CHAPTER 2

REVIEW OF LITERATURE

India is the second largest vegetable producer in the world with an annual production of 81 million tonnes from 5.1 million hectares of land [59]. During the last two decades considerable emphasis has been laid on increasing production of vegetable crops in India [60]. The major factor responsible for low production of solanaceous vegetables remains the diseases caused by phytopathogens. The disease development is so fast that whole crop is lost in a few days. Therefore, the problem deserves immediate and effective measures of control [61]. Lycopersicum sculentum is an important vegetable crop worldwide. Important fungal diseases limiting tomato production are early blight caused by Alternaria solani, late blight caused by Phytophthora infestans, Fusarium wilt caused by F. oxysporum fsp. lycopersici, verticilium wilt caused by Verticilium dahlea and septoria leaf spot caused by Septoria lycopersici [62].

2.1 Losses in Agriculture Due to Phytopathogens

Pathogenic fungi alone cause nearly 20 % reductions in the yield of major food and cash crops [63]. Among the pathogenic fungi causing root rots, Fusarium spp., Alternaria solani and Rhizoctonia solani are the major pathogens. These fungi are widely distributed in soil and can infect more than 400 host plants including cereals, pulses and fruit crops [64]. Poly and Srikanta (2012) [65] reported yield losses in tomato due to A. solani ranging from 0.75 to 0.77 t/ha for every 1% increase in disease severity for the two consecutive years in Gangetic plains of West Bengal. Based on evaluations on four genotypes of tomato including Patharkuchi (susceptible), Himsona (tolerant), Ajeet 11 and Arka Bikas for two consecutive years concluded that fruit loss due to Alternaria solani was pronounced in Ajeet-11
and Patharkuchi. The different species of *Alternaria* spp. also cause black spot or leaf spot disease in *Brassica* vegetables and solanaceae crops [66]. The damaged seeds usually show presence of fungus both internally and externally. The estimates of yield losses due to this disease vary between 35 and 70%. The yield losses due to infection have been reported to be in the range of 15-36% [67].

*Fusarium* spp. the main soil borne plant pathogens cause reductions in agricultural production all over the world [68, 69]. *Fusarium* spp., found worldwide in cereals and other food types for human and animal consumption are the most important toxigenic fungi. Several species of *Fusarium* cause essentially similar symptoms on different crops such as cortical decay of roots [70], root rot [71], wilting [72], yellowing [73], rosette [74] and premature death on infected plants [75]. Stem rot caused by *F. oxysporum* f.sp. *vanilla* has been known as one of important constraints for the vanilla cultivation and responsible for the decreasing of vanilla production [76-79]. Sudana et al. (1999) [80] reported death of 10 million banana plants because of wilt disease caused by *F. oxysporum* f.sp. *cubense*.

Late blight disease, caused by *Phytophthora infestans*, is one of the most serious threats to the tomato production worldwide [81]. The foliage and stem of the tomato can be killed by *P. infestans* which spreads through airborne asexual sporangia during the growing season [82]. The late blight also causes fruit rot either in the field or post harvest [83]. *Phytophthora palmivora* the cause of black pod disease on cocoa has emerged as an important obstacle in cocoa production [84]. *Rhizoctonia solani* is ubiquitous and cosmopolitan soil saprophyte and an opportunistic plant pathogen infecting over 500 host species [85]. High yield losses, up to 50% for sugar beet, up to 70% for field-grown lettuce, and about 20% for potato were reported [86, 87]. Over the last 15 years, the significance of *R. solani* has increased as an important causal organism of diseases such as black scurf on potato, late sugar beet rot, bottom rot on lettuce and damping-off diseases on various vegetable crops [88]. *Rhizoctonia solani* has a wide host range infecting more than 27 families in both monocots and dicots [89, 90].
Diseases caused by *Pythium* spp. are often considered as seedling diseases such as damping-off [91] however mature plants may also be infected [92]. Pathogenic species have been isolated from healthy looking roots, where their colonization causes a reduction in plant growth but not typical root rot symptom [93]. *Pythium* complex is also the cause for cavity spot, one of the most important soilborne diseases of carrot worldwide, characterized by the presence of sunken brown elliptical lesions on the surface of the tap root [94].

### 2.2 Use of Chemical Fungicides in Crop Improvement

The agrochemical industry with discoveries of the major classes of synthetic pesticides including organochlorines, organophosphates and carbamates provided agriculture with a vast array of crop protection chemicals such as fungicides, insecticides, nematicides, and herbicides [95]. Large numbers of potent fungicides possessing novel structure and mostly with systemic activity not found in the earlier products were introduced in the late 1960s and 1970s [96]. These include 2-amino-pyrimidines, benzimidazoles, carboxanilides, phosphorothiolates, morpholines, dicarboximides, phenylamides, and sterol demethylation inhibitors (DMIs) [97]. Introductions in the 1980s mainly were analogues of existing fungicides, particularly DMIs, with generally similar though sometimes improved properties. Over the past two decades, however, a number of novel compounds have been introduced commercially or have reached an advanced stage of development, including phenylpyrroles, anilinopyrimidines, quinone inhibitors, benzamides and carboxylic acid amides [98-100]. With the availability of many new fungicides in the market that provide very good control of many diseases, the use of foliar fungicides on crops has increased many fold [101].

### 2.3 Resistance to Chemical Fungicides

Resistance to fungicides in fungal pathogens is an important cause for poor disease control [102]. The development of fungicide resistance is influenced by complex interactions of factors such as the mode of action of the fungicide, the
biology of the pathogen, fungicide use pattern, and the cropping system. Various pathogens like, *Alternaria solani* [103], *Fusarium oxysporum*, *Colletotrichum capsici*, *Pythium* sp. and *Phytophthora* sp. *Gloeosporium amelophagum* [104, 105] have been reported to develop resistance against commonly used fungicides. Fungicide resistance problems in the field have been documented for more than 100 diseases and crop-pathogen combinations, and within about half of the known fungicide groups. Although many fungicides are marketed, any one major crop disease typically is well controlled by only three or four different types of fungicides, so that any fall in effectiveness of a previously reliable fungicide through resistance development can be a very serious matter for the grower [106].

### 2.4 Impact of Chemical Fungicides on Environment

Fungicides, sporicides or biocides have long been used to control, prevent and remediate fungal growth. Whilst a farmer’s objective is to apply fungicides to the agricultural crop/plant, unavoidably a proportion of the chemical spray will miss its target. Much of the lost chemical enters the soil surface where it will persist for a period of time and potentially migrate off-site due to leaching/or runoff. In addition, some of the agrochemicals applied on farm will migrate off-site due to aerial drift. Once agrochemicals have migrated off-site they can potentially enter nearby waterways and groundwater resources where they can cause adverse effects to aquatic organisms [107]. The fate and behaviour of agrochemicals in the environment is influenced by the properties of the chemical for e.g. ability to bind to soil, susceptibility to degradation and environmental factors such as soil type, rainfall, topography, agricultural management practices. The presence and persistence of fungicides in agricultural soils can cause adverse effects to soil organisms, such as biota, and the crucial functions these organisms are responsible for including the breakdown of organic matter, facilitating nutrient cycling. Thus, any negative impact caused by fungicide residues can have lasting impacts on the fertility of agricultural soils [108].
Currently public concern about the impact of pesticides, including fungicides, on human health is greater than ever before. Pesticides are essential in agricultural production, but they constitute a potential risk to humans who are exposed to them directly through various ways and indirectly through diet. Fungicide contaminants in the environment can enter the human body via the food chain and may cause adverse effects on human health [109]. The residues of these fungicides, have been recorded on harvested produce causing hazards to humans, animals and environment [110, 111].

2.5 Need for Botanical Fungicides

Owing to increase in public interest towards reduction of use of synthetic fungicides in agriculture, the use of botanical fungicides has emerged as the alternative choice. Plants possess inherent mechanisms for combating fungal infections. Plants produce numerous chemicals for defence and communication, and can elicit their own form of offensive chemical warfare for targeting the proliferation of pathogens [112, 113]. These are secondary metabolites and have general or specific activity against key target sites in bacteria, fungi and viruses. The metabolites can be extracted and used as sustainable alternative to the use of chemical fungicides [114]. According to Arif et al. (2002) [115] such products from higher plants have relatively broad spectrum of action and bio-efficacy, are non-toxic, systemic and biodegradable and hence environmentally safe [116-119]. Plant-derived compounds are regarded as novel leads for development of fungicides [120].

Phytotoxicity results when a substance or mixture of substances are sprayed or dusted onto plants and those plants suffer negative effects afterward. The effects can include death, abnormal growth, or discoloration of plants. Most botanical products are not phytotoxic, since these products are secreted by plants themselves as defence response to infection [121]. In addition plants possess a range of active defense mechanism that can be actively expressed in response to biotic stresses of various scales.
The mechanism of disease suppression by plant products have suggested that the active principles present in them may either act on pathogen directly or induce systemic resistance in host plants resulting in reduction of disease [122]. Botanicals are plant-derived materials generally short-lived in the environment, as they are broken down rapidly in the presence of light and air, hence minimise residual toxicity [123].

2.6 Use of Botanicals Against Phytopathogens

Use of plant products for the control of plant diseases is gaining importance [124]. Plant extracts have assumed special significance in the present day strategy of developing ecologically safe method of plant disease management [125, 126]. Antifungal activities of plant extracts have been reported by many workers. Table 2.1 summarizes the activities of various organic extracts of plants against fungal phytopathogens [127-132]. Among the higher plants, medicinal plants and their products have been shown to possess good efficacy against fungi, bacteria and viruses [133-136].
Table 2.1. Antimicrobial activity of botanical extracts against phytopathogens

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the plant</th>
<th>Plant Part Used</th>
<th>Extract</th>
<th>Target Phytopathogen (s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Acacia Arabica</em></td>
<td>Bark</td>
<td>Aqueous</td>
<td><em>Xanthomonas campestris</em></td>
<td>[137]</td>
</tr>
<tr>
<td>2.</td>
<td><em>Acacia nilotica</em></td>
<td>Leaves, Seeds and Fruits</td>
<td>Aqueous</td>
<td><em>Aspergillus spp.</em></td>
<td>[138]</td>
</tr>
<tr>
<td>3.</td>
<td><em>Allium sativum</em></td>
<td>Bulbs and Leaves</td>
<td>Ethanol</td>
<td><em>Curvularia lunata</em></td>
<td>[139]</td>
</tr>
<tr>
<td>4.</td>
<td><em>Aspilia Africana</em></td>
<td>Leaves</td>
<td>Aqueous</td>
<td><em>Cercospora</em> leaf spot of <em>Sesamum indicum</em></td>
<td>[140]</td>
</tr>
<tr>
<td>5.</td>
<td><em>Azadirachta indica</em></td>
<td>Seeds</td>
<td>Ethanol</td>
<td><em>Fusarium oxysporum</em> Alternaria alternata</td>
<td>[141]</td>
</tr>
<tr>
<td>6.</td>
<td><em>Cinnamomum cassia</em></td>
<td>Aerial parts</td>
<td>Methanol extract</td>
<td><em>Fusarium moniliforme</em> Phyllosticta caricae</td>
<td>[142]</td>
</tr>
<tr>
<td>7.</td>
<td><em>Curcuma longa</em></td>
<td>Rhizome</td>
<td>Acetone extract</td>
<td>Phyllosticta caricae</td>
<td>[142]</td>
</tr>
<tr>
<td>8.</td>
<td><em>Datura metel</em></td>
<td>Leaves</td>
<td>Methanol : Aqueous (1:2)</td>
<td><em>Fusarium oxysporum</em> f. sp. cubense</td>
<td>[143]</td>
</tr>
<tr>
<td>9.</td>
<td><em>Eucalyptus globules</em></td>
<td>Leaves</td>
<td>Aqueous extract</td>
<td><em>Cercospora moricola</em> Cooke (leaf spot of Mulberry)</td>
<td>[144]</td>
</tr>
<tr>
<td>10.</td>
<td><em>Inula viscosa</em></td>
<td>Leaves</td>
<td>Acetone and Hexane (1:10)</td>
<td>*Pseudoperonospora cubensis Phytophthora infestans Blumeria graminis Puccinia helianthi</td>
<td>[145]</td>
</tr>
<tr>
<td></td>
<td>Plant Name</td>
<td>Part</td>
<td>Extract Type</td>
<td>Pathogen Name</td>
<td>Reference</td>
</tr>
<tr>
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<tr>
<td>11</td>
<td><em>Lippia scaberimma</em></td>
<td>Aerial part</td>
<td>Essential oil</td>
<td><em>Botryosphaeria parva</em>&lt;br&gt;<em>Colletotrichum gleosporioides</em></td>
<td>[146]</td>
</tr>
<tr>
<td>12</td>
<td><em>Masea lanceolata var. goukangensis</em></td>
<td>Stem Bark</td>
<td>Methanolic extract</td>
<td><em>Phytophthora cryptogea</em>&lt;br&gt;<em>Trichoderma virens</em>&lt;br&gt;<em>Aspergillus niger</em>&lt;br&gt;<em>Phoma sp.</em>&lt;br&gt;<em>Cochliobolus heterostrophus</em>&lt;br&gt;<em>Fusarium oxysporum</em>&lt;br&gt;<em>Sclerotium rolfsii</em>&lt;br&gt;<em>Pyrenophora teres</em></td>
<td>[147]</td>
</tr>
<tr>
<td>13</td>
<td><em>Mentha spicata</em> L.</td>
<td>Aerial parts</td>
<td>Essential oil</td>
<td><em>Fusarium oxysporum f. sp. radicis cucumerinum</em></td>
<td>[148]</td>
</tr>
<tr>
<td>14</td>
<td><em>Metaseuioia glyptostroboides</em></td>
<td>Leaves</td>
<td>Essential oil</td>
<td><em>Fusarium oxysporum</em>&lt;br&gt;<em>Fusarium solani</em>&lt;br&gt;<em>Phytophthora capsici</em>&lt;br&gt;<em>Colletotrichum capsici</em>&lt;br&gt;<em>Sclerotinia sclerotiorum</em>&lt;br&gt;<em>Botrytis cinerea</em>&lt;br&gt;<em>Rhizoctonia solani</em></td>
<td>[149]</td>
</tr>
<tr>
<td>15</td>
<td><em>Origanum syriacum var bevanii</em></td>
<td>Leaves</td>
<td>Essential oil</td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>[150]</td>
</tr>
<tr>
<td>No.</td>
<td>Plant</td>
<td>Part</td>
<td>Extract Type</td>
<td>Fungi</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td>16.</td>
<td><em>Solanum xanthocarpum</em> and <em>Datura metel</em></td>
<td>Leaves</td>
<td>Methanolic extract</td>
<td><em>Aspergillus fumigatus</em>&lt;br&gt;<em>A. flavus</em>&lt;br&gt;<em>A. niger</em></td>
<td>[151]</td>
</tr>
<tr>
<td>17.</td>
<td><em>Spilanthes acmella</em></td>
<td>Flower head</td>
<td>Petroleum ether extract</td>
<td><em>Fusarium oxysporum</em>&lt;br&gt;<em>F. moniliiforme</em>&lt;br&gt;<em>Aspergillus niger</em>&lt;br&gt;<em>A. parasiticus</em></td>
<td>[152]</td>
</tr>
<tr>
<td>18.</td>
<td><em>Xylopia aethiopica</em></td>
<td>Seeds</td>
<td>Aqueous extract</td>
<td><em>A. niger</em>,&lt;br&gt;<em>A. flavus</em>&lt;br&gt;<em>F. oxysporum</em></td>
<td>[153]</td>
</tr>
<tr>
<td>19.</td>
<td><em>Zingiber officinale</em></td>
<td>Aerial parts</td>
<td>Methanol extract</td>
<td><em>Fusarium oxysporum</em>,&lt;br&gt;<em>Pythium aphanidermatum</em>&lt;br&gt;<em>Rhizoctonia solani</em></td>
<td>[154]</td>
</tr>
<tr>
<td>20.</td>
<td><em>Zizyphus jujube</em></td>
<td>Leaves</td>
<td>Methanol extracts</td>
<td><em>Rhizoctonia solani</em></td>
<td>[155]</td>
</tr>
</tbody>
</table>
Ravikumar et al. (2013) [156] evaluated aqueous extracts of 39 plants for antifungal potential against *Alternaria solani*. Among the various extracts, showed significant growth in hibition of *Crotalaria trichotoma*, *Azadirachta indica*, *Capsicum annum*, *Datura metel*, *Polyalthia longifolia* and *Citrus aurantifolia*. Enikuomehin and Oyedeji (2010) [157] reported mycelial growth of *Macrophomina phaseolina* and *Fusarium verticillioides* by extracts of *Moringa oleifera*.

Satish et al. (2009) [158] screened aqueous extracts of 46 plants belonging to 32 different families for antifungal activity against eight species of *Fusarium* viz., *Fusarium equiseti*, *F. moniliforme*, *F. semitectum*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. lateritium*. Significant antifungal activity was recorded in the petroleum ether extract of leaves of *Polyalthia longifolia*. Rani and Murthy (2006) [152] evaluated the effectiveness of *Spilanthes acmells* against *Fusarium oxysporum*, *F. moniliforme*, *Aspergillus niger* and *A. parasiticus*. Among the test organisms, high inhibition zones were observed in *F. oxysporium* (2.3 cm) and *F. moniliforme* (2.1 cm) followed by *A. niger* (2.0) and *A. paraciticus* (1.8 cm). Effective control of *F. oxysporum* sub sp. *ciceris* was obtained by aqueous extract of *Allium sativum* [159]. Mishra and Tiwari (1990) [160] reported the toxicity of extracts from the leaves of *Calotropis procera*, *Azadirachta indica*, and *Datura stramonium* to *Pyricularia oryzae*, *Rhizoctonia solani*, *Curvularia lunata* and *Fusarium moniliforme*. Extracts of *Adhatoda vasica*, *Jatropha curcas*, *Sapindus emarginatus* and *Vitex negunda* showed similar inhibitory effects against *Fusarium oxysporum* f. sp. *Melongenae* [161].

Singh et al. (1980) [162] reported inhibition in growth of *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* in liquid medium by various extracts of leaf, trunk, bark, fruit pulp and oil of *Azadirachta indica*. Of these four extracts, neem oil showed maximum inhibitory effect. Sundarraj et al. (1996) [163] reported reduction in mycelial growth of *Rhizoctonia solani* in aqueous extract of *Prosopis juliflora* and *Allium sativum* which was on par with bavistin. Inhibition in the mycelial growth and spore germination in *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *tracheiphilum* were recorded by Shivpuri et al. (1997) [164] on treatment with extracts of *Abutilon indicum*, *Delonix regia* and *Acalypha indica*. 
Kurucheve et al. (1997) [165] screened 13 plant species in vitro for their fungitoxicity against Rhizoctonia solani. Out of these, maximum inhibition of mycelial growth was observed in cold water extract of Prosopis juliflora (75 %). They also reported that cold water extract could better inhibit the mycelial growth than air dried, hot water and autoclaved extracts. Thiribhuvanamala and Narasimhan (1998) [166] reported that the leaf extracts of Delonix regia, Pongamia glabra and Acacia nilotica significantly reduced the spore germination, mycelial growth and spore production of the seed borne pathogens of sunflower namely Alternaria helianthi, Macrophomina phaseolina and Fusarium solani. Hot water and oil extracts of Azadirachta indica and Xylopia aethiopica applied before or after infection of cowpea plants with Colletotrichum lindemuthianum were effective in reducing the size of the pathogen induced lesions [167].

Karade et al. (2001) [168] studied the inhibition of mycelial growth and spore germination in solvent extracts of 18 medicinal plants. Of the plants tested, the crude extract of Allium sativum cloves completely inhibited the mycelial growth and spore germination of Alternaria alternata, Fusarium udum and Sclerotium rolfsii. The minimum concentrations of A. sativum that completely inhibited mycelial growth of A. alternata, F. udum and Sclerotium rolfsii were 35, 25 and 35 % respectively and for the inhibition of spore germination of A. alternata and F. udum were 25 % each. Joy et al. (2004) [169] assessed the effect of cashew leaf, fruit and shell extracts on the growth of Phytophthora palmivora, Alternaria solani, Rhizoctonia bataticola, Sclerotium rolfsii, Pellicularia filamentosasa, Macrophomina phaseolina and Phomopsis vexans and reported significant inhibition of all the tested fungi.

Crude cold water extracts of seven spices, viz. cardamom, chilli, coriander, onion, garlic, ginger, and galangale showed significant antifungal activity against the three rosette pathogens i.e. Phoma exigua, Fusarium nygamai and Rhizoctonia solani using the poisoned food technique [170]. Acorus tatarinowii was inhibitory to hyphal growth of Thielaviopsis paradoxa, Pestalotia mangiferae, Fusarium oxysporum f. sp. niveum, Alternaria alternate, Colletotrichum musae, Sphaceloma fawcettii, and Mycosphaerella sentina [171].
Dellavalle et al. (2011) [172] reported that the extracts of *Salvia sclarea*, *S. officinalis* and *Rosmarinus officinalis* could be considered as potential sources of antifungal compounds for treating diseases caused by *Alternaria* spp. These extracts showed maximum activity, even at very low concentrations, and the same fungicide effects as chemical fungicide. Lee et al. (2007) [142] reported that the leaf extract of *Cinnamomum cassia* was active against *F. moniliforme* and *Phylosticta earicae* *Aspergillus niger*, *Botrytis cinerea* and *Glomerella cingulata*. Ethanol extract of *Aquapanthus africanus* was active against a wide variety of plant pathogens like *Pythium ultimum*, *Fusarium oxysporum*, *Alternaria alternata*, *Sporisorium cruentum*, *Sporisorium sorghi* and *Mylophthora pinodas* [173]. All fenugreek plant parts showed antifungal potential and the high magnitude of their inhibitory effects against *Botrytis cinerea*, *Fusarium graminearum*, *Alternaria spp.*, *Pythium aphanidermatum*, and *Rhizoctinia solani* [174]. Singh and Srivastava (2012) [175] reported that ethanol and acetone leaf extract of *Lantana camera* resulted in complete inhibition of *Alternaria alternata* isolated from *Solanum tuberosum* and *Lycopersicon esculentum*. Activity of methanol extract of *Verbascum thapsus* against *Fusarium graminearum* and *Macrophomina phaseolina* was studied by Vogt et al. (2010) [176].

Bhaskar et al. (2002) [177] studied the effect of five botanicals and two commercial products at different concentrations against *Rhizoctonia solani* causing dry corm disease of *Amorphophallus* and showed that the percent disease incidence was significantly reduced in botanical extract treatments. No disease incidence was observed for the treatments receiving neem leaf extract, *Pongamia glabra* leaf extract, *Annona squamosa* leaf extract, garlic bulb extract, neem seed kernel extract, Proneem and Neem Gold. Similar results were reported earlier by Lakshmanan et al. (1990) [178] in *Phaseolus aureus* with neem leaf and garlic bulb extracts against collar rot disease caused by *R. solani*. Gautam et al. (2003) [179] evaluated *in vitro* fungitoxic activity of twenty four botanicals against *Rhizoctonia solani* Kuhn. Out of these botanicals, *Xanthium strumarium* and *Blumea mobilis* were most effective.
2.7 Secondary Metabolites as Antifungal Agents

Plant secondary metabolites consist of low-molecular weight compounds that are regarded as non essential for sustaining life, but are crucial for the survival of the producing organism [180]. These compounds are frequently accumulated by plants in smaller quantities than primary metabolites [181, 182]. Plant secondary metabolites are synthesized in specific pathways and sites of production can vary between kinds of compounds as well as between plant species. Moreover, some molecules can be synthesized by all plant tissues, whereas others are produced in a specific tissue or even cell-specific fashion [119]. The most important building blocks employed in the biosynthesis of secondary metabolites are derived from acetyl coenzyme A, shikimic acid, mevalonic acid, and 1-deoxyxylulose-5-phosphate and, these are utilized respectively in the acetate, shikimate, mevalonate, and deoxyxylulose phosphate pathways [181, 183].

While some secondary metabolites, such as terpenoids, give plants their odours, many colored compounds such as quinines are responsible for pigmentation while others including phenols and phenolic acids serve in plant defense against predation by microorganisms, insects, and herbivores [184]. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection and are often found effective in vitro as antimicrobial substance against a wide array of microorganisms. Tannins are polymeric phenolic sub-stance possessing the astringent property. Coumarins are phenolic substances made of fused benzene and \( \alpha \)-pyrone rings [184]. Phenolic toxicity to microorganisms results from the position and number of hydroxyl groups present in the compound. Dubey et al. (1983) [185] stated that essential oil extracted from leaves of *Chenopodium ambrasioides* exhibited strong fungitoxicity against the mycelial growth of *Rhizoctonia solani* which causes damping off disease in *Phaseolus aureus*. They found 1000 ppm as the minimum inhibitory concentration of the fungitoxic constituent present in essential oil. Singh et al. (2001) [186] reported the effectiveness of essential oils of eucalyptus and garlic against plant pathogenic fungi such as *Phytophthora infestans, Rhizoctonia solani* and *Penicillium digitatum*. 
Tzortzakis and Economakis, (2006) [187] reported a dose dependent retardation of sporulation, spore germination and germ tube length in *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum* and *Rhizopus stolonifer* by Lemongrass (*Cympopogon citratus* L.) oil. Sharma and Kumar, (2009) [188] worked on the development of ecofriendly antifungal compounds for controlling plant diseases caused by *Fusarium oxysporum* and reported that different extracts of three weed plants, namely *Capparis decidua*, *Lantana camara* and *Tridax procumbens*, showed potential antifungal activity against mycelia growth and spore germination of *Fusarium oxysporum*. Their results revealed that the free flavonoids and sterols of *T. procumbens* (flower) and bound flavonoids of *C. decidua* (fruit and stem) totally inhibited spore germination of the fungi (100%). Lignans from *Myristica fragrans* efficiently suppress *A. alternata*, *B. cinerea*, *Colletotrichum coccodes*, *C. gloeosporioides*, *F. oxysporum*, *R. solani* and *M. oryzae* with IC$_{50}$ values ranging between 24 and 100 µg/mL [189].

The major mechanism of antifungal activity of saponins, triterpenoids, steroids or steroidal alkaloids is apparently due to their ability to complex with sterols in fungal membranes and to cause loss of membrane integrity [190-192]. Pezet and Pont (1990) [193] reported the activity of pterostilbene a phenolic compound present in grape berries of *Vitis vinifera* against dormant conidia of *B. cinerea*. Modifications of the endocellular membrane system causes the rapid destruction of endoplasmic reticulum, and of nuclear and mitochondrial membranes leading to complete cessation of respiration. The cytoplasm coagulated into numerous vacuoles and complete disorganization of mitochondria was recorded leading to disruption of the plasma membrane. Luo et al. (2002) [194] proposed that the monoterpene citral isolated from *Litsea cubeba* oil had a strong antifungal action against *Aspergillus flavus* by penetrating cell wall. It irreversibly damaged plasma membrane and it also inhibited spore germination. Kurita et al. (1979) [195] also reported that citral a terpenoid compound, forms charge transfer complexes with tryptophan, a good electron donor. The antifungal actions of the citral, was due to its capacity to form charge transfer complexes with electron donors as well as to its reactivity with SH groups.
The tetracyclic triterpenoids, cucurbitacins I and D are inhibitors of the induction of extracellular laccase formation of *Botrytis cinerea* [196]. Extracts of *Ecballium elaterium* or cucurbitacin I applied to cucumber fruits or plants or to cabbage leaves, prior to inoculation with *B. cinerea*, prevented infection of the tissue, the infecting fungus being restricted to the site of infection. The protective effect is due to the ability of cucurbitacin I to inhibit induction of laccase formation by *B. cinerea* [197, 198]. Examples of the secondary metabolites reported for with antifungal activity against phytopathogens are listed in Table 2.2.

### 2.8 Effect of Botanicals in the Control of spore germination and Mycelial growth of fungal phytopathogens

Extracts of leaves of *Thymus vulgaris* inhibited mycelial growth and cause degeneration of hyphae of *Pythium ultimum* and *Colletotrichum lindemuthianum* [214]. Lignans from *Myristica fragrans* efficiently suppressed *A. alternata, B. inerea, Colletotrichum coccodes, C. gloeosporioides, F. oxysporum, R. solani* and *M. oryzae* with IC$_{50}$ values ranging between 24 and 100 µg/mL [208]. Akhter et al. (2006) [215] reported that *Vinca rosea, Piper betel* and *Azadirachta indica* extracts have inhibitory (100%) effect against spore germination of *Bipolaris sorokiniana*.

Begum et al. (2010) [216] reported *Azadirachta indica* leaf 80% ethanolic extracts at 20% concentration inhibited the mycelial growth of *Fusarium oxysporium* f. sp. *capsici* (65.30%), *Rhizopus arthrospori* (89%) and *Alternaria tenuis* (77%). The inhibitions of spore germination against these pathogens were 87%, 100% and 82% respectively. Mohammedi and Atik (2013) [217] reported that methanolic extracts of *Haloxylon scoparium* (aerial part), *Arthrophytum schmittianunand* (aerial part), *Daphne gnidiun* (leaves) caused significant inhibition of mycelium growth and inhibition of spore germination inhibition.
Table 2.2. Activity of bioactive compounds against phytopathogens

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Plant</th>
<th>Plant Part Used</th>
<th>Extract</th>
<th>Bioactive compound (s) isolated</th>
<th>Pathogens</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Acorus gramineus</em></td>
<td>Rhizome</td>
<td>Hexane fraction</td>
<td>Asaraldehyde and α-asarone</td>
<td><em>Phytophthora infestans</em> <em>Rhizoctonia solani</em></td>
<td>[199]</td>
</tr>
<tr>
<td>2</td>
<td><em>Bulnesia sarmientoi</em></td>
<td>Aerial parts</td>
<td>Essential oil</td>
<td>Bulnesol, hanamyol</td>
<td><em>Fusarium solani</em> <em>Fusarium moniliforme</em></td>
<td>[200]</td>
</tr>
<tr>
<td>3</td>
<td><em>Calocedrus macrolepis var. formosana</em></td>
<td>Heart wood</td>
<td>Ethanol extract</td>
<td>β-Thujaplicin and γ-thujaplicin</td>
<td><em>Lenzites betulina</em> <em>Trametes ersicola</em> <em>Schizophyllum commune</em> <em>Pycnoporus coccineus</em> <em>Laetiporus sulphuratus</em> <em>Phaeolus schweinitzii</em> <em>Gloeophyllum trabeum</em></td>
<td>[201]</td>
</tr>
<tr>
<td>4</td>
<td><em>Catalpa ovate</em></td>
<td>Stem</td>
<td>Methanol extract</td>
<td>Dehydro-α-Lapachone</td>
<td><em>Botrytis cinerea</em>, <em>Colletotrichum spp.</em> <em>Magnaporthe oryzae</em> <em>Pythium ultimum</em></td>
<td>[202]</td>
</tr>
<tr>
<td>5</td>
<td><em>Celastrus hypoleucus</em></td>
<td>Root</td>
<td>Acetone extract</td>
<td>Pristimerin and cestrol</td>
<td><em>Glomerella cingulata</em>, <em>B. cinerea</em>, <em>R. solani</em></td>
<td>[203]</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Part(s)</td>
<td>Extract/Extractant</td>
<td>Active Constituent(s)</td>
<td>Fungi</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>6</td>
<td><em>Curcuma longa</em></td>
<td>Rhizome</td>
<td>Methanol extract</td>
<td>Demethoxycurcumin</td>
<td><em>Magnaporthe grisea</em></td>
<td>[204]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Phytophthora infestans</em></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Hypericum heterophyllum</em></td>
<td>Aerial parts</td>
<td>Essential oil</td>
<td>β-Caryophyllene oxide and α-terpineol</td>
<td><em>Fusarium</em> species</td>
<td>[205]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Rhizoctonia solani</em></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Laurus nobilis</em></td>
<td>Aerial parts</td>
<td>Essential oil</td>
<td>Linalool, terpineol acetate, methyl eugenol, linalyl acetate, eugenol, sabinene, β-pinene, α-terpineol.</td>
<td><em>Botrytis cinerea</em></td>
<td>[206]</td>
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<td></td>
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<td></td>
<td></td>
<td><em>Monilinia laxa</em></td>
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<td></td>
<td></td>
<td></td>
<td><em>Penicillium digitatum</em></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Malva sylvestris</em> L.</td>
<td>Stem</td>
<td>Acetone extract</td>
<td>Malvone A (2-methyl-3-methoxy-5,6-dihydroxy-1,4-naphthoquinone)</td>
<td><em>Verticillium dahliae</em></td>
<td>[207]</td>
</tr>
<tr>
<td>10</td>
<td><em>Myristica fragrans</em></td>
<td>Seeds</td>
<td>Methanol extract</td>
<td>Erythro-austrobailignan-6,meso-dihydroguaiaretic acid and nectandrin-B</td>
<td><em>Alternaria econdite</em></td>
<td>[208]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Botrytis cinerea</em></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Colletotrichum coccodes</em></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td><em>Colletotrichum gloeosporioides</em></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Fusarium oxysporum</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>R.solani</em></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Magnaporthe oryzae</em></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2. (Continued)

<table>
<thead>
<tr>
<th></th>
<th><strong>Phyllanthus amarus</strong></th>
<th>Whole plant</th>
<th>Methanol extract of the whole plant</th>
<th>Securinine and Allosecurinine</th>
<th>Alternaria spp. Heterosporium spp. Curvularia spp.</th>
<th>[209]</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td><em>Piper angustifolium</em></td>
<td>Leaf</td>
<td>Essential oil</td>
<td>Camphene</td>
<td>Trichophyton mentagrophytes Aspergillus flavus Aspergillus fumigates</td>
<td>[210]</td>
</tr>
<tr>
<td>13</td>
<td><em>Rhamnus triquetra</em></td>
<td>Bark</td>
<td>Emodin</td>
<td>Alternaria spp. Fusarium spp. Fomes annosus</td>
<td>[211]</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td><em>Thymbra spicata</em></td>
<td>Leaf</td>
<td>Petroleum ether extract</td>
<td>Thymol and carvacrol</td>
<td>Fusarium moniliforme Rhizoctonia solani Sclerotinia sclerotiorum</td>
<td>[212]</td>
</tr>
<tr>
<td>15</td>
<td><em>Wedelia biflora</em></td>
<td>leaves</td>
<td>Chloroform extract</td>
<td>3’-Formyl-2’-4’,6’-trihydroxydihydrochalcone</td>
<td>Phytophthora ultimum Rhizoctonia solani</td>
<td>[213]</td>
</tr>
</tbody>
</table>
Tzortzakis and Economakis (2007) [187] investigated the antifungal activity of lemongrass (*Cymbopogon citratus*) oil against *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum* and *Rhizopus stolonifer*. The results showed that fungal spore production was inhibited up to 70 to 100% at 25 to 500 ppm of lemongrass oil.

Investigation for antifungal activity of *Aloe vera* against postharvest fruit pathogens was undertaken by Saks and Barkai-Golan (1995) [218]. They assayed the antifungal activity of *A. vera* leaf pulp on four postharvest fruit pathogens *Penicillium digitatum, Penicillium expansum, Botrytis cinerea, and Alternaria alternata*. The spore survival of all tested fungi was reduced by 15 to 20% at 1 to $10^3$ µg/L of leaf pulp. The *P. digitatum* and *A. alternata* species were the most sensitive against *A. vera* leaf pulp [218].

*In vitro* experiments carried out by Camele et al. (2012) [219] indicated that *Penicillium italicum* did not show any mycelium growth in presence of thyme essential oils. Moreover the essential oils were very effective reducing the spore germination and mycelial growth of *Botrytis cinerea*. In vitro efficacy of different plant extracts viz. bitter guard, turmeric, garlic and black pepper was tested for control of *Fusarium udum, F. oxysporum f.sp. ciceri* causing wilt in pigeonpea and chickpea respectively. All the plant extracts showed considerable diminution in the growth of pathogens. Mycelial growth of *F. udum* has been reduced by 15% concentration of turmeric (89.2%) followed by garlic (88.26%) and black pepper (82.22%) [220]. Hassanein et al. (2008) [221] observed that 5%, 10%, 15% and 20% concentration of neem extract effectively suppressed the mycelial growth of *Alternaria solani* (causing early blight) and *Fusarium oxysporum f.sp. lycopersici* (causing wilt disease) in tomato. 20% concentration of ethanol and ethyl acetate extracts of neem totally inhibited the growth of *Alternaria solani*. At 100% aqueous neem leaf extract caused complete inhibition of spore germination of *Fusarium* spp. whereas the mycelial growth inhibition rate increased with increasing concentration [222].
Joseph et al. (2008) [223] tested different concentration i.e. 5%, 10%, 15% and 20% extract of different plants viz., Artemesia annua, Eucalyptus globulus, Ocimum sanctum and Rheum emodi and found significant reduction in growth of Fusarium solani f. sp. melongenae. Azadirchta indica was found most effective at 20% concentration followed by Rheum emodi, Eucalyptus globulus, Artemesia annua and Ocimum sanctum. Mycelial growth of various Fusarium species were inhibited by plant extracts of Adhatoda vasica, Azadirachta indica, Cinnamomum camphora and Ocimum sanctum [224], Agave Americana, Cassia nodosa [225], Azadirchta indica [226].

2.8.1 Antifungal Effect of Secondary Metabolites

The efficacy of osthol, a coumarin derivative isolated from dried fruits of Cnidium monnieri, in controlling powdery mildew was evaluated by Wang et al. (2009) [227]. The treatment showed significant reduction in the mildew leaf area in young pumpkin plants. Osthol also strongly inhibited spore germination and mycelial growth of Sphaerotheca fuliginea in vitro causing damage to the cell wall and the organelles of the pathogen. Singh et al. (1990) [228] demonstrated that ajoene a sulphur containing phenolic constituent of garlic Allium sativum exhibited 100% inhibition of spore germination of Alternaria solani, Fusarium oxysporum at a concentration of 100 µg/mL.

Three coumarins, imperatorin (furanocoumarin), 7-prenyloxy coumarin and aurapten were isolated from the Zosima absinthifolia seeds by Razavi et al. (2010) [229]. Imperatorin exhibited more fungitoxic activity than the other two compounds. An addition of 1 mg/mL of imperatorin to the PDA medium entirely inhibited the mycelial growth of Sclerotinia sclerotiorum. The same concentration of auraptene and 7-prenyloxycoumarin reduced the mycelia growth of S. sclerotiorum to 30 and 25% respectively.

Sesquiterpenoid components T-murolol and a-cadinol possess antifungal activities against a broad spectrum of plant pathogenic fungi. These two compounds strongly inhibited the growth of Rhizoctonia solani and Fusarium oxysporum, with
the IC$_{50}$ values less than 50 $\mu$g/mL. These compounds also efficiently inhibited the mycelial growth of *Colletotrichum gloeosporioides*, *Pestalotiopsis funerea*, *Ganoderma australe* and *F. solani* [230].

Crude alkaloid, phenol and tannins isolated from the flowers of *Ammi visinaga* completely inhibited the mycelial growth and spore germination of *Helminthosporium* sp. at a concentration of 0.04 mg/mL of the alkaloid and phenol whereas tannins failed to inhibit the growth of mycelium [231]. Caryophyllene oxide and $\alpha$-terpineol of *Hypericum hyssopifolium* and *H. heterophyllum* were inhibitory to the growth of *Fusarium* spp. and *Rhizoctonia solani* [205]. Asaraldehyde and $\alpha$-asarone derived from rhizome of *Acorus gramineus* were active against *Phytophthora infestans* and *R. solani* with mycelial inhibition values of 50-100% [199]. Singh et al. (2008) [209] reported complete inhibition of spore germination in *Alternaria* spp., *Heterosporium* spp. and *Curvularia* spp. by securinine and allosecurinine isolated from *Phyllanthus amarus*. Nor-securinine was fungicidal to *Helminthosporium frumentacei* [232]. Alkaloids neoeveratalines A and B (MICs about 200 $\mu$g/mL), veramitaline, stenophylline B, stenophylline B-3-O-$\beta$-D-glucopyranoside, veramiline-3-O-$\beta$-D-glucopyranoside, jervine, and jervine-3-O-$\beta$-D-glucopyranoside isolated from rhizomes of *Veratrum taliense* exhibited strong antifungal activity against *Phytophthora capsici* and *Rhizoctonia cerealis* [233].

Emodin, physcion, and rhein isolated from *Cassia tora* showed activity against *Botrytis cinerea*, *Blumeria graminis* f. sp. *hordei*, *Phytophthora infestans*, and *R. solani* with IC$_{50}$ values in a range of 46–375 $\mu$g/mL. [234]. Chrysophanol, parietin (physcion), and nepodin isolated from roots of *Rumex crispus* showed activity against *B. graminis* f. sp. *hordei* and synergistic activity against fungus *Sphaerotheca fuliginea* with IC$_{50}$ values of 4.7, 0.48 and 20 $\mu$g/mL, respectively [235, 236]. Ciryneol A, Ciryneol C and 1-heptadecene-11,13-diyn-8,9,10-triol from roots of *Cirsium japonicum* inhibited the mycelial growth of *B. cinerea*, *Colletotrichum* spp., *Magnaporthe oryzae* and *Pythium ultimum* at a concentration of 50 $\mu$g/mL [237].
Curcumin was fungicidal to *Phytophthora infestans*, *Puccinia recondita*, and *Rhizoctonia solani*. The antifungal activity of curcumin, demethoxycurcumin and bisdemethoxycurcumin against red pepper anthracnose in a range of 0.4–100 µg/mL [238]. Lignans erythro-austrobailignan-6, meso-dihydroguaiaretic acid and nectandrin-B from *Myristica fragrans* efficiently suppressed *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum coccodes*, *C. gloeosporioides*, *Fusarium oxysporum*, *Rizoctonia solani* and *Magnaporthe oryzae* with IC$_{50}$ from 24 to 100 µg/mL [189]. Four neolignans from *Magnolia obovata* such as magnolol, honokiol, 4-methoxynonokiol and obovatol showed potent mycelial growth inhibition with IC$_{50}$ from 7.1 to 95 µg/mL against *Alternaria alternata*, *C. coccodes*, *F. oxysporum*, *M. oryzae*, *Phytophthora capsici*, *Phytophthora infestans*, *Pythium ultimum* and *Rizoctonia solani* [239]. Hederagenin aglycone was reported to be active against *Rhizoctonia bataticola* and *Sclerotium rolfsii* [240, 241]. Quinonoid triterpenes pristimerin and celastrol from the roots of *Celastrus hypoleucus* inhibited the mycelial growth of *Glomerella cingulata*, *B. cinerea*, *R. solani* and *M. oryzae* [203].

Marcel and Alina (2011) [242] reported that bark extract of *B. vulgaris*, induced large-scale damage to *B. cinerea* conidia, causing large scale disappearance of the surface protuberances from the organism. Hydration and redrying caused these protuberances to disappear. Transmission electron microscopic observations revealed disruption of the conidial cell wall of *B. cinerea*, with an electron dense external layer shrunk and detached the plasmalemma and the cytoplasm and partial destruction of organelles and nucleus. Similar changes in the conidia of *B. cinerea* were observed in Berberine treatment caused as did *B. vulgaris* bark extract.

The antifungal activity of *Tagetes patula* extract was tested against *Pythium ultimum*. The extract proved to have a dose-dependent activity with a marked difference between treatments. SEM and TEM observations revealed the induction of alterations on fungal membranes with a photoactivation mechanism possibly involving the production of free radicals and leading to a premature aging of the mycelium [243]. Severe damage of the Colletotrichum sp. hyphae was observed in the form of large pores when incubated on 5 mg mL$^{-1}$ Ethanol extract of Propolis. The pores were clear within the hyphal matrix with raised outer edges. The pores were more severe and numerous in hyphae which were in direct contact with the agar [244].
The effects of osthol on morphology of *F. graminearum* were examined by transmission electron microscopy, 14C-labelling and enzyme activity detection. The results revealed that osthol could inhibit the hypha growth of *F. graminearum* by decreasing hyphal absorption of reducing sugar. The morphological observations showed cytoplasmic vacuolation and blurring of organelles and cell walls after treatment with osthol [245].

Lemon grass (*Cymbopogon citratus*) essential oil exhibited the strong antifungal effect followed by mentha (*Mentha piperita*) and eucalyptus (*Eucalyptus globulus*) essential oil. Scanning Electron Microscopy observation of *C. albicans* cells treated with lemon grass essential oil showed prominent shrinkage and partial degradation of the mycelia [246]. Leaf extracts *Eucalyptus* were found to inhibit mycelial growth of *Rhizoctonia solani*, *Phytophthora*, *F. oxysporum* and many other fungi in concentrations above 20% [247]. The leaf extract of *Clitoria ternatea* showed antifungal activity against *Aspergillus niger* at a minimum inhibitory concentration (0.8 mg/mL) and minimum fungicidal concentration (1.6 mg/mL). Further loss of cytoplasm in fungal hyphae, reduction in the thickness of hyphal wall leading to distortion and disruption of cell wall in addition to conidiophore alterations were observed [248].

**2.9 Coumarins as Antifungal Agents**

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine for thousands of years. Antifungal [249], anticancer, anti-HIV, anticoagulant, spasmolytic and antibacterial activities [250, 251] activities of coumarins are reported. These widespread simple phenolic compounds originate from the phenylpropanoid pathway, appear to function in different capacities in various plant defense mechanisms against insects, herbivores and fungi. They contribute essentially to the persistence of plants by being involved in processes such as defense against phytopathogens [252]. It is believed that these cyclic compounds behave as natural pesticidal defense compounds for plants and they represent a starting point for the exploration of new derivatives possessing a range of improved antifungal activity.
Some furanocoumarins are potent phytoalexins [253] and allele chemical compounds [254, 255]. Due to the structural relationship of pyranocoumarins with furanocoumarins, a role as phytoalexins may be assumed for them [252].

2.9.1 Classification of Coumarins

Coumarins are classified based on the substitution in benzene and pyrone rings are tabulated in Table 2.3 (Aoife and Richard, 2004) [256] and pathways in plants leading to synthesis of coumarins are shown in Figure 2.1.

**Table 2.3 Classification of Coumarins**

<table>
<thead>
<tr>
<th>Classifications</th>
<th>Features</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIMPLE COUMARINS</td>
<td>Hydroxylated, alkoxylated or alkylated on benzene ring</td>
<td></td>
</tr>
<tr>
<td>FURANOCOUMARINS</td>
<td>5-membered furan ring attached to benzene ring. Linear or Angular</td>
<td></td>
</tr>
<tr>
<td>PYRANOCOUMARINS</td>
<td>6-membered pyran ring attached to benzene ring Linear or Angular</td>
<td></td>
</tr>
<tr>
<td>PYRONE-SUBSTITUTED COUMARINS</td>
<td>Substitution on pyrone ring. Often at 3-C or 4-C positions</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.1 Pathways in plants leading to synthesis of coumarins [252]
Coumarins are found free or as heterosides in many dicotyledonous families, including Apiaceae, Asteraceae, Fabaceae, Moraceae, Rosaceae, Rubiaceae, Rutaceae and Solanaceae [257, 258]. Many monocotyledonous plants, especially the Gramineae and Orchidaceae, also contain large amounts of coumarins. Although mainly synthesised in the leaves, coumarins occur at the highest levels in the fruits, followed by the roots and stems. In addition, seasonal changes and environmental conditions may affect the occurrence in various parts of the plant.

The distribution of biologically active coumarins in a wide range of plants seems to correlate with their ability to act as phytoalexins, which are hydroxylated derivatives of coumarins. They are formed as a response to traumatic injury, during the wilting process, by plant diseases or through drying, they accumulate on the surface of the leaves, fruits and seeds, and they inhibit the growth and sporulation of fungal plant pathogens and act as repellents against beetles and other terrestrial invertebrates [257, 258]. Coumarins are leached from the roots of some plants, such as wild Avena, into the soil, where they provide a defence against hostile microorganisms. Coumarins are also active in plant metabolism, taking part in growth regulation phytoalexins are produced in carrots in response to fungal infection [257-259].

Scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, and pinoresinol isolated from the seed kernels of Melia azedarach enhanced activity against Fusarium verticillioides [260]. In sweet potato, major coumarins including umbelliferone and scopoletin, and the minor ones esculetin and the two β-D-glucosides such as scopolin and skimminn secreted at an early stage against the pathogen Ceratosystis fimbriata [261-264]. Several authors have been reported that scoparone, 6,7-dimethoxycoumarin is the main metabolite involved in inducing defense in Citrus against Phytophthora parasitica [265], Guignardia citricarpa [266], Penicillium digitatum [267] and Diaporthe citri [268].

Coumarins such as 1, 2-Benzopyrone, found in a variety of plants such as clover, sweet woodruff and grasses possess antifungal activity against Macrophomina phaseolina and Pythium spp. This halogenated coumarin derivative remained active for extended periods of time displaying 100% inhibition of fungal growth for more than 3 weeks in vitro [269].
Poli et al. (1995) [270], localized two different coumarin compounds Xanthotoxol and 4-methyl-umbelliferone in Smyrnium perfoliatum in various parts like leaves, stem, fruits, thin and tuberous roots. The highest concentration of these compounds reported in the roots was related to their role against microbial attack and inhibiting the germination of neighbouring plants [271]. Johann et al. 2007 [272] reported activity 6, 7-Dimethoxycoumarin isolated from the hexane extracts of Citrus limon peels against Penicillium digitatum, Colletotrichum sp. and Curvularia sp. The presence of 6, 7-dimethoxy-coumarin in citrus fruits after 6 days of storage was observed by [273]. These authors suggested that this compound is produced when fruits are either under stress conditions or at their senescent stage. Citrus aurantium, C. limon, C. paradise, C. sinensis, Poncirus trifoliata, and Troyer citrange showed gradual increase of 6,7-dimethoxy-coumarin content during the course of infection by Phytophthora citropahtora, indicating that this process is part of the plant’s response to invading pathogens [265].

Osthoh, 7-methoxy-8-[3-methylpent-2-enyl] coumarin, extracted from dried fruits of Cnidii monnieri showed a wide activity against Rhizoctonia solani, Colletotrichum musae and Phytophora capsici Botrytis cinerea, Sclerotinia sclerotiorum, and Fusarium graminearum [274]. Johri et al. 1992 [275] evaluated fungicidal activity of coumarins such as calophyllolide, byakangelicin, xanthotoxin methoxsalen and karanjin of plant origin to help prevent crop losses of Piper betel caused by Colletotrichum capsici and Phytophora palmivora. Methoxsalen showed activity similar to that of the synthetic fungicides Blitox and streptocycline.

Venugopal et al. (2013) [249] investigated, number of coumarins for their antifungal activity, and found psoralen, imperatorin and ostruthin to be most effective, Psoralen a fouranocoumarin, has displayed strong antifungal activity against plant pathogen fungi like Sclerotinia sclerotiorum, Alternaria brassicicola and Cercospora carotae [276]. Bergapten, 5-methoxy psoralen, was found to have antifungal effects against Alternaria brassicicola, Penicillium expansum and Cercospora petrosetini [276, 277]. Bergapten, coumarin, herniarin, umbelliferone, xanthotoxin and scopoletin evaluated by Ojala et al. (2000) [278] indicated retarded the growth of Botrytis cinerea due to Bergapten while scopoletin, umbelliferone, xanthotoxin, and herniarin exhibited strong inhibition of Fusarium culmorum.
Ayapin is the most potent antifungal coumarin against *Sclerotinia sclerotiorum*. In Sunflower, ayapin, scopoletin and scopolin were produced in response to pathogen attack in the plant bracts and corollas at high they were transported to leaf surface to avoid phytotoxicity and to induce resistance against fungal pathogens such as *Sclerotinia sclerotiorum* and *Puccinia graminis* [279]. Ag (I)-coumarin complex against pathogenic yeast *Candida albicans* have been observed and the results showed a lowered ergosterol content of the fungal cell walls and increased transmembrane leakage of aminoacids [280].

2.10 *Psoralea corylifolia* L

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Magnoliopsida  
Order : Fabales  
Family : Fabaceae  
Subfamily : Faboideae  
Tribe : Psoraleeae  
Genus : *Psoralea*  
Species : *corylifolia*  
Common Name : Bachi, latakasturi, sugandha, kantak, kala gija, bakhuchi

*Psoralea corylifolia*, (Fabaceae) is a medicinal plant (Plate 2.1a) widely distributed in India, China and Southeast Asian countries. The purple seed pods contain dark elongated seeds (Plate 2.1b). Seeds of *Psoralea*, which are harvested in the fall are large, solid, and black and they are pungent and bitter [281]. *Psoralea corylifolia* has traditionally been used for the treatment of leucoderma and other skin
diseases, pollakiuria, nephritis, asthma, osteoporosis, hypertension and cardiovascular diseases. The active fraction isolated from fruits, seeds and roots possesses antibacterial, antioxidative and immunomodulatory properties [282]. Seeds possess anthelmintic, diuretic, stomachic properties and are used for treatment of leprosy, febrile condition, skin diseases and scorpion or snake bites. Psoralen and isopsoralen isolated from *P. corylifolia* were anti cancerous [283].

Corylifolean, corylifolin, corylifolinin, bakuchicin, psoralidin, isopsoralidin, bavachin, isobavachin, bavachinin, bavachalcone, isobavachalcone, 7-o-methyl bavachin, bavachromanol, corylin, corylidin, corylinal, 4-o-methyl bavachalcone, neobavaisoflavone, bavachromene, neobavachalcone are flavanoids isolated from the seeds of the *P. corylifolia* [284]. Neobavaisoflavone (7-hydroxy-3[4-hydroxy-3(3-methylbut-2-enyl) phenyl]-chromen-4-one) belongs to the isoflavones, a subclass of the flavonoids was first isolated from the seeds of *P. corylifolia*. Neobavaisoflavone significantly inhibited the production of ROS, RNS, and cytokines: IL-1β, IL-6, IL-12p40, IL-12p70, TNF-α in activated RAW 264.7 macrophages, demonstrating the anti-inflammatory activity of this compound [282]. Isovabachalcone, 4'-o-methylbavachalcone, isobavachromene, corylifolin, and bavachinin have inhibitory potencies towards monophenolase activity of mushroom tyrosinase were investigated [285].

Bakuchiol a natural phenol and 3-hydroxybakuchiol a meroterpene were isolated from *Psoralea corylifolia*. Bakuchiol has shown activity against numerous Gram-positive and Gram-negative oral pathogens. It was able to inhibit the growth of *Streptococcus mutans* [286]. Bakuchiol has broad-spectrum antioxidant activity and effectively quenches superoxide-, hydroxy-, peroxyl-, peroxynitrite radicals and singlet oxygen non-radicals in addition to inhibiting lipid peroxidation, bakuchiol effectively reduce lipid peroxidation, protect mitochondria from NADPH-dependant and ascorbate-induced lipid peroxidation, and protect mitochondrial respiratory enzyme activities against both NADPH-dependant and dihydroxyfumarate-induced peroxidation injury. Squalene is particularly prone to photooxidation during sun exposure, and bakuchiol protects squalene and other skin lipids from oxidation due its excellent lipid peroxidation inhibitory activity [287].
The seeds contain an essential oil (0.05%), a nonvolatile terpenoid oil, a dark brown resin (8.6%), and traces of alkaloidal substance. The essential oil of *Psoralia corylifolia* contains limonene, α-elemene, γ-elemene, 4-terpineol, β-caryophylleneoxide, linalool and geranylacetate. Essential oil has a distinct stimulatory action on voluntary muscles in high dilutions. Resin acids along with glycerides of oleic, stearic, palmitic, myristic, myristolic, linoleic, and linolenic acids are also extracted from the petroleum ether extract of the seeds [288].

2.11 Isolation of Metabolites from Botanicals using Column Chromatography

Column chromatography is suitable for the physical separation of gram quantities of material. A solvent acts as the mobile phase while a finely divided solid surface acts as the stationary phase. The stationary phase will adsorb the components of the mixture to varying degrees. As the solution containing the mixture passes over the adsorbent, the components are distributed between the solvent and adsorbent surface. The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute. During the entire chromatography process the eluent is collected in a series of fractions. Fractions can be collected automatically by means of fraction collectors. The productivity of chromatography can be increased by running several columns at a time. Several authors followed this method for the collection of compounds [289-292].

2.12 Spectroscopic Studies for Analysis of Coumarins

2.12.1 Gas Chromatography Mass Spectroscopy (GC/MS)

The GC-MS analysis of the hexane extract of *Citrus aurantifolia* fruit peel led to the identification of 44 volatile components, including monoterpenes (16.00%), sesquiterpenes (6.55%), coumarins (27.37%), fatty acids (9.78%), and some other oxygenated aromatic and non-aromatic compounds (40.30%). The main components were identified as 5, 7-dimethoxycoumarin (15.79%), 3-methyl-1,2-
cyclopentanedione (8.27%), 1-methoxy-cyclohexene (8.0%), corylone (6.93%), palmitic acid (6.89%), 5,8-dimethoxypsoralen (6.08%), α-terpineol (5.97%), and umbelliferone (4.36%) [293]. Distilled oil of fruit peels of *Citrus aurantifolia* from southern Florida was studied by GC-MS detecting between 50 to 60 volatiles with limonene (32.6%), α-terpineol (12.5%), and β-pinene (6.3%) as the main compounds [294]. On the other hand, an Australian research group reported the analysis of the essential oil of Mexican peel lime with limonene (30.5%) and γ-terpinene (19.2%) as the main components, together with some minor constituents as geranial (5.9%), 7-methoxycoumarin (3.3%), 5,7-dimethoxycoumarin (6.6%), and bergapten (2.9%). These reports showed that limonene and γ-terpinene are the most common and abundant components of the essential oil of *C. aurantifolia* [295].

*Mirabilis jalapa* whole plant methanol extract by GC-MS analysis clearly showed the presence of six compounds. The results revealed that -3, 3’- Methylenebis (4- hydroxycoumarin) (17.07), N-D-alpha- Phenylglycine (38.76), laminaribiitol (7.753), 3-(4-(dimethylamino) cinnamoyl)-4-hydroxycoumarin (16.89), unkown (5.284), unknown (10.26) and unknown (3.956) [296]. Mass spectrum of the coumarin compound isolated from the *Ammi majus* L. suggested its molecular mass to be 206 in agreement with the formula C_{11}H_{10}O_{4}, which shows fragments at m/z193 and 162, suggesting that fragmentation is occurring in the manner associated with coumarin nucleus [297]. Pomelo (*Citrus maxima*), grapefruit (*Citrus paradise*), and orange (*Citrus sinensis*), as citron (*Citrus medica*) were used to find the occurrence of Osthole in various parts [298]. All extracts from pomelo, grapefruit, and citron showed well separated peaks with retention times identical with the osthole standard solution and mass spectra showing very high match as with the osthole standard in GCMS. The general chromatographic profile was different for particular fruit, but the abundance of the osthole peak was always quite high. The absolute recovery was 91% at the concentration level of 1 μg/g, and 106% at 10 μg/g. Osthole was present mainly in the peel of pomelo, grapefruit, and citron in the range of 7.0-78.5 mg/kg whereas orange was practically free of this compound.
Phytochemical evaluation of *Litsea glutinosa* bark methanolic extract was carried out by Parikh and Rangrez (2012) [299]. GC-MS analysis of the total methanolic extract showed the presence of Oliec acid, tricosene, erucic acid, tetradecanoic acid, pyrrolidinone, piperidine, eicosanoic acid like major phytochemicals. Alkaloid fraction was found to be rich in therapeutically potential compounds like Eicosane, Pieprizine, pyridine, thiocoumarin, tetrahydroisoquinoline. Apart from this various Androstane, Androstratrione, pregnene like phytoestrogens were also observed in this plant.

Essential oils of cinnamon and frankincense were isolated and identified using analytical gas chromatography mass spectrum (GC-MS) by Shareef, (2011) [300]. GC/MS analysis of cinnamon essential oil identified nine phytochemicals as constituents of these cinnamaldehyde was the major compound (41.62%) followed by acetic acid 1- octyl acetate (13.58%), Eugenol (7.1%), coumarin (4.49%), Pregnane- 11, 20 – dione, 3,17-dihydroxy (3.46%).

### 2.12.2 UV-visible Spectroscopy

Ultraviolet-visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Coumarins absorb ultraviolet light and emits as blue fluorescence. Some of the energy absorbed is lost to the solvent. The molecule returns to the lower state by emitting a photon with less energy than was absorbed, called fluorescence.

The UV spectrum of the compounds isolated from the petroleum ether extract of *Clausena pentaphylla* exhibited the absorption maxima at 215, 287 and 332 nm, suggesting that the compound belong to the coumarin family [301]. A coumarin compound isolated from the *Ageratum conyzoides* leaves showed UV absorption bands at 273 and 311 nm, attributed to the benzene and pyrone rings respectively [302]. Rao et al. (2009) [303] reported characteristic absorbance for the
coumarin moiety at 335, 263, 253 and 242 nm in methanol solution of coumarin diol 6-(2',3'-dihydroxy-3-methylbutyl)-8-prenylumbelliferone isolated from Chloroxylon swietenia. The methanolic solution of coumarins isolated from the flowers Aesculus hippocastanum showed UV absorption $\lambda_{\text{max}}$ for esculetin at 231.7, 334.8, for scopoletin $\lambda_{\text{max}}$ at 232.9, 297.8, 342.9 and for fraxetin $\lambda_{\text{max}}$ at 232.9 and 337.3 [304].

2.12.3 Fourier Transform Infra Red Spectroscopy

Beccamarin was isolated as a yellowish solid from the hexane extract of Mesua beccariana. The FTIR spectrum gave absorptions of chelated hydroxyl (3,400 cm$^{-1}$), carbonyl (1,741 cm$^{-1}$), saturated C-H stretch (2971 cm$^{-1}$) and aromatic ring (1,466 and 1605 cm$^{-1}$), which reflected similarity to typical IR bands for coumarins [305]. Four coumarins viz auraptene, gleinadiene, 5, 7-dimethoxy-8-(3-methyl-2-oxo-butyl) coumarin and toddalenone were extracted by chromatographic fractionation of the leaf extracts of Murraya paniculata by Aziz et al. (2010) [289]. The IR spectrum of gleinadiene showed peak at 1728 cm$^{-1}$ assigned as C=O group, while signal at 1618 cm$^{-1}$ was due to the presence of double bonds in this coumarin compound.

From the aerial parts of Cynanchum acutum coumarins scopoletin and scoparone were isolated. The IR spectrum of scopoletin showed absorption bands at 3396 cm$^{-1}$, due to a hydroxylic group; 1713 cm$^{-1}$, corresponding to carbonyl group ($\delta$-lactone), 1611 cm$^{-1}$, corresponding to CH=CH group, 1565 and 1514 cm$^{-1}$, corresponding to aromatic benzene ring. The IR spectrum of Scoparone showed 1713 cm$^{-1}$ (CO $\delta$-lactone), 1611 cm$^{-1}$ (CH=CH), 1565 cm$^{-1}$ and 1514 cm$^{-1}$ (aromatic benzene ring) [306]. Xanthotoxin (8- methoxypsoralen) was isolated from the fruits of the Heracleum persicum by Sajjadi and Noroozi et al. (2007) [307]. The IR spectrum showed peaks at 1725 (Coumarin carbonyl), 1590, 1350 (a, $\beta$ – Unsaturated lactone) and 1130 cm$^{-1}$ (C-O strtching) confirms the skeleton of xanthotoxin.
A terpenoidal coumarin namely, hekumarone, was isolated from *Clausena anisum-olens* Merr. IR bands at 1,753, 1,748 and 1,730 cm\(^{-1}\) were indicative of the presence of three carbonyl groups suggesting a ketonic group and a lactone in the side chain [308]. Acetylenic thiophene, isolated from the hexane extract of *Artemisia absinthium* showed IR bands at 1545, 1632 and 2233 cm\(^{-1}\) indicative respectively of the presence of an aromatic group, a carbonyl group conjugated to a double and an internal triple band [309].

2.12.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance is a branch of spectroscopy in which radio frequency waves induce transitions between magnetic energy levels of nuclei of a field. It is a powerful tool for investigating nuclear structure. Scopoletin, a coumarin derivative compound isolated from methanol extract of *Macaranga gigantifolia* Merr. leaves using chromatography methods. Chemical structure determination carried out based on NMR spectroscopic data. The \(^1\)H-NMR spectrum showed four aromatic protons (δH6.2, 6.70, 6.82, and 7.97 ppm), and one methoxy group (δH3.83 ppm), combined with ten carbons from 13C-NMR, and HMQC/HMBC correlations showed the chemical structure of Scopoletin [310]. From the aerial part of Pineapple weed *Matricaria matricarioides* three coumarin compounds viz, 7-methoxycoumarin (Herniarin), umbelliferone and 7-methoxy-3, 4–dihydrocoumarin were isolated [311]. \(^1\)H NMR, after deduction of the umbelliferone signals, displayed two sets of triplet signals at δ2.41 (2H, t, J=7.5Hz) and 2.66 (2H, t, J=7.5Hz), attributed to H-3 and H-4. A singlet signal integrated for 3 protons appeared at δ3.65 (3H, s, OCH3). A pattern of 1, 2, 4-tri-substituted benzene signals appeared at δ6.29 (1H, dd, J=3.0, 8.0 Hz), 6.36 (1H, d, J=3.0 Hz), 6.93 (1H, d, J= 8.0 Hz).

Investigation of the aerial parts of *Ammi majus* L. led to isolation of 6-hydroxy-7-methoxy-4 methyl coumarin and 6-hydroxy-7-methoxy coumarin by Selim and Ouf, (2012) [297]. The \(^1\)H NMR of the compound showed that no band was typical of H-4 of a coumarin and singlet at δ 6.25 was assignable to H-3, indicating that methyl group was attached at position 4. Another doublet was
observed at δ6.62, which could be H-5 of a coumarin. There was a singlet at δ6.43 and 6.82 for two protons which represented H-6 and H-8 of the nucleus. The $^{13}$C NMR spectrum showed resonance for all 11 carbon atoms in the molecule. The spectra revealed the presence of two methyl, three methane and six quaternary carbon atoms. The two downfield quaternary carbon signals at δ162.5 (C-3) and 143.7 (C-6) showed the presence of ketonic and one hydroxyl functionality in the molecule. In the $^1$H NMR of the compound 6-hydroxy-7-methoxy coumarin showed a doublet at δ6.72 which was typical of 5.35 which could be H-5 of a coumarin. There was a singlet at δ 6.25 and 6.80 for two protons which represented H-6 and H-8 of the nucleus.

2.13 Phytopathogenic Enzymes - Docking Studies

Interaction between bio molecules are fundamental to all biological processes. Using these interactions, living organisms maintain complex regulatory and metabolic interaction networks that together constitute the process of life. Experimental work and computer simulation and analysis are main tools for understanding these processes and for finding molecules that can be utilized as bioactive substances to modify and control them [312]. Computational techniques are one of the vital processes to understand interaction between the proteins and drugs [313].

The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced Molecular docking is the optimal process in way of fast alternatives and also inexpensive method to design a novel scaffolds [314]. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. It is useful for predicting both the strength and type of signal produced with an aim to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized [315].
Soundararajan et al. (2011) [316], carried out homology modeling of the protein FGB1 due to the absence of X-ray crystal structure of FGB1 in Protein Data Bank (PDB, www.rcsb.org) and found its tertiary structure. The G protein’s, α subunit (FGA1) and β subunit (FGB1) have partially overlapping functions in the regulation of development and pathogenicity in F. oxysporum [317]. Disruption of gene encoding a heterotrimeric G-protein-β-subunit (FGB1), led to decreased intracellular cAMP levels, reduced pathogenicity, colony morphology, and germination.

The plant defense protein, Nicotiana alata defensin (NaD1) displays potent antifungal activity against a variety of agronomically important filamentous fungi. Molecular modeling and docking studies to find vital amino acids which can interact with various antifungal compounds using Discovery Studio v2.5 and GRAMMX, respectively were carried out by Soundararajan et al. (2011) [316]. The docking results from FGB1-NaD1 and FGB1-antifungal complexes, revealed the vital amino acids such as His64, Trp65, Ser194, Leu195, Gln237, Phe238, Val324 and Asn326, and suggested that the anidulafungin is a the good antifungal compound. The predicted interaction can greatly assist in understanding structural insights for studying the pathogen and host-component interactions.

As fungal pathogens have an enormous impact on plant production worldwide, the strategies they use to infect plants and to cause disease are a topic of great interest [318]. Knowledge of the pathogenicity/virulence factors essential for fungal infections is very important because it represent the targets that researches must attack in the fight against these pathogens [319]. Pathogenicity gene is defined as those necessary for disease development but not essential for pathogen to complete its lifecycle in vitro. Pathogenicity genes are of interest not only to increase our overall knowledge of disease process, but also because any such gene could became a target for disease control. The types of genes essential for pathogenesis depend on the infection process of a particular fungus. Some fungi degrade the cuticle and cell wall to enter the plant; others form specialized structures, such as appressoria, to penetrate the epidermis, while others enter the host through wounds or natural openings [320].
The plant pathogen interactions begin with the secretion of plant cell wall degrading enzymes. They secrete extracellular degradative enzymes capable of evading or suppressing the defense mechanism of the plant and proving it important for pathogenesis. Pectin lyases catalyze the transelimination of pectin and play important role in the plant tissue maceration, loss in electrolytes and cell death. Hence it is an important pathogenicity factor in the fungal-plant interaction. \[321,322\]. *Alternaria brassicicola* breaches the plant cuticles during the penetration of plant surfaces secreting two cutinase isozymes under saprophytic and parasitic stages of the fungus \[323\]. Endopolygalacturonase catalyzes the fragmentation and solubilization of homogalacturonan, results in the transient formation of elicitor-active oligogalacturonides with degrees of polymerization between 9 and 15. These oligogalacturonides are rapidly converted to smaller, biologically inactive fragments by the endopolygalacturonase \[324\].

Number of fungal mechanisms and molecules have been shown to contribute to fungal pathogenicity or virulence, understood as the capacity to cause damage in a host, in absolute or relative terms. Among them, cell wall degrading proteins, inhibitory proteins, and enzymes involved in the synthesis of toxins are included. These virulence factors are typically involved in evolutionary arms races between plants and pathogens \[325\]. The plant cell wall is a complex structure of polymers which surrounds the cell containing cytoplasm \[326\]. Small proteins encoded by fungal genes involved at various stage of infection, alter host cell structure and function facilitate infection. These proteins are often cysteine rich \[327\]. The enzymes produced by pathogens affect chemistry of cell wall which is accompanied by cell wall degradation \[328\]. For the degradation of cell wall, plant pathogens have been found to secrete a range of enzymes like cellulolytic, hemicellulolytic, pectolytic and proteolytic enzymes which are capable of attacking each of the major polymeric components \[329, 330\].

Most pathogens have the capacity to produce more cellulolytic than pectolytic enzymes \[331\]. The correlation between stalk rot and cellulase production was significant in maize \[332\]. Apart from cell wall degrading enzymes secreted by a wide variety of saprophytic and phytopathogenic microorganisms \[333, 334\], the
pectinases also play an important role in the entry of the plant pathogen intra and intercellularly into the host tissues thus blocking the conducting vessels resulting in the development of wilt [335]. Lipases and proteases are important enzymes in pathogenesis which attack the plasmalemma after the degradation of cell wall by proteases along with pectolytic and cellulolytic enzymes [336, 337].

Antifungal targets-site are extremely diverse. However, substances that act on these target-sites needs to fulfill several prerequisites such as antifungal activity in vivo and lack of effects on the host cells. Methods in the prediction of fungal-target like proteins can also be enhanced from new progress in learning algorithms and sequence descriptors also by genomic, proteomics, pathogenesis and morphogenesis studies [338]. The use of natural products or related compounds as specific enzymes inhibitors is an archetype, as they would be species specific and the environmental impact would be reduced to a minimum [339].

2.14 Activation of Phenyl Propanoid Pathway

The plants utilize their own defence mechanism for restriction of pathogen development by activating the phenylpropanoid pathway. As markers of resistance, physiological changes always appear in certain intervals after application of the biotic and abiotic inducers against pathogens [340]. Plants accumulate a great diversity of natural products, many of which confer protective effects against phytopathogenic attack. Kagale et al. (2004) [341] demonstrated that the leaf extracts of Zizyphus jujuba and Ipomoea carnea can inhibit the in vitro mycelial growth of Rhizoctonia solani, and effectively reduce the incidence of sheath blight disease in rice. The foliar application of the aqueous leaf extracts of Z. jujuba and I. carnea followed by challenge inoculation with R. solani induced systemic resistance in rice as evident from significantly increased accumulation of pathogenesis-related proteins such as chitinase, β-1,3-glucanase and peroxidase, as well as defense-related compounds such as phenylalanine ammonia-lyase and phenolic substances. Thus, the enhanced sheath blight resistance in rice seedlings treated with leaf extracts of Z. jujuba or I. carnea was attributed to the direct inhibitory effects of these leaf extracts as well as their ability to elicit systemic resistance against R. solani [155].
In pumpkin leaves with challenge inoculation of *Sphaerotheca fuliginea* and treatment with osthol and coumarin compound induced the accumulation of chitinase and peroxidase and PAL activity and induced resistance response in the plant against powdery mildew [274]. Tomato plants treated with *Trichoderma virens* followed by challenge inoculation of *F. oxysporum* fsp. *Lycopersici* enhance induction of defence related enzyme such as PO, PPO and PAL than other isolates which could be very effective in the control of *Fusarial* wilt of tomato [342].

Aqueous leaf extract of neem (*Azadirachta indica*) provided the control of *Alternaria* leaf spot pathogen (*Alternaria sesami*) of *Sesamum indicum*. Treatment with this extract led to the changes in plant metabolism as leaves of the treated plants exhibited significantly high level of enzymes of phenylpropanoid pathway namely phenylalanine ammonialyase (PAL), peroxidase (PO) and content of phenolic compounds. It is therefore, suggested that, protection of sesame plants against *A. sesami* by neem extract might be due to stimulation of plants natural defence response [343]. The expression of induced systemic resistance in banana fruits upon treatment with aqueous extracts of *Solanum torvum*, Zimmu and *Allium alliaceum* for five minutes and inoculated with conidial suspensions ($10^6$/mL) of *Colletotrichum musae* by pin prick method. The Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia-lyase (PAL) activity was significantly increased both in peel and pulp of the inoculated fruits dipped in leaf extracts as compared to inoculated fruits alone. Activity of defense enzymes were highest six day after inoculation declined in subsequent days [344].

Latha et al. (2009) [345] formulated talc based formulation using *pseudomonas fluorescens* (Pf1 and Py15), *Bacillus subtilis* (Bs16) and Zimmu leaf extract. The formulation was successful in reducing the early blight disease incidence and induction of defense enzymes, such as peroxidase (PO) and polyphenol oxidase (PPO) phenylalanine ammonia-lyase (PAL), chitinase and $\beta$-1,3-glucanase and accumulation of phenolics in tomato. This revealed the probable influence of plant growth promotion and induced systemic resistance in enhancing the disease resistance in tomato plants against early blight disease.
Satya et al. (2007) [346] applied aqueous leaf extract of Zimmu to first and second leaves cotton plants that induced systemic resistance in third and fourth leaves and reduced the number of lesions by up to 73% after challenged infection with *Xanthomonas campestris* pv. *Malvacearum* compared with water-treated control plants. Roth et al. (2000) [347] studied the effects of aqueous extract of *Lychnis viscaria* L. seeds that contains brassinosteroids against *Phytophthora infestans*. Its application enhanced resistance to tobacco, cucumber, and tomato by increasing PR-proteins, peroxidase, chitinase, and β-1, 3-glucanase. The disease-controlling ability of *Datura metel* against plant pathogens and as an inducer of resistance against *Ascochyta rabiei* [348], *Pennisetum glaucum*, and *Sclerospora graminicola* in pearl Millet [349].

### 2.15 Increase in Vigour Index Due to Botanicals

Preparation and application of botanicals for crop protection are linked to the folklores and tradition of the farmers [350]. Phytopesticde materials range from whole fresh plants to purely isolated bioactive phytochemicals or their formulations which are effective against pests and pathogens [351]. Prior to discovery of organochlorine and organophosphate compounds, botanicals were used as important products in pest and disease management in industrialized nations [352]. The formulations of winter green oil as emulsifiable concentrate (EC) was tested for its storability at room temperature for different periods showed that they retained their antifungal effect up to 60 days [353]. Bharathi (2004) [354] reported that Lowfolin 40 EC formulation was effective up to 90 days after preparation. Storable nature of the formulation up to 3 months at room temperature has got much practical utility as its effect was retained during the storage period [355].

Seeds of *Phaseolus aconitifolius* treated with formulations of *Ocimum canum* and *Brassica juncea* increased the germination percentage and vigour of seedlings due to presence of protein, amino acids, nitrogen and precursor of plant hormones [356]. The beneficial effects of botanical formulations in inhibiting the fungal pathogens and increasing the seedling vigour in ground nut [357] and paddy [358] have been reported. Dongzhi (2004) [359] reported *Aloe vera* contained natural plant growth regulators and it promoted shoot and root length of turnips.
Effect of root extracts of *Hemidesmus indicus* on seedling emergence, shoot and root length, shoot and root rot of maize was reported by Sangvikar et al. (2012) [360]. Maximum root and shoot lengths were recorded in alcoholic extracts (root length- 4.8 cm and shoot length) followed by ethyl acetate extract (root length- 4.3 cm and shoot length- 6.0 cm). In alcoholic and ethyl acetate extract treated seeds did not show any rot symptoms. Sesame seeds were treated separately with recommended dosages of Neem leaf powder (NLP), Dress force powder (DFP), Dry pepper powder (DPP) each treatment placed in air-tight container and stored in a wooden cabinet at average ambient conditions of 26.5°C for a period of 18 weeks. Observations showed that NLP and DPP treatments had better mean seed germination of 89.53% and 82.35% respectively compared to DFP (46.47%) and control (80.76). NLP also enhanced better seedling vigour index throughout the storage time compared to DFP [361].

Three different botanicals were used for evaluating the preservative effect for maintaining the quality of lentil seeds in storage viz., leaf powder of neem (*Azadirachta indica*), dholkalmi (*Ipomoea sepiara*) and bishkatali (*Polygonum hydropiper*). The germination was maximum in the seeds stored with neem leaf powder. There was no significant effect of botanicals on root length of lentil. The shoot length of lentil seeds after germination differed significantly due to application of botanicals [362]. Savitri et al. (1994) [363] reported that neem leaf powder gave higher germination (65.7%) of sorghum seeds.

2.16 Effect of Botanicals in Plant Disease Control *in vivo*

Abdel-Monaim et al. (2011) [364] studied the effect of water extract and organic solvents from some plant species against *F. oxysporum* f. *sp. Lupine* casual agent of damping-off and wilt diseases of lupine plants. Their experiments revealed that solvent extracts of *Eugenia jambalaya*, *Nerium oleander* and *Citrullus colocynthis* are most effective against *F. oxysporum* f. *sp. lupini*. Amongst the tested organic solvents, the butanolic and ethereal extracts were highly effective in reducing diseases than the other tested extracts. Under field conditions, ethereal and butanolic extracts of *N. oleander* and *E. jambolana* leaves and *C. colocynthis* fruits significantly reduced the percentage of wilt severity as well as improved plant growth parameters and increased seed index that is total seed yield/hectare compared with control.
**Lycopersicon esculentum** on challenge inoculation with *Phytophthora infestans* and then treated with various methanolic plant extracts revealed strong antifungal activity in extracts of *Saussurea lappa*, *Agastache rugosa*, *Lysimachia foenum-graecum* and *Curcuma longa*. Essential oils obtained from *Origanum syriacum* and *Foeniculum vulgare* showed effective control over *Sclerotinia sclerotinum* even in soil [150, 365].

Essential oil of *Magnolia liliflora* displayed potent *in vivo* antifungal effect against one of the selected plant pathogens *Phytophthora capsici* on greenhouse-grown pepper plants and could be used as natural alternatives to synthetic fungicides [366]. *In vivo* fungicidal activities have been reported in methanol extracts of *Piper nigrum*, *Rheum coreanum*, *Lysimachia. foenum-graecum*, *Evodia officinalis*, *Glycyrrhiza uralensis*, *Paonia moutan*, *Nardostachys chinensis* and *Curcuma longa* showed moderate activity (60 – 80%) [367]. Essential oils marketed as botanical fungicides for organic farming include oils extracted from Jojoba (*Simmondsia californica*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), clarified hydrophobic extract of neem (*Azadirachta indica*) and cottonseed (*Gossypium hirsutum*) [368].

The oregano and lemon oils were very effective in controlling disease severity of infected fruit by *B. cinera* in tomatoes, strawberries and cucumbers. In tomatoes, grey mould caused by *B. cinerea* was completely inhibited by oregano essential oils at 0.30 μL/mL. Lemon essential oils induced a significant reduction of grey mould disease severity. In strawberries, grey mould was completed inhibited by lemon essential oils at 0.05 μL/mL. In addition, lemon essential oils at 0.05 μL/mL showed 39% reduction of infected cucumber fruits by *B. cinerea*. These results indicate that essential oils after suitable formulation could be used for the control of diseases caused by *Botrytis* and *Penicillium* pathogens [369]. The efficacy of the essential oil from flowers of *Cestrum nocturnum* L. was evaluated for controlling the growth of *Phytophthora capsici* fungi. Further, the oil displayed remarkable *in vivo* antifungal effect up to 82.4-100% disease suppression in greenhouse-grown pepper plants. The initial concentration of 1000 μg/mL the oil exhibited 100% antifungal effect against leaf spot/scorch of pepper caused by *Phytophthora capsici* [370].
Combined application of botanical formulation and biocontrol agents reduced the wilt incidence of *Fusarium oxysporum* f. sp. *cubense* significantly under greenhouse (63%) and field conditions (65.23%) in banana. Reduction in disease incidence was positively correlated with the induction of defense-related enzymes peroxidase (PO) and polyphenol oxidase (PPO) [123]. Spraying tomato plants with 20% aqueous neem leaf extracts lowered the disease incidence of *Alternaria solani* to 42.54% while spray and irrigation reduced disease incidence to 39.49%. Spray and irrigation with 20% aqueous extracts from neem leaves seemed to work in a synergism in controlling the disease [141].

Aqueous leaf extracts of three medicinal plants (*Podophyllum hexandrum*, *Withania somnifera* and *Xanthium strumarium*) against late blight of potato caused by *Phytophthora infestans* was studied by Majeed et al. (2011) [371]. Foliar sprays of 25% (w/v) leaf extracts of the three medicinal plants at a 3 day interval significantly reduced disease severity and resulted in higher tuber yield and biological yield compared to control. Leaf extracts of *Podophyllum hexandrum* were more effective in minimizing the disease incidence and producing better biological yield/plant (24.92 g) and tuber yield (14.93 t/ha) when compared to other two extracts. Foliar application *P. hexandrum* against late blight could be used for minimum disease incidence and better yields.

In greenhouse experiment carried out by Nashwa and Abo-Elyousr (2012) [372], in tomato plants challenged with *Alternaria solani*, 71.7% and 68.2% reduction of disease severity was achieved by the *Allium sativum* and *Datura stramonium* extracts at a concentration of 5% respectively. In field studies both the extract showed disease reduction of 57.6% and 54.2% respectively. Treatment with *Allium sativum* extract resulted in fruit yield of 3.5 t/ha and *Datura stramonium* extract resulted in 3.7 t/ha which were significantly higher than the control treatments. Various plant extracts were tested in field by [373] Pattnaik et al. (2012) in *Lycopersicon esculentum* for controlling diseases caused by them. Aqueous extract of *Ageratum conyzoides* was found most effective in reducing the *Alternaria* canker disease by 78.20%. Aqueous extract *Azadirachta indica* reduced the early blight and leaf spot disease by 53.84% and 40.78% respectively. Aqueous extract of
Aegle marmelos reduced the fruit spot disease by 61.29%. Pongamia pinata and Brassica campestris reduced the blossom end rot disease by 86.95% and 82.17%. Ageratum conyzoides and Pongamia pinata reduced the sunscald disease by 90.08% and 76.85% respectively in Lycopersicum esculentum.

### 2.17 Influence of Botanicals on Growth and Yield of Crops

Plants sprayed with D. stramonium and A. sativum at 5% concentration increased the fruit yield of tomato by 76.2% and 66.7%, respectively, compared to the nontreated control. In contrast, O. basilicum, A. indica, E. chamadulonsis and N. oleander treatments increased the fruit yield moderately, in the range between 28.6% and 38.1% compared to the infected control [372]. Dougdoug et al. (2007) [374], studied about the effect of phyto-antivirus against tomato yellow leaf curl geminivirus (TYLCV). Using water extract of Khella (0.3%) and black cumin (3.0%) led to elimination of TYLCV which was confirmed by PCR tests. They also confirmed that using of the plant extracts, increased the plant growth (plant height, leaf area, number of branches, fresh and dry weight and chlorophyll content) as well as yield (number of flowers and fruits per tomato plant. Patil et al. (2001) [375], found that incidence of tomato early blight caused by A. solani was affected by a botanical like neem seed extract with increased fruit yield between 156.43 and 168.56q / ha.

The marked reduction in the severity of early blight pathogen was observed when plants were treated with aqueous neem leaf extract. The irrigation of uninfected tomato seeds with 20 % aqueous neem extract (2 L /pot) after 4, 6 and 8 weeks of sowing for both pathogens, highly improved the germination with significant increase in various growth parameters of treated tomato plants especially after the 8 week followed by infected seeds irrigated with the extract [376]. Ageratum conyzoides was found most effective in reducing the Alternaria canker disease by 78.20% and Azadirachta indica reduced the early blight and leaf spot disease by 53.84% and 40.78% respectively, Aegle marmelos reduced the fruit spot disease by 61.29%.
*Pongamia piniata* and *Brassica campestris* reduced the Blossom end rot disease by 86.95% and 82.17% and *Ageratum conyzoides* and *Pongmia piniata* reduced the sunscald disease by 90.08% and 76.85% respectively in *Lycopersicum esculentum*. Coincidentally plant extracts increased all growth parameters including yield along with reduction of plant diseases [373].

Zimmu leaf extract showed the inhibition of mycelial growth of *P. aphanidermatum* (13.7 mm). The pot culture studies revealed that seed treatment with combined application of *T. viride* and *P. fluorescens* and Zimmu leaf extract was superior in reducing the pre and post-emergence damping-off incidence (8.3 and 17.0%, respectively), and increased the plant growth and yield (shoot length and root length of 13.7 and 6.3 cm, 146 g/plant, respectively) of chilli when compared to control [377].