *Pestalotiopsis* sp., *Diaporthe* sp., *Xylaria* sp., *P. aphanidermatum*, *Pythium oligandrum*, *Pythium ultimum*, *Pythium aphanidermatum*, *Phytophthora cypogea*, *Phytophthora cactorum*, *Phytophthora infesta*, *Fusarium solani*, *Aspergillus fumigatus*, *Mycosphaerella fijiensis*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Phytophthora erythroseptica* (Shan et al. 2018; Costa et al. 2013; Igarashi et al. 2002; Tian et al. 2004; Zin et al. 2007) and are protecting a large number of cereals and other important cash crops from being affected by these common contaminants. Endophytic actinobacteria directly counteract with fungal plant pathogens not only by producing bioactive compounds but also by enhancing the plant's growth through

Table 9.6 Mode of action of antibiotics against fungal crop pathogens

Active component	Trade name	Source	Effective against	Mode of action	References
Kasugamin TM	Kasugamycin	<i>Streptomyces kasugaensis</i>	Rice blast and other diseases in Japan (<i>Magnaporthe oryzae</i>)	Inhibition of protein synthesis by t-RNA and ribosome assembly interference	Tanaka et al. (1966) and Zaker (2016)
Polyoxin B and polyoxorim	Polyoxin Z, Polyoxin AL	<i>Streptomyces cacaoi</i>	Fungal pathogens	Protein synthesis inhibitor	Isono and Suzuki (1979)
Validamycin	Validacin, Valimun, Sheathmar, Mycin	<i>S. hygroscopicus</i>	Rhizoctonia root rot	Knockout of an enzyme necessary for glucose generation on hyphal tips	Karneda et al. (1987) and Dayan et al. (2009)
Mildiomyacin	Mildiomyacin	<i>Streptovercillium rimofaciens</i> (soil actinomycete)	Powdery mildews in Japan	Inhibition of protein synthesis by blockage of peptidyl transferase	Om et al. (1984)
Blasticidin S	Bla-S	<i>S. griseochromogenes</i> (soil actinomycete)	Rice blast disease in Eastern Asia	Inhibition of protein synthesis	Kimura and Yamaguchi (1996)
Natamycin	Delvolan (amphoteric macrolide)	<i>S. natalensis</i> or <i>S. chattanoogenensis</i>	Fungal disease of ornamental plants	Membrane dysfunction by binding to membrane ergosterol	te Welscher et al. (2008)

Table 9.7 Antifungal activity of different species of *Streptomyces*

Actinomycetes	Antifungal against	References
<i>Streptomyces griseus</i>	<i>Rhizoctonia solani</i>	Merriman et al. (1974)
<i>Streptomyces kasugaensis</i>	<i>Fusarium</i> sp.	De Vasconcellos and Cardoso (2009)
<i>Streptomyces</i> sp.	<i>Alternaria brassicae</i> , <i>Colletotrichum gloeosporioides</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora capsici</i>	Srividya et al. (2012)
<i>Streptomyces</i> sp.	<i>Sclerotium rolfsii</i>	Gholami et al. (2014)
<i>Streptomyces griseus</i> E44G	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Al-Askar et al. (2015)
<i>Streptomyces sanglieri</i>	<i>Ganoderma boninense</i>	Azura et al. (2016)
APA2 <i>Streptomyces longisporoflavus</i> , AAH53 <i>Streptomyces mutabilis</i> , APC70 <i>Streptomyces griseus</i>	<i>Alternaria solani</i> , <i>Colletotrichum coccodes</i> , <i>Fusarium oxysporum</i>	Dávila et al. (2016)
	Wilt disease of banana caused by <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> (FOC)	Dewi et al. (2017)
<i>Streptomyces</i> strain KX852460	<i>Rhizoctonia solani</i> AG-3 KX852461	Ahsan et al. (2017)
<i>Streptomyces</i> 12-09-4 and 12-09-11	<i>Botrytis cinerea</i> , <i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Wang et al. (2018)

the production of plant growth promoters and making the plant less susceptible to pathogenic invasion. They are efficient agent of reducing the symptoms that arise due to exposure to environmental stress (Shimizu 2011). Enhanced production of indole acetic acid (IAA) was mediated by *Streptomyces* sp. (isolated from *Centella asiatica*) and *Nocardiopsis* sp. (Dochhil et al. 2013; Shutsrirung et al. 2014; Gangwar et al. 2014). Experimental trials on cucumber indicate positive result as the isolates *Actinoplanes campanulatus*, *Micromonospora chalcea*, and *Streptomyces spiralis* enhanced plant growth and improved yield conditions (El-Tarabily et al. 2010). Other than auxin, auxin-like similarly functioning molecules named as peridic acids A and B are found to be inducers of adventitious root proliferation in kidney bean plants at very minute concentrations of 1 mM (Igarashi et al. 2002).

9.7.2.3 Siderophores and Chitinases

Chitin is a major fungal cell wall polysaccharide (the second most abundant polysaccharide in nature after cellulose) component and is the first line of defense of fungal cells. Actinobacteria antagonize the fungal cell by producing chitinases (an enzyme capable of hydrolyzing fungal cell wall) and break the glycosidic bonds in chitin and lead to the death of the pathogenic cell. Endophytic *Kitasatosporia* sp.

Table 9.8 Antifungal compounds from bacterial origin and their common targets

Bacillomycin D (<i>B. subtilis</i> AU195), bacillomycin and fengycin (<i>B. amyloliquefaciens</i> FZB42), Zwittermicin A (<i>B. cereus</i> UW85), geldanamycin (<i>S. hygroscopicus</i> var. <i>geldanus</i>), chaetomin (<i>Chaetomium globosum</i>), gliotoxin (<i>T. virens</i>), 2,4-diacetylphloroglucinol (<i>Pseudomonas fluorescens</i> F113)	<i>Aspergillus flavus</i> , <i>Fusarium oxysporum</i> , <i>Phytophthora medicaginis</i> , <i>R. solani</i> , <i>P. ultimum</i> , <i>Pyricularia oryzae</i>	DeBoer et al. (1970), Shanahan et al. (1992), Smith et al. (1993), Moyné et al. (2001), Wilhite et al. (2001), Koumoutsis et al. (2004) and Anitha Murugesan (2005)
Pyrrolnitrin, pyoluteorin, pseudane (<i>Burkholderia cepacia</i> , <i>P. fluorescens</i> Pf-5), phenazines (<i>P. fluorescens</i> 2–79 and 30–84)	Damping off (<i>Phytophthora medicaginis</i> , <i>P. aphanidermatum</i>), damping off and rice blast (<i>R. solani</i> , <i>Pyricularia oryzae</i> , <i>Pythium ultimum</i>), take-all (<i>Gaeumannomyces graminis</i> var. <i>tritici</i>)	Howell and Stipanovic (1980), Homma et al. (1989), Thomashow et al. (2002) and Smith et al. (1993)
Harpin proteins (<i>Erwinia amylovora</i>), trade name: Harpin $\alpha\beta$ (ProAct)	Induction of systemic acquired resistance (SAR) and less susceptibility to fungal and bacterial disease	Wei et al. (1992)
Strobilurin and oudemansin (members of basidiomycete grows on dead wood) Commercial synthetic analogues: azoxystrobin and kresoxim-methyl	Fungal pathogens (blockage of mitochondrial respirations by blocking of ubiquinone receptor)	Kraiczky et al. (1996)
Mycosubtilin (<i>B. subtilis</i> BBG100), iturin A (<i>B. subtilis</i> QST713), herbicolin (<i>Pantoea agglomerans</i> C9-1), xanthobaccin A (<i>Lysobacter</i> sp. strain SB-K88),	Damping off (<i>Pythium aphanidermatum</i> , <i>Botrytis cinerea</i>), root rots (<i>R. solani</i>), fire blight (<i>Erwinia amylovora</i>), damping off (<i>Aphanomyces cochlioides</i>)	Sandra et al. (2001), Kloepper et al. (2004), Leclere et al. (2005), Islam et al. (2005) and Paulitz and Belanger (2001)

(isolate of *Catharanthus roseus*) and *Kibdelosporangium* sp. (isolate of *Achillea fragrantissima*) are reported to be chitinase producers (El-Shatoury et al. 2009; Mini Priya 2012). *Actinoplanes missouriensis* isolated from *Lupinus* sp., a member of Fabaceae family, produces chitinase causing hyphal cell lysis and reducing the conidial germination rate and protects the plant from pathogenic attack of *Plectosporium tabacinum*, the causal agent of lupin root rot in Egypt (El-Tarabily 2003; El-Tarabily and Sivasithamparam 2006). Siderophores are soluble, small, high-affinity iron carriers produced by bacterial or fungal members and are involved in the transportation of iron (Fe^{3+}) across the cell membrane. They have caught sudden attention due to their involvement in plant growth promotion as well as antagonistic ability against phytopathogens (Cao et al. 2005; Tan et al. 2006; Rungin et al. 2012). Endophytic actinobacteria from *Aloe vera*, *Mentha arvensis*, and *Ocimum sanctum* are known to be producers of hydroxymate type and catechol type of siderophores, and the isolate *Saccharopolyspora* O9 is known to be the potent inhibitor

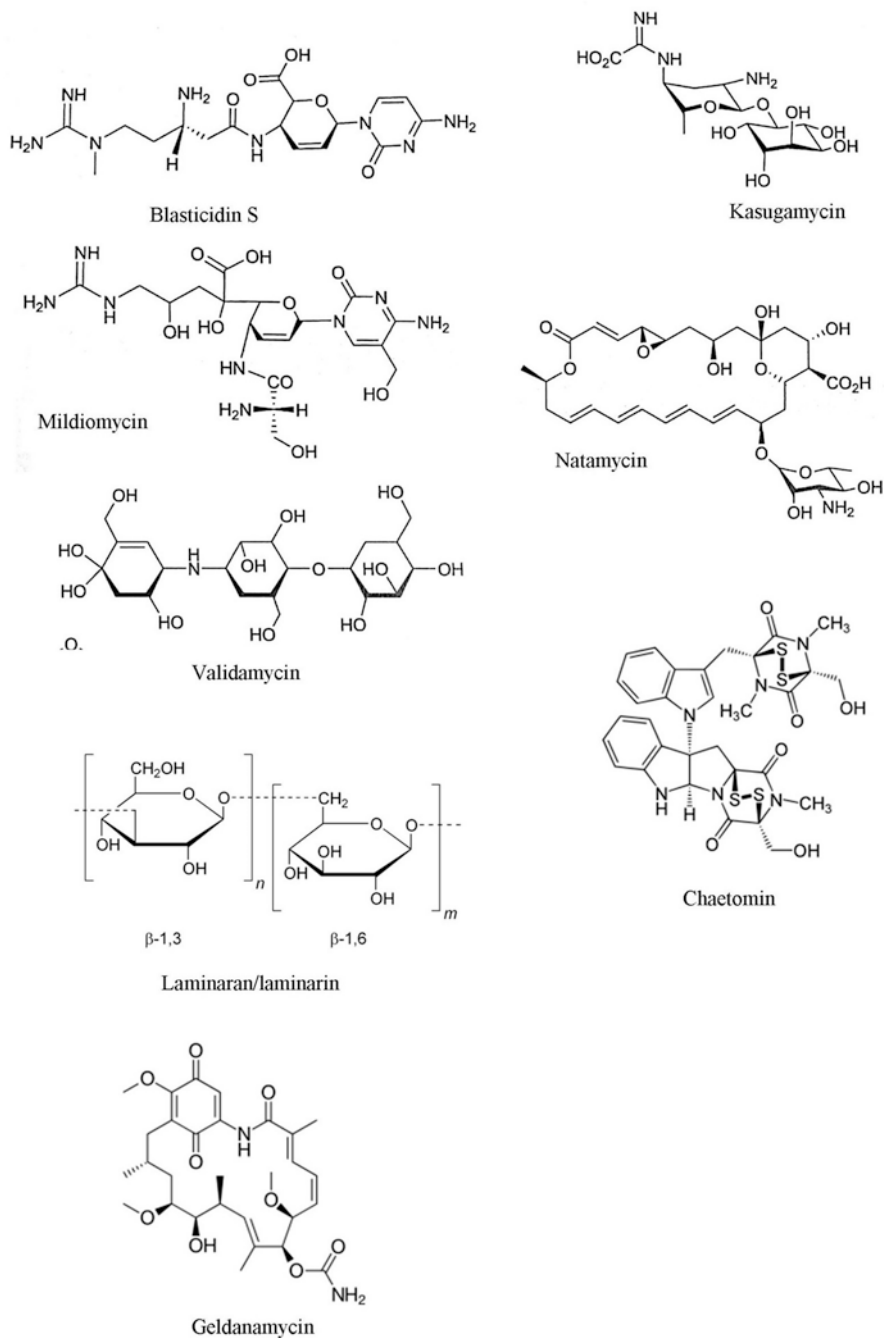


Fig. 9.6 Antibiotics and polysaccharides with plant protective ability

of *Alternaria brassicicola*, *Botrytis cinerea*, and *Fusarium oxysporum* (Gangwar et al. 2014; El-Shatoury et al. 2009).

9.7.2.4 Biocontrol Activity

Endophytic isolates of *Cucumis sativus* (cucumber), identified as *Actinoplanes campanulatus*, *Micromonospora chalcae*, and *Streptomyces spiralis*, are reported to control the growth and development of damping off, crown rot, and root rot pathogen *Pythium aphanidermatum*. They are known to promote plant growth and to protect seedlings and mature plants. A novel bioactive compound identified as 6-prenylindole was isolated from endophytic *Streptomyces* sp. showing strong antifungal activity against a broad range of phytopathogens: *Alternaria brassicicola* and *Fusarium oxysporum* (Igarashi 2004). Another new prenylated indole derivative from endophytic actinobacterial source inhibited the growth of *Colletotrichum orbiculare*, *Phytophthora capsici*, *Corynespora cassiicola*, and *Fusarium oxysporum* (Zhang et al. 2014). Naphthomycins A and K isolated from *Streptomyces* sp. CS have antifungal activity against *Penicillium avellaneum* (Lu and Shen 2003, 2007). Biocontrol ability of fistupyronone has made it a useful tool to minimize the crop loss of *Brassica* due to black leaf spot disease caused by *Alternaria brassicicola* (Igarashi 2004). Interest on actinomycetes of endophytic origin as an alternative tool for antifungal agent is increasing day by day (Table 9.9).

9.8 Plant Extracts as the Prime Source for Antifungals

Since the beginning of human civilization, whenever human race has faced any turbulence in its path of existence, they have rushed to their green friends, trees, for the ultimate solution. Search for bioactive products of medical importance has been a thirst area from time immemorial. Whether it is a concern of human or plant health, trees have given answers in all aspects. In the recent past, phytopathogenic infection has pushed the agricultural productive parameters to a real challenge, and plant extracts in its crude and purified form are applied as biocontrol methods (Table 9.10). The existing synthetic chemicals are facing problem of immediate or delayed drug resistance and also issues of nephrotoxicity (the gold standard; amphotericin B), biomagnification, or quality assurance of the food products and thus are inconsistent in their business (Goa and Barradell 1995; Cuenca-Estrella et al. 2000). So green plant extracts are the novel, safest, and the best effective treatment tool in this arena. Plants are mysterious in their chemical nature and in respect to their secondary metabolite production. The faith is consistent on green plants due to the fact that plants protect themselves from fungal or bacterial diseases specially for the taxa that occur in marshy shady or water-logged or stress conditions (Gurgel et al. 2005). So the search is primarily made on the wild native taxa or invasive species that have higher potential of antimicrobial production. The knowledge of ethnobotany comes in this context, and tribal people are imitated for the gathering of crude knowledge. The problem of fungal pathogenesis is mainly faced by plants of economic importance, that is, cash crops. A single event of pathogenic attack can

Table 9.9 Endophytic actinomycetes as antifungal agents

Actinomycetes	Host plant	Antifungal against	References
<i>Streptomyces</i> sp.	Rhododendron	<i>Phytophthora cinnamomi</i> , <i>Pestalotiopsis sydowiana</i>	Shimizu et al. (2000)
<i>Streptomyces</i> NRRL 30562	Snake vine medicinal plant (<i>Kennedia nigriscans</i>)	<i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora cinnamomi</i> , <i>Geotrichum candidum</i> , <i>Sclerotinia sclerotiorum</i>	Castillo et al. (2002), (2006)
<i>Streptomyces</i> sp.	<i>Allium fistulosum</i>	<i>Alternaria brassicicola</i> on Chinese cabbage seedlings	Igarashi et al. (2002)
<i>Streptomyces</i> sp. AOK-30	Mountain laurel (<i>Kalmia latifolia</i>)	<i>Pestalotia rhododendri</i>	Nisimura et al. (2002)
<i>Streptomyces</i> sp. CS	<i>Maytenus hookeri</i>	<i>Penicillium avellaneum</i> UC-4376	Lu and Shen (2003), (2007)
<i>Streptomyces</i> sp. TP-A0595, <i>Streptomyces</i> sp. TP-A0569	<i>Allium tuberosum</i>	<i>Alternaria brassicicola</i>	Igarashi (2004)
<i>Streptomyces</i> sp. MSU-2110	<i>Monstera</i> sp.	<i>P. ultimum</i> , <i>Phytophthora cinnamomi</i> , <i>Geotrichum candidum</i> , <i>F. solani</i> , <i>Rhizoctonia solani</i>	Ezra et al. (2004a, b)
<i>Streptomyces griseofuscus</i> , <i>Streptomyces hygrosopicus</i> , <i>Streptomyces globisporus</i> , <i>Streptomyces aureus</i> , <i>Streptomyces albosporus</i>	Rice (<i>Oryza sativa</i>)	<i>Magnaporthe grisea</i> , <i>Rhizoctonia solani</i> , <i>Xanthomonas oryzae</i> , <i>Fusarium moniliforme</i>	Tian et al. (2004)
<i>Streptomyces aureofaciens</i> CMU Ac130, <i>Streptomyces</i> sp. Tc022	<i>Zingiber officinale</i>	<i>Fusarium oxysporum</i> , <i>Colletotrichum musae</i>	Taechowisan et al. (2006), (2007)
<i>Streptomyces</i> sp., <i>Streptoverticillium</i> sp., <i>Streptosporangium</i> sp.	<i>Musa paradisiaca</i>	<i>Fusarium</i> sp. wilt pathogen	Cao et al. (2005)

(continued)

Table 9.9 (continued)

Actinomycetes	Host plant	Antifungal against	References
<i>Streptomyces fulvoviolaceus</i> , <i>Streptomyces caelestis</i> , <i>Streptomyces coelicolor</i>	<i>Thottea grandiflora</i> , <i>Mapania</i> spp., <i>Polyalthia</i> spp.	<i>Fusarium solani</i> , <i>Aspergillus fumigatus</i> , <i>Mycosphaerella fijiensis</i> , <i>Pythium ultimum</i> , <i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora erythroseptica</i>	Zin et al. (2007)
<i>Microbispora</i> sp., <i>Nonomuraea</i> sp., <i>Streptomyces</i> sp.	<i>Solanum lycopersicum</i>	<i>Rhizoctonia solani</i> , <i>Pythium irregulare</i> , <i>A. solani</i> , <i>P. parasitica</i>	Inderiati and Franco (2008)
<i>Streptomyces</i> sp., <i>Nocardia</i> sp., <i>Streptosporangium</i> sp., <i>Streptoverticillium</i> sp.	<i>Azadirachta indica</i>	<i>Pythium oligandrum</i> , <i>Pythium ultimum</i> , <i>Pythium aphanidermatum</i> , <i>Phytophthora cypogea</i> , <i>Phytophthora cactorum</i> , <i>Phytophthora infestans</i>	Verma et al. (2009)
<i>Promicromonospora cymbopogonis</i> , <i>Nonomuraea roseola</i> , <i>Micromonospora chokoriensis</i> , <i>Streptomyces ochraceoscleroticus</i> , <i>S. aurantiacus</i> , <i>S. griseocameus</i> , <i>S. chryseus</i> , <i>S. albobrisesolus</i>	<i>Juncus effusus</i> L., <i>Ainsliaea henryi</i> , <i>Stellera chamaejasme</i> , <i>Salvia miltiorrhiza</i> , <i>Lysimachia fortune</i> , <i>Senecio declouxii</i> , <i>Potentilla discolor</i> , <i>Achyranthes aspera</i> , <i>Cynanchum auriculatum</i>	<i>Verticillium dahliae</i> , <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> f. sp. <i>niveum</i> , <i>Colletotrichum orbiculare</i> , <i>Fusarium graminearum</i> , <i>Exserohilum turcicum</i> , <i>Curvularia lunata</i> , <i>Botrytis cinerea</i>	Zhao et al. (2011)
<i>Streptomyces</i> sp. Hedaya-48	<i>Aplysina fistularis</i>	<i>F. oxysporum</i>	El-Gendy and El-Bondkly (2010)
<i>Actinoplanes campanulatus</i> , <i>Micromonospora chalcea</i> , <i>Streptomyces spiralis</i>	Cucumber (<i>Cucumis sativa</i>)	<i>Pythium aphanidermatum</i>	El-Tarabily et al. (2010)
Cr-12 and Cr-20 unidentified isolates	<i>Catharanthus roseus</i>	<i>Curvularia lunata</i> , <i>Fusarium oxysporum</i> , <i>Fusarium solani</i> , <i>Rhizoctonia solani</i>	Kafur and Khan (2011)
<i>Streptomyces coelicolor</i>	<i>Rhizophora apiculata</i> , <i>Avicennia marina</i>	<i>A. niger</i> , <i>A. flavus</i> , <i>Penicillium</i> sp., <i>A. fumigatus</i>	Gayathri and Muralikrishnan (2013)

(continued)

Table 9.9 (continued)

Actinomycetes	Host plant	Antifungal against	References
<i>Streptomyces</i> sp. 16R3B	<i>Zea mays</i>	<i>P. aphanidermatum</i> , causal agent of damping off in cucumber (<i>Cucumis sativa</i>)	Costa et al. (2013)
<i>Streptomyces</i> sp. neau-D50	Soybean (<i>Glycine max</i>)	<i>Phytophthora capsici</i> , <i>Corynespora cassiicola</i> , <i>Fusarium oxysporum</i> , <i>Colletotrichum orbiculare</i>	Zhang et al. (2014)
<i>Streptomyces cinereus</i> AR16	<i>Embllica officinalis</i>	<i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i> , <i>Aspergillus niger</i> , <i>Alternaria brassicicola</i> , <i>Phytophthora dreselea</i>	Gangwar et al. (2015)
<i>Streptomyces</i> sp., <i>Leifsonia xyli</i> , <i>Microbacterium</i> sp., <i>Streptomyces</i> sp., <i>Brevibacterium</i> sp.	<i>Mirabilis jalapa</i> , <i>Clerodendrum colebrookianum</i>	<i>Rhizoctonia solani</i> , <i>Fusarium graminearum</i> , <i>Fusarium oxysporum</i> , <i>Fusarium proliferatum</i> , <i>Fusarium oxysporum</i> f. sp. <i>ciceris</i> , <i>Colletotrichum capsici</i>	Passari et al. (2015)
<i>Streptomyces</i> sp.	<i>Schima wallichii</i>	<i>Colletotrichum</i> sp., <i>Alternaria</i> sp., <i>F. oxysporum</i> f. sp. <i>ciceris</i> , <i>F. proliferatum</i> , <i>F. culmorum</i> , <i>F. graminearum</i>	Passari et al. (2016)
<i>Melia toosendan</i> (<i>chinaberry</i>)	<i>Rhodococcus</i> sp., <i>Tomitella</i> sp.	<i>Colletotrichum orbiculare</i> , <i>Fusarium oxysporum</i> , <i>Alternaria solani</i> , <i>Magnaporthe grisea</i> , <i>Curvularia lunata</i> , <i>Gibberella saubinetii</i>	Zhao et al. (2018)
<i>Streptomyces levis</i> NBRC 15423(T), <i>Streptomyces gilvifuscus</i> T113(T), <i>Micromonospora olivasterospora</i> DSM 43868(T), <i>Actinomadura geliboluensis</i> A8036(T), <i>Streptomyces djakartensis</i> NBRC 15409(T), <i>Streptomyces griseoaurantiacus</i> NBRC 15440(T), <i>Nocardiopsis dassonvillei</i> NBRC 13392(T)	<i>Camellia sinensis</i>	<i>Magnaporthe oryzae</i> , <i>Fusarium graminearum</i> , <i>F. oxysporum</i> f. sp. <i>lycopersici</i> , <i>F. oxysporum</i> f. sp. <i>cubense</i> , <i>F. verticillioides</i> , <i>Colletotrichum</i> sp., <i>Pestalotiopsis</i> sp., <i>Diaporthe</i> sp., <i>Xylaria</i> sp.	Shan et al. (2018)

Table 9.10 Bioactive products with their common sources and targets

Bioactive product	Source	Effective against	References
Carvone	Dill and caraway seed	Inhibit the growth of storage pathogen	Mozeelaar et al. (1999)
GAMMA aminobutyric acid (GABA)	Any type of plant and animal source	Prevent powdery mildew on grapes, brown rot and shot hole of stone fruit; enhances growth of almond, broccoli, onions	Copping (2004)
Cinnamaldehyde from cinnamon leaf essential oil (mixture of α -methyl cinnamaldehyde, (E)-2-methylcinnamic acid, eugenol, isoeugenol, citral, geraniol oil), trade name: Vertigo and Cinnacure	<i>Cinnamomum osmophloeum</i> Alternate source: seeds of the weed <i>Cassia obtusifolia</i>	Dry bubble (caused by <i>Verticillium fungicola</i>), dollar spot (<i>Sclerotinia homoeocarpa</i>), pitch canker (<i>Fusarium moniliforme</i> var. <i>subglutinans</i>), wood rot fungi (<i>Coriolus versicolor</i> and <i>Laetiporus sulphureus</i> , <i>Aspergillus fumigatus</i> and <i>Trichophyton rubrum</i>)	Copping (2004), Wang et al. (2005), Cheng et al. (2008) and Khan and Ahmad (2011)
Jojoba oil (vegetable oil)	<i>Simmondsia chinensis</i> (Simmondsiaceae)	Powdery mildew for ornamental plants and grapes and applied as ground spray	Copping (2004)
Milsana (ethanolic extracts of plant)	<i>Reynoutria sachalinensis</i> (Polygonaceae)	Powdery mildew of wheat and grape (<i>Uncinula necator</i> , <i>Sphaerotheca fuliginea</i>) by induced resistance, used as a spray	Copping (2004)
Pink plume poppy blood root	<i>Macleaya cordata</i> <i>Sanguinaria Canadensis</i> (Papaveraceae)	Alkaloids; sanguinarine effective against <i>Rhizoctonia solani</i> by systemic acquired resistance (SAR)-mediated accumulation of endogenous phenolics	Liu et al. (2009a, b)

(continued)

Table 9.10 (continued)

Bioactive product		Source	Effective against	References
Sporan		Rosemary oil (<i>Rosmarinus officinalis</i>)	<i>Botrytis cinerea</i>	Zaker (2016)
Promax		Thyme oil (<i>Thymus vulgaris</i>)	<i>Botrytis cinerea</i>	Zaker (2016)
Trilogy		Neem oil (<i>Azadirachta indica</i>)	<i>F. oxysporum</i> , <i>R. solani</i>	Zaker (2016)
GC-3™		Cottonseed (<i>Gossypium hirsutum</i>) and garlic (<i>Allium sativum</i>)	–	Zaker (2016)
Fatty Acids	Polyacetylenic acids, octadeca-9,11,13-triynoic acid, trans-octadec-13-ene-9,11-diyenoic acid	<i>Prunella vulgaris</i>	<i>M. oryzae</i> , <i>R. solani</i> , <i>P. infestans</i> , <i>S. sclerotiorum</i> , <i>F. oxysporum</i> , and <i>P. capsici</i> . Inhibits rice blast, tomato late blight, wheat leaf rust, and red pepper anthracnose	Yoon et al. (2010)
	Lipopeptides	<i>Bacillus</i> XT1 CECT 8661	<i>Botrytis cinerea</i>	Toral et al. (2018)
	Ginger oleoresin (GO)	Ginger	<i>Pestalotiopsis microspora</i> ; dominant pathogenic fungi causing rotten disease in harvested Chinese olive (<i>Canarium album</i> Lour.) fruits	Chen et al. (2018)

(continued)

Table 9.10 (continued)

Bioactive product	Source	Effective against	References
Alkaloids	Steroidal alkaloids; verazine type (veramitaline, stenophylline B, veramiline) and jerveratrum type (jervine)	<i>Veratrum taliense</i>	<i>Phytophthora capsici</i> Zhou et al. (2003)
	D-Calycanthin, L-folicanthine	<i>Chimonanthus praecox</i> (from seeds)	<i>Exserohilum turcicum</i> , <i>B. maydis</i> , <i>A. solani</i> , <i>S. sderotiorum</i> , <i>F. oxysporum</i> Zhang et al. (2006)
	Securinine	<i>Phyllanthus amarus</i>	<i>Alternaria alternata</i> , <i>A. brassicae</i> , <i>A. brassicicola</i> , <i>Curvularia lunata</i> , <i>C. maculans</i> , <i>C. pallenscens</i> , <i>C. musae</i> , <i>Helminthosporium echinoclova</i> , <i>H. spiciferum</i> Singh et al. (2008)
	Piperonaline, a piperidine alkaloid	<i>Piper longum</i>	<i>M. oryzae</i> , <i>R. solani</i> , <i>B. cinerea</i> , <i>P. infestans</i> , <i>P. recondite</i> , <i>B. graminis</i> Yoon et al. (2013)
Glycosides	Hemoiedemosides A, B (sulfated triterpene),	Patagonian sea cucumber (<i>Hemoiedema spectabilis</i>)	<i>Cladosporium cucumerinum</i> Chludil et al. (2002)
	Triterpenic saponins,	<i>Sapindus mukorossi</i> , <i>Diploknema butyracea</i>	Saha et al. (2010)
	Pregnane (caudatin glycosides)	<i>Cynanchum wilfordii</i> (roots)	Barley powdery mildews and strawberry powdery mildew (<i>Sphaerotheca humuli</i>) Yoon et al. (2011)

affect seriously the demand and supply ratio; thus the sustainability is lost, and restoring the good health of crops is a basic need of agricultural sectors but in an efficient way not hampering the soil health, ecosystem characters, and human health and also should be budget friendly. The search is strictly focused on plants of ethno-medicinal importance as history indicates the ability of medicinal plant extracts in human and animal mycoses and antifungal ability (Mathias-Mundy and McCorkle

1995). The statistics of the World Health Organization (WHO) states that 80% of the world's population in underdeveloped or developing countries depend on plants of ethnomedicinal importance for their primary healthcare issues.

9.8.1 Antifungal Properties of Secondary Metabolites of Plants

Secondary metabolites are plants' best weapon against phytopathogenic invasion. Several plant extracts have been assessed for their antifungal activity against a variety of phytopathogens of serious agricultural threats (Table 9.11). The metabolites are divided into terpenoids, saponins, phenolic compounds, flavones, flavonoids, flavonols, alkaloids, and coumarins (Table 9.12). Plant extracts are primarily tested for antifungal efficacy and further are purified by solvent extraction and chromatographic procedures leading to discovery of new antifungal agents. Terpenoids, also called as isoprenoids (under the chemical subclass of prenillipids), are known to be the oldest group of widespread molecular compounds produced by plants. Scher et al. (2004) reported a variety of six sesquiterpenes of antifungal importance against the causal organisms of bunch rot (*Botrytis cinerea*) on grapes, scab of cucurbits (*Cladosporium cucumerinum*), potato blight (*Phytophthora infestans*), rice blast (*Pyricularia oryzae*), and blotch of wheat (*Septoria tritici*). Sesquiterpene isolated from *Polygonum punctatum* (dotted knotweed of knotweed family Polygonaceae) named after the chemical polygodial is an effective control agent of *Zygosaccharomyces bailii* (a common food spoilage yeast). Scab of cucurbits is a common and devastating fungal pathogenic disease in agricultural fields, and this disease is to some extent prevented by the use of clerodane diterpenes extracted from *Detarium microcarpum*, a plant of Leguminosae family (Cavin et al. 2006). Skaltsa (2000) reported fungi inhibitory (*Cunninghamella echinulata*) activity of costunolide and eudesmane derivatives isolated from *Centaurea* plants. Other than terpenes, saponins (triterpene and steroidal saponins) are also effective antifungals reported from plant sources. Tea is one of the most vital cash crops in terms of foreign money earning and the most popular beverage having antioxidative properties. Pathogenic infection by *Pestalotia longiseta* causes a huge loss of tea production. Nagata et al. in the year 1985 isolated triterpenoid saponins camelids I and II from the leaves of *Camellia japonica* (Japanese camellia) that inhibited the tea pathogen *P. longiseta*. Cucurbitacins I, A, B, Q, and E isolated from cucurbitacins (*Ecballium elaterium*) have antifungal activity against *Botrytis cinerea* (Har-Nun and Meyer 1990). Phenolics are odorous compounds having antifungal compounds and are also responsible for the plant pigment production. Phenolics cover a large number of chemical compounds, for example, alkylated phenols, anthraquinones, coumarins, phenolic acid, phenols, phenylpropanoids, quinines, xanthenes, hydroxycinnamic acid, p-coumaric acid, ferulic acid, and chlorogenic acid. Phenol derivatives like crassinervic acid (*P. crassinervium*), aduncumene (*P. aduncum*), hostmaniane (*P. hostamannianum*), and gaudichaudanic acid (*P. gaudichaudianum*) are effective against strawberry blossom blight pathogen *Cladosporium cladosporioides* (Lago et al. 2004). 3-Acetyl-4-acetoxyacetophenone showed antifungal activity against

Table 9.11 Bioactive phytochemicals against common plant diseases of crop plants

Name of the disease	Plant taxa and chemical class of the compound	References
Scab of cucumber (<i>Colletotrichum cucumerinum</i>)	Naphoxirenes from bark of <i>Sesamum angolense</i> of Pedaliaceae family, xanthone from roots of <i>Polygala nicaeensis</i> (Polygonaceae), sakurasosaponin from leaves of <i>Rapanea melanophloeos</i> , 5-methylcoumarins, mutisicoumarones C and D from <i>Mutisia friesiana</i> (Asteraceae), mollugenol A from <i>Mollugo pentaphylla</i> (Molluginaceae), flavone and flavonol of <i>Helichrysum decumbens</i> (Asteraceae), triterpenoid saponin from roots of <i>Dolichos kilimandscharicus</i> (Leguminosae), Clerodane diterpene from <i>Detarium microcarpum</i> , E-triticine, P-triticine, puroindoline from <i>Triticum aestivum</i> (Poaceae)	Spendley et al. (1982), Potterat et al. (1987), Martson et al. (1988), Marston et al. (1993), Viturro et al. (2004), Cavin et al. (2006a), and Dhatwalia et al. (2009)
Disease of woody plant (<i>Melampsora medusae</i>)	Pinocembrin from leaves of <i>Populus deltoides</i> (Salicaceae)	Shain and Miller (1982), Hoof et al. (2008)
<i>Cladosporium</i> fruit and leaf rot and bitter root (<i>Cladosporium gloeosporioides</i>)	Long-chain alcohol from peels of young fruit of <i>Persea americana</i> from Lauraceae, methylripariochromene A from roots of <i>Eupatorium riparium</i> (Asteraceae)	Prusky et al. (1983), Ratnayake Bandara et al. (1992)
Pine needle pathogen (<i>Dothistroma pini</i>)	Stearic acid from needles of <i>Pinus radiata</i> (Pinaceae)	Franich et al. (1983)
Pathogen of corn, sorghum, apple (<i>Helminthosporium carbonum</i>)	Luteone and wighteone from leaf surface of <i>Lupinus albus</i> (Leguminosae)	Ingham et al. (1983)
Black and brown spot of banana (<i>Colletotrichum musae</i>)	Dopamine from unripe banana fruit (<i>Musa</i> sp.)	Muirhead and Deverall (1984)
Powdery mildew of grains (<i>Erysiphe graminis</i>)	Gramine from leaves of <i>Hordeum vulgare</i> (Poaceae)	Wippich and Wink (1985)
Leaf spot, rots, and blights (<i>Alternaria alternata</i>), disease of cereal (<i>Penicillium verrucosum</i>)	Alizarin and emodin from root of <i>Rubia tinctorum</i> of Rubiaceae, alkylated phenols of peel and pulp of <i>Mangifera indica</i> (Anacardiaceae)	Cojocarú et al. (1986), Manojlovic et al. (2005)
Maize rot (<i>Fusarium moniliforme</i>), epidemic outbreak of glume and kernel discoloration (<i>Curvularia lunata</i>)	Flavan-4-ols of root bark of <i>Sorghum</i> cultivars of Poaceae	Jambunathan et al. (1986)

(continued)

Table 9.11 (continued)

Name of the disease	Plant taxa and chemical class of the compound	References
Rice blast disease (<i>Pyricularia oryzae</i>)	Jasmonic acid, hydroxybenzoic acid from leaves of <i>Oryza officinalis</i> ; pisiferic acid from leaves of <i>Chamaecyparis pisifera</i> (Cupressaceae); gingerenones A, B and C; isogingerenone B from <i>Zingiber officinale</i>	Kobayashi et al. (1987), Endo et al. (1990), Cho et al. (1998)
Blue mold of tobacco (<i>Peronospora tabacina</i>)	Diterpenoids from <i>Nicotiana tabacum</i> of Solanaceae	Reuveni et al. (1987)
Leaf and fruit pathogen (<i>Cladosporium cladosporioides</i>)	Canaliculatol from bark of <i>Stemonoporus canaliculatus</i> and long-chain alcohol from <i>Persea americana</i> , phenylethanone from <i>Euodia lunuankenda</i> , sinharine and methylsinharine from <i>Glycosmis cyanocarpa</i> , Illukumbin from <i>Glycosmis mauritiana</i> (Rutaceae), phenylethanone from <i>Euodia lunuankenda</i> (Lauraceae), benzoquinone from <i>Croton lacciferus</i> (Euphorbiaceae)	Bokel et al. (1988), Ratnayake Bandara and Wimalasiri (1988), Kumar et al. (1990), Greger et al. (1992), Pacher et al. (2001), Springob and Kutchan (2009),
Butt rot on conifers; Douglas fir, spruce, fir, hemlock, pine, and larch (<i>Phaeolus schweinitzii</i>)	Astringin and rhaponticin from <i>Picea sitchensis</i> (Pinaceae)	Woodward and Pearce (1988)
Sudden death of soybean (SDS) pathogen, damping off, corn rot, root rot, wilting, and necrotic spots (<i>Fusarium solani</i>)	Tomatine from <i>Lycopersicon esculentum</i> (Solanaceae)	Defago and Kern (1988)
Gray mold of grapes (<i>Botrytis cinerea</i>)	Surangin B from <i>Mammea longifolia</i> (Clusiaceae), cucurbitacin I of <i>Ecballium elaterium</i> (Cucurbitaceae), chalcone from <i>Bauhinia manea</i> (Leguminosae)	Achenbach et al. (1988), Har-Nun and Meyer (1990), Deng and Nicholson (2005)
Witches' broom of cocoa tree (<i>Crinipellis perniciososa</i>)	Polymeric procyanidin from <i>Theobroma cacao</i> of Sterculiaceae	Brownlee et al. (1990), Duke (2004)
Fusarium wilt of pathogen (<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>)	Jodrellin B and oerodin from <i>Scutellaria woronowii</i> and <i>S. violacea</i> of Lamiaceae	Cole et al. (1991)
Collar rot, root rot, damping off, wire stem, primarily the pathogen of herbs (<i>Rhizoctonia solani</i>)	Dihydrochalcone from twigs and leaves of <i>Psidium acutangulum</i> (Myrtaceae), coumarin from <i>Mammea longifolia</i> (Clusiaceae), dolichin of <i>Dolichos lablab</i> (Fabaceae), methylquercetin from leaf of <i>Wedelia biflora</i>	Miles et al. (1991), Miles et al. (1993), Lee et al. (2003), Deng and Nicholson (2005) and Yoganandam et al. (2009)

(continued)

Table 9.11 (continued)

Name of the disease	Plant taxa and chemical class of the compound	References
Spoilage of fruit and vegetables (<i>Cladosporium sphaerospermum</i>)	Nonglycosidic iridoid from <i>Alibertia macrophylla</i> (Rubiaceae)	Young et al. (1992)
Late blight of potato or tomato (<i>Phytophthora infestans</i>), bbrown rust (<i>Puccinia recondita</i>), rice blast disease (<i>Pyricularia grisea</i>)	Emodin from <i>Cassia tora</i> (Leguminosae)	Kim et al. (2004)
Pathogen of Rhododendron (<i>Pestalotia guepinii</i>), cortical stem rot (<i>Fusarium avenaceum</i>), Drechslera leaf spot (<i>Drechslera</i> spp.)	Trifolin and hyperoside from <i>Camptotheca acuminata</i> (Cornaceae)	Li et al. (2005a, b)
Strawberry pathogen (<i>Cladosporium fragariae</i>) and anthracnose of <i>Lupin</i> sp., <i>Cladosporium acutatum</i>	Alkaloid (findersine, anhydroevoxine, haplamine) from <i>Haplophyllum sieversii</i> (Rutaceae)	Cantrell et al. (2005)
Pre- and postharvest fungal disease of cereal grains, legumes, and tree nuts (<i>Aspergillus flavus</i>)	Alkaloids from <i>Datura metel</i>	Dabur et al. (2005)
Early blight of potato and rice (<i>Alternaria solani</i>)	Meliacin-type of nortriterpenoid from <i>Chisocheton paniculatus</i> (Meliaceae)	Yang et al. (2009)
Take-all fungus in wheat, barley, rye, oat, turf grass (<i>Gaeumannomyces graminis</i>)	Avenacins from <i>Avena sativa</i> (Poaceae)	De Bertoldi et al. (2009)

Sclerotinia sp. Phenolic structures when contain a carbonyl group are known to be flavones, and the addition of an extra 3-hydroxyl group indicates flavonol. Flavonoids are also known to hydroxylated phenolics but occurring as a C6–C3 unit linked to aromatic ring. Not only plant samples directly but also plant derivatives like porpulis (galangin isolated from the bee glue or resinous mixture produced as a result of the mixture of tree buds, sap, botanical extracts, and bee exudates) are shown to be antifungal against green rot or mold of tangerine (pathogens *Penicillium digitatum*, *P. italicum*) and also control postharvest disease of cereal grains, legumes, and tree nuts caused by *A. flavus* (Afolayan and Meyer 1997). Flavones (6,7,4'-trihydroxy-3,5'-dimethoxyflavone, 5,5'-dihydroxy-8,2',4'-trimethoxyflavone) from *Artemisia giraldi* are effective against *A. flavus* infections (Cowan 1999). Leaf wax of *Arrabidaea brachypoda* (Brazilian medicinal plant from Bignoniaceae) contains

Table 9.12 Direct extracts of plants tested for antifungal potency

Plant species	Fungal pathogen	References
<i>Reynoutria sachalinensis</i>	<i>Uncinula necator</i> (the causal pathogen of grapevine (powdery mildew))	Herger et al. (1988) and Abdu-Allah and Elyours (2017)
<i>Cassia tora</i> (dealcoholized extract of leaves)	<i>Aspergillus niger</i>	Mukherjee et al. (1996)
Thymol, carvacrol, citronellol, geraniol, citral, perillyl, menthol, eugenol, 1,8-cineole, Y-terpinene, p-cymene, anethole from several plants like <i>Thymus vulgaris</i> , <i>T. spicata</i> , <i>T. pulegioides</i> , <i>Cymbopogon citratus</i> , <i>Cymbopogon martini</i>	<i>Fusarium moniliforme</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora capsici</i> , <i>Monilinia fructicola</i> , <i>Botrytis cinerea</i> , <i>Curvularia lunata</i>	Mueller-Riebau et al. (1995), Krishna Kishore et al. (2007)
<i>Phlomis fruticosa</i> (Jerusalem sage)	<i>Aspergillus niger</i> , <i>Penicillium ochrochloron</i> , <i>Trichoderma viride</i> , <i>Fusarium tricinctum</i> , <i>Phomopsis helianthi</i> , <i>Cladosporium cladosporioides</i> , <i>Aspergillus ochraceus</i>	Ristic et al. (2000)
<i>Vernonia tenoreana</i>	<i>Aspergillus niger</i> ; <i>A. flavus</i>	Ogundare et al. (2006)
<i>Tagetes erecta</i>	<i>Fusarium wilt</i> (<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>)	Du et al. (2017)
Garlic (<i>Allium sativum</i> L.), neem (<i>Azadirachta indica</i> L.)	Narrow brown leaf spot (<i>Cercospora oryzae</i>), sheath blight (<i>Rhizoctonia solani</i>), sheath rot (<i>Sarocladium oryzae</i>), false smut (<i>Ustilaginoidea virens</i>)	Mahmud et al. (2018)
Neem seed and eucalyptus extract	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (FOL) tomato crop causing wilt disease	Poussio et al. (2018)
Stilbene and resveratrol	<i>Plasmopara viticola</i>	Krzyzaniak et al. (2018)
<i>Zingiber officinale</i> , <i>Piper nigrum</i> , <i>Azadirachta indica</i> , <i>Nicotiana tabacum</i> , <i>Carica papaya</i>	<i>P. expansum</i> pathogenic of yam tubers (<i>Dioscorea rotundata</i>)	Gwa et al. (2018)
Neem (<i>Azadirachta indica</i>), lantana (<i>Lantana camara</i>), and eucalyptus (<i>Eucalyptus globulus</i>)	<i>Pyricularia oryzae</i>	Wasimfiroz et al. (2018)
Onion (<i>Allium cepa</i>), garlic (<i>Allium sativum</i>), lantana (<i>Lantana camara</i>), marigold (<i>Tagetes erecta</i>), dathura (<i>Datura stramonium</i>), tulasi <i>Ocimum sanctum</i> , eupatorium (<i>Eupatorium rugosum</i>), parthenium (<i>Parthenium hysterophorus</i>), neem (<i>Azadirachta indica</i>)	<i>Botrytis oryzae</i> (brown leaf spot of paddy)	Channakeshava and Pankaja (2018)

(continued)

Table 9.12 (continued)

Plant species	Fungal pathogen	References
Methanol extracts of <i>Eucalyptus tereticornis</i> , <i>Ammi visnaga</i> , <i>Azadirachta indica</i> , <i>Rheum Palmatum</i> , <i>Adansonia digitata</i>	<i>Rhizoctonia solani</i> (root rot of maize)	Rashad et al. (2018)
<i>Lawsonia inermis</i> (henna), <i>Acalypha wilkesiana</i> (acalypha), <i>Melia azedarach</i> (chinaberry), <i>Punica granatum</i> (pomegranate), <i>Lantana camara</i> (lantana)	<i>Puccinia triticina</i> (leaf rust fungus)	Draz et al. (2019)
<i>Azadirachta indica</i>	<i>Sclerospora graminicola</i> (pearl millet downy Mildew)	Atri et al. (2019)

cirsiliol, cirsimaritin, and hispidulin and is showed to be effective against *Cladosporium sphaerospermum* (Alcerito et al. 2002). Galeotti et al. (2008) studied the antifungal activity of kaempferol 3-O- β -d-glucopyranosyl(1-2)-O- β -d-glucopyranosyl(1-2)-O-[α -1-rhamnopyranosyl-(1-6)]- β -d-glucopyranoside isolated from *Dianthus caryophyllus* (carnation, a member of Caryophyllaceae family) against *Fusarium oxysporum* f. sp. *dianthi* (Galeotti et al. 2008). *Fusarium culmorum*, a serous pathogen of seedling blight, foot rot, ear blight, stalk rot, and common rot of cereals and grasses, is found to be inhibited by six commercial coumarins: bergapten, herniarin, umbelliferone, xanthotoxin, and scopoletin. *Tithonia diversifolia*, the source of tithoniamarin, is effective against the anther smut fungus *Microbotryum violaceum*, earlier known as *Ustilago violacea* (Yemele-Bouberte et al. 2006). Berberine and jatrorrhizine (alkaloids) are isolated from *Mahonia aquifolium* (a plant of Berberidaceae family commonly called as Oregon grape and native to western North America) and are effective against human pathogenic *Candida* species. Pathogens of mango (*C. gloeosporioides*), anthracnose of lupin species, postbloom fruit drop of citrus, Valencia and navel oranges in Florida (caused by *C. acutatum*), and strawberry (caused by *Colletotrichum fragariae*) are inhibited by findersine, anhydroevoxine, and haplamine (Cantrell et al. 2005). Roots of *Cyathobasis fruticulosa* are source of beta-carboline, tryptamine, and phenylethylamine-derived alkaloids and are antifungal in nature (Bahceevli et al. 2005).

9.8.2 Essential Oil

Essential oils (EOs) of aromatic and medicinal plant origin are reported to possess antifungal properties and are of wide spectrum in their application for the control of agricultural pathogen (Table 9.13). EOs are mainly categorized under the plants' secondary metabolites and may fall under the category of terpenes, ketones, esters, aromatic phenols, ethers, alcohols, oxides, etc. (Fig. 9.7). They act by inhibiting the fungal hyphal growth either by accumulating in the fungal cell membrane or by

Table 9.13 Common sources of popular essential oils with antifungal property

Sl. no.	Name of the plant	Family
1	<i>Anethum graveolens</i>	Apiaceae
2	<i>Aniba rosaedora</i>	Lauraceae
3	<i>Artemisia absinthium</i>	Asteraceae
4	<i>Boswellia thurifera</i>	Buseraceae
5	<i>Brassica nigra</i>	Brassicaceae
6	<i>Bunium persicum</i>	Apiaceae
7	<i>Cestrum nocturnum</i>	Solanaceae
8	<i>Calocedrus macrolepis</i> var. <i>formosana</i>	Cupressaceae
9	<i>Cananga odorata</i>	Annonaceae
10	<i>Carum carvi</i>	Apiaceae
11	<i>Cedrus deodara</i>	Pinaceae
12	<i>Chenopodium ambrosioides</i>	Amaranthaceae
13	<i>Cinnamomum jensenianum</i>	Lauraceae
14	<i>Cinnamomum zeylanicum</i>	Lauraceae
15	<i>Cicuta virosa</i>	Apiaceae
16	<i>Citrus limon</i>	Rutaceae
17	<i>Cuminum cyminum</i>	Apiaceae
18	<i>Cymbopogon citratus</i>	Poaceae
19	<i>Cymbopogon martini</i>	Poaceae
20	<i>Cymbopogon winterianus</i>	Poaceae
21	<i>Daucus carota</i>	Apiaceae
22	<i>Echinophora spinosa</i>	Apiaceae
23	<i>Eucalyptus citriodora</i>	Myrtaceae
24	<i>Eugenia caryophyllata</i>	Myrtaceae
25	<i>Foeniculum sativum</i>	Apiaceae
26	<i>Foeniculum vulgare</i>	Apiaceae
27	<i>Helichrysum arenarium</i>	Asteraceae
28	<i>Hypericum perforatum</i>	Hypericaceae
29	<i>Illicium verum</i>	Liliaceae
30	<i>Juniperus excelsa</i>	Cupressaceae
31	<i>Laurus nobilis</i>	Lauraceae
32	<i>Lavandula intermedia</i>	Lamiaceae
33	<i>Lavandula officinalis</i>	Lamiaceae
34	<i>Lippia rugosa</i>	Verbenaceae
35	<i>M. albanica</i>	Lamiaceae
36	<i>M. thymifolia</i>	Lamiaceae
37	<i>Majorana hortensis</i>	Lamiaceae
38	<i>Matricaria chamomilla</i>	Asteraceae
39	<i>Mentha arvensis</i>	Lamiaceae
40	<i>Mentha piperita</i>	Lamiaceae
41	<i>Mentha spicata</i>	Lamiaceae
42	<i>Micromeria dalmatica</i>	Lamiaceae
43	<i>Monarda</i> spp.	Lamiaceae
44	<i>Myrrhis odorata</i>	Apiaceae

(continued)

Table 9.13 (continued)

Sl. no.	Name of the plant	Family
45	<i>Nepeta rtanjensis</i>	Lamiaceae
46	<i>Ocimum basilicum</i>	Lamiaceae
47	<i>Ocimum majorana</i>	Lamiaceae
48	<i>Ocimum sanctum</i>	Lamiaceae
49	<i>Origanum vulgare</i>	Lamiaceae
50	<i>Origanum onites</i>	Lamiaceae
51	<i>Portenschlagiella ramosissima</i>	Apiaceae
52	<i>Rosmarinus officinalis</i>	Lamiaceae
53	<i>Seseli annuum</i>	Apiaceae
54	<i>Seseli globiferum</i>	Apiaceae
55	<i>Seseli montanum</i> subsp. <i>tommasinii</i>	Apiaceae
56	<i>Seseli rigidum</i>	Apiaceae
57	<i>Seseli scardica</i>	Apiaceae
58	<i>Salvia brachyodon</i>	Lamiaceae
59	<i>Salvia fruticose</i>	Lamiaceae
60	<i>Salvia officinalis</i>	Lamiaceae
61	<i>Salvia pomifera</i> subsp. <i>Calycina</i>	Lamiaceae
62	<i>Salvia sclarea</i>	Lamiaceae
63	<i>Sardinian desoleana</i>	Lamiaceae
64	<i>Sassafras albidum</i>	Lauraceae
65	<i>Satureja hortensis</i>	Lamiaceae
66	<i>Satureja montana</i>	Lamiaceae
67	<i>Satureja thymbra</i>	Lamiaceae
68	<i>Seseli montanum</i> subsp. <i>tommasinii</i>	Apiaceae
69	<i>Syzygium aromaticum</i>	Myrtaceae
70	<i>Tagetes patula</i>	Asteraceae
71	<i>Thymbra spicata</i>	Lamiaceae
72	<i>Thymus pulegioides</i>	Lamiaceae
73	<i>Thymus vulgaris</i>	Lamiaceae
74	<i>Trachyspermum ammi</i>	Apiaceae
75	<i>Zataria multiflora</i>	Lamiaceae

crossing the cell membrane and entering into the eukaryotic cell. Being lipophilic in their chemical nature, they can easily cross the cell and interrupt in sterol biosynthesis leading to growth retardation and finally cell death. As sterols are the maintenance, compounds of cellular integrity treatment with EO cause fungal cell death. Metabolic processes like respiration, replication, transcription, and translation are inhibited. Membrane permeability is drastically changed as they cause swelling and disruption of protein–lipid–protein membrane. Leakage of useful ions like Ca^{2+} and K^+ causes cell death. Thymol, carvacrol, eugenol, and related phenolic compounds cause H^+ and K^+ leakage and water imbalance and deplete intracellular high-energy molecule (ATP). Essential oils are extracted from almost every parts of a plant, for example, roots, fruits, barks, twigs, leaves, seeds, and flowers, by several extraction procedures that include hydro and steam distillation, cold pressing, and

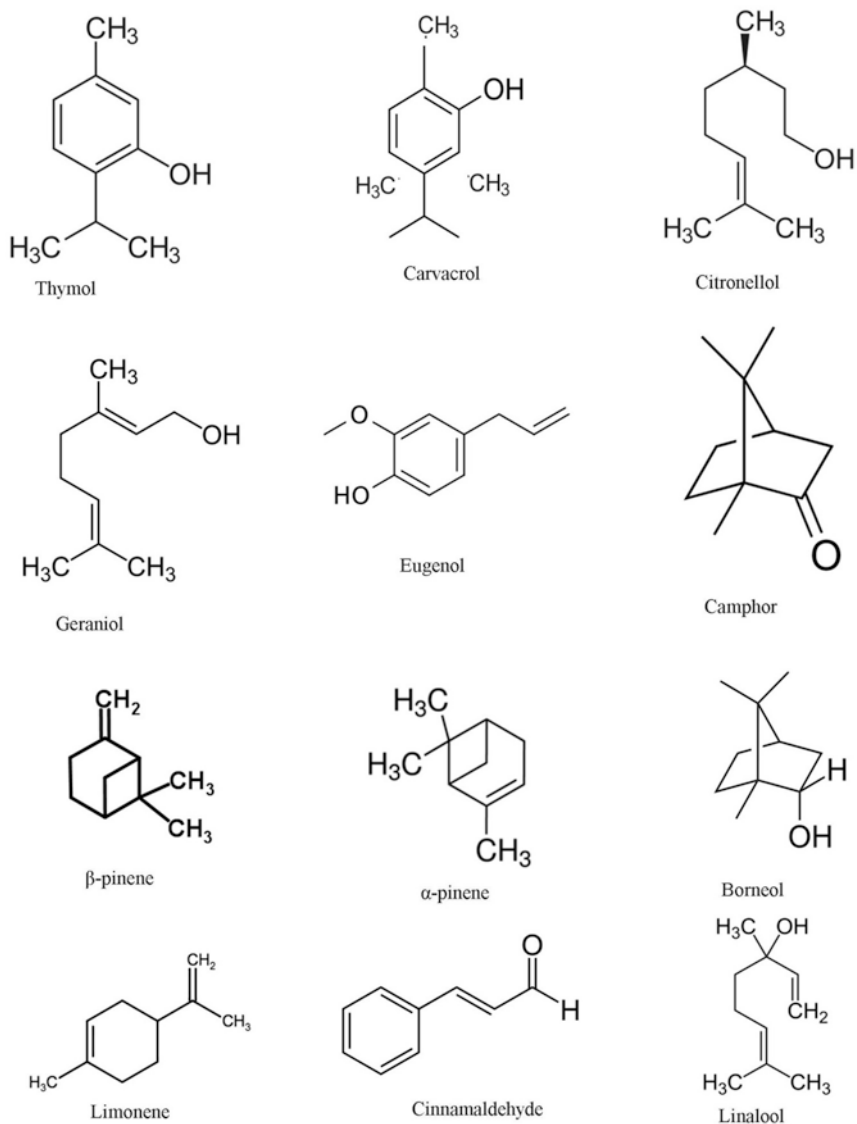


Fig. 9.7 Common ingredients of essential oils of antifungal importance

fermentation. The antifungal efficacy is checked by direct contact of the essential oil components and fungal hypha and poison food method, following micro or broth dilution techniques, or in vivo fumigation assay is also performed in case of field trials.

Essential oils from leaves of *Chenopodium ambrosioides*, a member of Amaranthaceae family, are effective against storage fungi *Aspergillus flavus*, *A. glaucus*, *A. niger*, *A. oryzae*, *Colletotrichum gloeosporioides*, *C. musae*, *Fusarium oxysporum*, and *Fusarium semitectum* (Jardim et al. 2008). Lemongrass oil from *Cymbopogon citratus* and *Cymbopogon martini* are potent inhibitors of *Botrytis cinerea*, *Rhizoctonia solani*, *Aspergillus tamari*, *A. fumigatus*, and *A. conicus* (Tzortzakis and Economakis 2007; Mishra et al. 2015). The members of Lamiaceae family are well known for their pungent odor and are tested for their antifungal activity by agar and broth dilution methods (Roby et al. 2013; Omidbeygi et al. 2007). Essential oils extracted from *Laurus nobilis*, *Syzygium aromaticum*, and *Origanum vulgare* are effective antifungal compounds against two pathogens of rice, *Fusarium culmorum* and *Fusarium verticillioides* (Rosello et al. 2015). Essential oils from *Cymbopogon* exhibited antifungal activities against rot molds (Soundharrajan et al. 2003). Antifungal activities of peppermint and sweet basil were tested against plant pathogenic fungi *S. sclerotiorum*, *Rhizopus stolonifer*, and *Mucor* sp. (Edris and Farrag 2003). Antifungal activity of β -dolabrin, γ -thujaplicin, and 4-acetyltropolone was tested against *Pythium aphanidermatum* IFO 32440 (Morita et al. 2004). Boyraz and Ozcan (2006) tested the antifungal activity of the essential oils isolated from wild Turkish summer savory (*Satureja hortensis*). Essential oils (carvacrol, thymol, p-cymene) extracted from *Origanum acutidens* are effective against phytopathogens. Growth of *A. humicola*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, and *Phytophthora cactorum* was inhibited by the essential oil of *Asarum heterotropoides* var. *mandshuricum* (Dan et al. 2010). Though there are several reports of essential oils being potent anti-phytopathogenic (*Penicillium purpurogenum*, *Rhizopus stolonifer*, *Spondylocadium austral*, *Penicillium digitatum*, *Penicillium luteum*, *Monilinia laxa*, *Curvularia lunata*, etc.) in nature, still there are some problems regarding their maximum use and optimum effectivity. That includes their volatile natures, requirement of close systems, and degradation of EOs by oxidation due to presence of extreme amount of hydrogenated compounds (Kim et al. 2003).

9.9 Conclusion and Future Prospects

We are nourished by Mother Nature. So it is our prime duty to keep up the normal equilibrium of natural parameters. But in a way to seek solutions, some steps taken toward success may have negative impact on our environment. To fight against the fungal pathogens for the ensuring of better crop productivity, use of chemical fungicide is just another example of that fact. But we must emphasize on products from direct natural origin over the chemically synthesized one. Natural products are the best weapon to fight fungal pathogenic diseases on economically important crop species. They are less toxic, stable, and of no side effects when used in crop fields. The crying need of modern era is obtaining pathogen-free crop species in one hand and assurance of environmental sustainability on the other. Fungal and bacterial products are already used in large scales followed by the plants' secondary

metabolites. Phytoalexins as internal molecules are the plants' own defense system. The detailed biochemical analysis of the phytoalexins and study of their regulatory mechanisms are opening up new horizons for universal use of phytoalexin inducing elicitors as plant defense enhancers. Mycorrhizae provide the basic line of physical barrier against pathogenic invasion, and reports include their ability to enhance plant growth, thus making the plant nonsusceptible to fungal attack. Endophyte on the other hand can enhance the plants' defense system by direct incorporation and open up popular angles of green immunization or plant vaccination. Researches on these fields are still scanty, but in the near future, they could lead to the ultimate solution of fungal pathogenic crop loss.

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