2. Literature review

2.1. General aspects of Host-pathogen Interaction

Host pathogen interaction is one of the important areas of research. Although a variety of pathogen attack plants, some pathogens are considered to be of virulent in nature. Response of plants to fungal attack leads to either resistance or susceptibility depending upon interaction between plant and the pathogen. Pathogenesis and disease resistance are closely related. Compatible interactions lead to successful establishment of the pathogen which leads to susceptibility while resistance is related to incompatible ones. Application of exogenous inducers (may be biotic or abiotic) induce defense enzymes and its production at a threshold level leads to resistance of plants. Study of defense enzymes is one of the important areas which have been studied by several workers in the last three decades. Molecular basis of the defense genes particularly in tea have recently being initiated in different laboratories. In the present study defense related enzyme of tea has been proposed to be induced by abiotic inducers. Further, it has also been proposed to study the regulation (up and down) of selected defense related transcripts of selected defense related enzymes. Hence, it has been considered to review the works of the earlier workers. The studies, reports and observations of the previous workers in concord with the present line of investigation are being presented in the following paragraphs, in a selective rather than in a comprehensive manner. The present review, for convenience, has been grouped in to some aspects. The aspects of the review are as follows:

1. Diseases of tea
2. Induction of defense related enzymes and disease assessment
3. Molecular aspects of defense related genes (identification and expression analysis)

4. Management of pathogenic microorganisms by some botanicals and biocontrol agents.

2.2. Diseases of tea

Foliar fungal diseases of tea are main obstacle and also have adverse effect on quality and productivity of tea in all over the world. The fungus, *Colletotrichum sp.* and *Curvularia eragrotidis* are the main causal agent of brown blight disease and leaf spot disease of tea respectively in north-east India (Dasgupta *et al.* 2005).

Gray blight disease of tea caused by *Pestalotiopsis theae* is another important foliar disease of all tea growing countries of the world. The disease appears on mature foliage, bare stalks as well as in young shoots of the tea bushes. The disease symptoms are generally circular, with upper surface concentrically zoned with different colors (Chen and Chen, 1982; Baby and Sanjay, 2006; Premkumar *et al.*, 2012; Kumhar *et al.*, 2016; Pallavi *et al.*, 2012).

Alternaria alternata, is also a foliar fungal pathogen of tea in North Bengal, India. Disease symptoms first appear as grayish brown patches around tips and margins of young leaves which extend towards the midrib resulting to leaf curl, death and defoliation (Chakraborty *et al.*, 2006).

Black rot is an important disease of the mature leaf and stem caused by fungi *Corticium theae* and *Corticium invisum.* They attack the maintenance leaves, causing gradual deterioration in the health of the bush and consequent loss of crop. The infected leaves turn black as they rot during the wet weather. The dead leaves, which are detached from the bush but remain suspended on the bush and the mycelial chords come out from the infected leaves hold the bush. Then the fungal sclerotia embed themselves in the cracks and crevices of the stem and bark (Chen and Chen, 1982; Singh *et al.*, 2006).

2.3. Induction of defense related enzymes and disease assessment

Pajot *et al.* (2001) reported about two elicitors [(DL-*β*-amino butyric acid (BABA) and Phytogard (K_2HPO_3)] which induced systemic resistance in lettuce against *Bremia lactucae* causal organism of downy mildew disease and protected the plants. Among the two elicitors Phytogard reduced spore germination completely but BABA reduced partially. They also reported that BABA increased the activity of PR proteins like β-1,3-glucanase, peroxidase etc. but Phytogard did not induce this PR proteins.

Amzalek and Cohen (2007) studied the effect of SAR inducer like BABA, BTH, INA, NaSA, AABA and GABA to control sunflower rust caused by *Puccinia helianthi*. They applied all the compounds in sunflower plants as a foliar spray, root dip or leaf disc assay techniques. From the foliar spray results they found BABA was more effective and NaSA was least effective to induce resistance against rust but in leaf disc assay BTH, BABA and INA were fully protective. NaSA and AABA were less effective against rust but GABA did not have any potential effect. They also reported that BABA did not have any effect to defense compounds accumulation and on spore germination but suppressed mycelial colonization in the mesophyll.

Hassan and Abo-Elyousr (2013) pre-treated tomato plants with DL-3-aminobutyric acid (BABA) by soil drenching and studied the ability of BABA to protect tomato plants as well as the accumulation of total phenolic compounds, SA and the activity of PPO, CAT against bacterial wilt caused by *Ralstonia solanacearum*. They showed that, plants treated with BABA reduced disease incidence and also reduced leaf wilting index and vascular browning index. According to them BABA treated tomato plants showed increased activity of PPO, SA and total phenolic compound but decreased the activity of CAT.

Aleandri *et al.* (2010) studied the effect of three resistance inducers methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH) and dipotassium hydrogenphosphate (K_2HPO_4) to control root rot and vine decline disease of melon caused by *Monosporascus cannonballus*. They applied the inducers either by foliar application or as seed soaking technique. Plants treated

with MeJA and BTH reduced disease severity in case of artificially inoculated soil or by naturally infected greenhouse soil by *M. cannonballus* but K_2HPO_4 did not reduced disease severity under similar condition. They also reported that Different PR-proteins and their isozymes were increased in root system treated with resistance inducers.

Hukkanen *et al.* (2007) reported that, benzothiadiazole (BTH) enhanced the accumulation of soluble and cell-wall-bound phenolics in strawberry leave and also improved the resistance to powdery mildew infection under greenhouse conditions. Most pronounced change was seen in the levels of ellagitannins, which increased up to 2- to 6-fold in 4 days post BTH application, but persisted only in the inoculated plants. The induction of phenolic metabolism by BTH was also reported in the fruits. Basal salicylic acid (SA) content was high in strawberry leaves, but increased in a similar fashion like other phenolics following treatments. The several new compounds have been identified by the authors for the first time in strawberry such as ellagic acid deoxyhexose, three agrimoniin-like ellagitannins, sanguiin H-10- and lambertianin C-like ellagitannins in the leaves, ellagic acid, p-coumaric acid, gallic acid, and kaempferol hexose in the cell-wall-bound fraction of the leaves, and kaempferol malonylglucoside in the fruits. According to them BTH enhanced the accumulation of phenolics in strawberry plants which might have been involved in the BTH-induced resistance against powdery mildew.

According to Romanazzi *et al.* (2009) studied phytoplasma caused diseases of grapevine. They evaluated the effectiveness of field treatments with resistance inducers (Chito Plant, Aliette, Kendal, Bion, and Olivis) to promote recovery in Bois noir (BN) infected grapevines. Treatments consisted of weekly sprays in spring-summer 2007 (seven applications) and 2008 (thirteen applications). All treatments increased the number of recovered plants but best results were obtained with Kendal, Olivis and Bion. Phytoplasma were reported to be absent in induced and recovered plants.

Percival (2010) used a detached leaf bioassay to evaluate systemic inducing resistance (SIR) agents, biostimulant products and one triazole fungicide (myclobutanil) on apple scab (*Venturia inaequalis*) development under laboratory conditions. They found that SIR agents (potassium phosphonate, potassium phosphite, harpin protein, salicylic acid, salicylic acid derivative) and myclobutanil inhibited germination and subsequent formation of appressoria of conidia and also found reduced leaf scab severity.

Tamm *et al.* (2011) focussed on the use of DL-β-aminobutyric acid (BABA) and an aqueous extract of *Penicillium chrysogenum* (Pen) as elicitors. In their studies, BABA as well as Pen could contribute to control diseases caused by *Rhizoctonia solani*.

According to Cohen *et al.* (2011) DL-3-amino-butyric acid (BABA) induced local and systemic resistance against disease in numerous plant species. In their study they showed that preventive application of BABA to lettuce (*Lactuca sativa*) plants induced resistance against downy mildew caused by the oomycete *Bremia lactucae* by callose encasement of the primary infection structures of the pathogen. They also showed that post-infection application of BABA to the foliage or the roots, even at progressive stages of disease development, is highly protective against *B. lactucae*. Resistance induced by BABA is manifested in multiple microscopic forms, depending on the time of its application. When applied at 1 day post inoculation (dpi) BABA induced HR in penetrated epidermal cells; at 2 dpi it caused massive encasement with callose of the primary haustoria; and, at 3 or 4 dpi it enhanced the accumulation of H_2O_2 in the developing mycelia runners and altered their colour to red. The pronounced change in the colour of the mycelium was visually apparent to the naked eye. In all cases the pathogen failed to sporulate on the treated plants.

Abdel-Kader *et al.* (2012) carried out an experiment to evaluate the efficacy of some plant resistance inducers against downy and powdery mildew of cucumber plants. They evaluated foliar spray treatment at a schedule of four times (at fifteen days intervals) starting at thirty days after

transplanting. They found two treatment mix resulted in the highest reduction in foliar Downy and Powdery mildew disease incidence reported increased production the under plastic houses conditions. Treatment mixture 1) was Calcium chloride (20mM) + *S. cerevisiae* 10x1010cfu/mL (10ml/L) + Chitosan (0.05mM) and Treatment mixture 2) was Potassium bicarbonate (20mM) +Thyme oil (5ml/L).

Gilardi *et al.* (2013) worked to control downy mildew of sweet basil (*Ocimum basilicum* L.), incited by *Peronospora belbahrii* Thines. According to them greatest reduction in disease incidence and severity was found with treatments that included metalaxyl-M+copper hydroxide, a mineral fertilizer 'Alexin', mandipropanid, and azoxystrobin. The glucohumates activator complex and acibenzolar-S-methyl also provided significant disease control ($P<0.05$). The mineral fertilizer Alexin, the glucohumates activator complex and acibenzolar-S significantly reduced disease incidence and severity after 20 days of the last treatment. They reported that effective control could be achived by either using a rotation of fungicides with compounds that can induce resistance, or by using rotation with different resistance-inducing compounds on their own.

El-Mougy *et al.* (2013) also evaluated foliar spray treatments in cucumber, pepper and tomato plants to control foliar diseases, powdery and downy mildews. Their treatment schedule was four times with fifteen days intervals starting at thirty days after transplanting. Among the different treatments, treatment mixture (1) contained Calcium chloride (20mM) + *S. cerevisiae* 10x1010cfu/mL (10ml/L) + Chitosan (0.05mM)] but Treatment mixture (2) contained Potassium bicarbonate (20mM) + Thyme oil (5ml/L) reduced diseases mentioned above as well as early and late blight diseases. The activity of Peroxidase, Polyphenol oxidase, Phenylalanine ammonia-lyase, chitinase and β-1,3-Glucanase enzymes significantly escalated defense response in cucumber, pepper and tomato plants resulting to reduced disease symptoms.

Gilardi *et al.* (2014) performed an experiment to control crown and root rot of tomato incited by *Phytophthora nicotianae*. Five different

treatments were made under greenhouse condition to test the efficacy of spray programmes. The disease reduction achieved with a single application of azoxystrobin and metalaxyl-M. Partial disease control was found by other four Treatments such as Phosetyl-Al and the biocontrol agents *Glomus* spp. + *Bacillus megaterium* + *Trichoderma*, *B. subtilis* QST713, *B. velezensis* IT45 and the mixture of *T. asperellum* ICC012 + *T. gamsii* ICC080.

Alkahtani *et al*. (2011) studied effect of six abiotic elicitors (Oxalic acid, Potassium oxalate, and salicylic acid, Bion, Fungastop and Photophor) for inducing reisitance in cucumber (*Cucumis sativus* L.) against powdery mildew (caused by *Sphaerotheca fuliginea*) disease. They studied disease severity to evaluate inducers efficiency and measured the biochemical changes in both PR-related protein and phytoalexins accumulation in treated plant and compaired with the control plants. All the elicitors they tested showed decreased powdery mildew disease. Among the six elicitors Bion was most effective and potassium oxalate showed lowest effectiveness in both single and booster spray. PR-proteins such as POX, PPO, CHI and ß-1, 3- glucanase was found to increase and accumulation of phytoalexin was also increased.

Altinok and Dikilitas (2014) reported Acibenzolar-S-methyl (ASM) as an abiotic plant activator. They applied the compound in eggplant seedlings and found increased resistance to *Fusarium oxysporum* f. sp. *melonis*, a virulent pathogen of brinjal wilt. ASM pretreated brinjal plants significantly reduce wilt disease. ASM pretreatment resulted hypersensitive reaction (HR) and callose formation and H_2O_2 synthesis was increased. ASM treated plants showed increased activity of catalase and polyphenol oxidase. They also applied a non-host isolate of the pathogen on eggplant as biotic inducer and got decreasd disease severity like the ASM application but abiotic inducers was found to better than the biotic inducer they used.

Amer *et al*. (2015) induced cucumber plants by three biotic inducers (*Trichoderma harazianum*, *Pseudomonas fluorescen* and *Ampelomyces*

quisqualis) against *Pseudoperonospora cubensis*, a downy mildew disease causing fungi. They reported that Peroxidase and β-1, 3-glucanase activities were increased by application of *Trichoderma harazianum* and downy mildew was controlled to a significant extent. Although *Pseudomonas fluorescen* also reduce the disease but it did not induced β-1, 3-glucanase significantly. When *Ampelomyces quisqualis* was applied as an inducers neither peroxidase nor β-1, 3-glucanase was increased but it gave a good results in SA signal pathway induction.

Sreedevi *et al.* (2011) reported that *Trichoderma harazianum* can induce systemic resistance in groundnut against *Macrophomina phaseolina*. Defense enzymes (POX and PPO) were induced by application of *T. harazianum*. Their observation let them too concluded that *T. harazianum* was capable of inducing systemic resistance against *Macrophomina phaseolinai* by triggering defense enzyme production.

Abhayashree *et al.* (2016) applied five abiotic elecitor such as L-isoleucine, L- leucine, L-methionine, L- phenylalanine and L- proline to induced resistance in chili against *Colletotrichum capsici*, an anthracnose disease causing fungi. They reported that 50 mM concentration of the elecitors performed well to induce defense and to control the disease significantly. The abiotic elicitors could induce activity of Phenylalanine ammonia lyase (PAL) and Peroxidase (POX) enzymes.

Acharya *et al.* (2011) used five abiotic elecitors (arachidonic acid, cupric chloride, chitosan, isonicotinic acid and salicylic acid) to induced systemic resistance in *Raphanus sativus* L. Significant increase of b-1,3 glucanase, peroxidase and polyphenoloxidase was reported alonghwith a remarkable increased of nitric oxide (NO). They suggested that NO might be a signaling molecule while inducing ISR in the host by abiotic elecitors.

Al-Sohaibani *et al.* (2011) used four organic acids (ascorbic acid, salicylic acid, oxalic acid and tannic acid); four different salts (KCL, K_2PO_4, NaCl and Na_2PO_4) and two growth regulators (Indole acetic acid and indole butyric acid) to control root rot disease of sweet basil caused by three different fungal pathogens such as *Macrophomina Phaseolina, Rhizoctonia*

solani and *Fusarium oxysporum* f. sp. *basilica*. Salicylic acid effectively decreased dumping off caused by *Macrophomina Phaseolina* and *Fusarium oxysporum* f. sp. *basilica*. While oxalic acid was best effective inducers against root rot disease caused by *Rhizoctonia solani*. They showed that inducers increased POX and CHI activity.

Baysal *et al*. (2003) applied three different defense activator BTH, ASM and Bion to induced resistance in tomato against *Clavibacter michiganensis* ssp. *michiganensis*, causal organism of bacterial canker of tomato. ASM pretreated plants showed reduction in disease severity upto 76.3%. In the resistance induced plants peroxidase and chitinase increased significantly.

Walters *et al*. (2011) reported that powdery mildew disease caused by *Blumeria graminis* f. sp. *hordei* and leaf scald disease caused by *Rhynchosporium secalis* of two spring barley varieties was controlled by the combined application of three resistance elicitors acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA) and cis-jasmone (CJ) in field conditions. They also showed the effect of combined application of those elicitors on greenhouse-grown barley leaves. The treated leaves activated the systemic acquired resistance (SAR) marker gene PR1-b and suppressed the jasmonic acid (JA) biosynthesis gene LOX2.

Romanazzi *et al*. (2009) studied the effect of five resistance inducers (Chito Plant, Aliette, Kendal, Bion, and Olivis) to reduce the Bois noir (BN) infected severe diseases of grapevine. Inducer treated plants decreased the disease severity in comparison to the control one. They sprayed the inducers by weekly manner in spring-summer 2007 (seven applications) and 2008 (thirteen applications). Among the inducers Kendal, Olivis and Bion showed the better results but on the other hand in the first year Aliette and Chito plant treatments showed better results than the second year.

Perez-de-Luque *et al*. (2004) studied the effect of foliar application of three SAR activator like, salicylic acid, glutathione and benzothiadiazole (BTH) to control the broomrape infected pea disease. They reported that the

broomrape infection was controlled under the growth chamber and greenhouse conditions by the application of BTH (0.6 to 1.0 mM), in the form of Bion 50 (50% a.i.).

Boro *et al.* (2011) studied the effects of abiotic inducer acibenzolar-S-methyl (ASM) and two *Xanthomonas* species extracted biotic agents, harpin protein and glycoproteins to control the bacterial leaf spot of yellow passion fruit. They applied all the inducers through seed immersion as well as by spraying and the seedlings were inoculated at four true leaves stage. They showed that ASM or harpin treated seed protect plants up to 90% and 47% and leaf treated with ASM or the glycoproteins from *Xanthomonas* spp protect plants up to 70% and 72% respectively against inoculation of *Xanthomonas axonopodis* pv. *Passiflorae*.

Chen *et al.* (1995) studied the role of salicylic acid (SA) in plant defense against pathogens. They found endogenous SA level increased in correlation with both resistance of tobacco to infection with tobacco mosaic virus and induction of defense-related genes [such as that encoding pathogenesis-related protein 1 (PR-1)]. Newly synthesized SA was conjugated to glucose to form SA β-glucoside. They also found a cell wall-associated S-glucosidase activity that releasesd SA from this glucoside. According to them SA β -glucoside served as an inactive storage form of SA. They purified a soluble SA-binding protein and isolated its encoding cDNA from tobacco. Finally they were able to further characterize the mechanism of SA signaling. The protein was a catalase, and binding of SA and its biologically active analogues inhibited catalase's ability to convert H_20_2 to 0_2 and H_20. Thus elevated levels of cellular H_20_2 appeared to induce PR-1 gene expression, perhaps by acting as a second messenger. Additionally, transgenic tobacco expressed an antisense copy of the catalase gene and exhibitied depressed levels of catalase also showed constitutive expression of PR-1 genes. They tested several abiotic inducers of PR gene expression and disease resistance for their ability to stimulate SA production. From their experiments it was found that levels of SA and its glucoside rose following application of all of the inducers except 2,6-dichloroisonicotinic

acid. 2, 6-Dichloroisonicotinic acid was found to bind catalase directly and inhibit its enzymatic activity.

Chen *et al*. (2000) reported that root and crown rot of cucumber caused by *Pythium aphanidermatum* could be suppressed by various plant growth-promoting rhizobacteria (PGPR). They treated cucumber roots with *Pseudomonas corrugata* 13 or *Pseudomonas aureofaciens* 63-28 which stimulated the activity of phenylalanine ammonia-lyase (PAL) in root tissues in 2 days and this activated accumulation lasted up to 16 days of bacterization. Peroxidase (POX) and polyphenol oxidase (PPO) activities were also increased in roots within 2-5 days. Isoperoxidase native PAGE (polyacrylamide gel electrophoresis) analysis indicated that the peroxidase isomer forms in cucumber roots induced by rhizobacteria were different from that in roots infected with *P. aphanidermatum*. Thus the activation mechanisms of PO by the rhizobacteria were thought to be different from those of pathogen infection.

Christ and mosinger (1989) reported increase of eleven acid soluble proteins (with apparent molecular masses ranging from 13-82 kD) in tomato (*Lycopersicon esculentum* Mill.) leaves following *Phytophthora infestans* or *Fulvia fulva* infection. Prominent changes in the protein pattern were also detected in the untreated leaves of infected plants which indicated systemic effects of the infection. Similar changes in the proteins were also induced either by moderate irradiations of the leaves with UV light (254 nm) or by injecting the leaves with chemical inducers [indole-3-acetic acid, 2-chloroethyl-phosphoric acid (ethephon), fusicoccin or an elicitor preparation from *Phytophthora megasperma* f.sp. *glvcinea*]. Acetylsalicylic acid (aspirin), kinetin, and abscisic acid did not induce detectable changes in protein pattern nor did they induce resistance.

Conrath *et al*. (1995) used 2,6-Dichloroisonicotinic acid (INA) and salicylic acid (SA) as potent inducers of plant defense responses including the synthesis of pathogenesis-related (PR) proteins and the development of enhanced disease resistance. They purified a SA receptor protein (a soluble SA-binding protein) from tobacco with an affinity and specificity of binding.

The protein has been reported to be a catalase whose enzymatic activity was inhibited by SA binding. They have proposed that increase in intracellular levels of reactive oxygen species plays a role in the induction of defense responses such as PR protein gene expression. The dose-response curves for inhibition of catalase by two compounds (INA and SA) are similar. Furthermore, the ability of both INA analogues and SA derivatives to bind and inhibit tobacco catalase correlates with their biological activity to induce PR-1 gene expression and which ultimately enhanced resistance to tobacco mosaic virus. Comparison of the structures of INA, SA, andtheir analogues revealed several common features that appeared to be important for biological activity.

Cortes-Barco *et al*. (2010) showed induced resistance in *Nicotiana benthamiana* against anthracnose caused by the hemibiotrophic fungus *Colletotrichum orbiculare.* The inducers were benzothiadiazole (BTH), (2R, 3R)-butanediol or PC1, an isoparaffin-based mixture. In disease assessment experiments, BTH, (2R, 3R)-butanediol and PC1 reduced the number of lesions per leaf area caused by *C. orbiculare* to a significant extent. They also reported that foliar application of BTH induced expression of genes for the acidic pathogenesis-related (PR) proteins, NbPR-1a, NbPR-3Q and acidic NbPR-5. In contrast, soil application of (2R, 3R)-butanediol or PC1 primed expression of genes for the basic PR proteins, NbPRb-1b, basic NbPR-2 and NbPR-5dB. These results are consistent with the activation of salicylic-acid-dependent systemic acquired resistance (SAR) by BTH and that of jasmonate/ethylene-dependent induced systemic resistance (ISR) by (2R, 3R)-butanediol or PC1, and show that (2R, 3R)-butanediol and PC1 can affect gene expression similarly to plant growth-promoting rhizobacteria. The effects of (2R, 3R)-butanediol and PC1 were not identical. In addition to priming, (2R, 3R)-butanediol induced expression of basic NbPR-2, whereas PC1 treatment induced expression of both NbPRb-1b and basic NbPR-2. Although a number of microbial products, such as (2R, 3R)- butanediol, have been shown to produce ISR, but the first demonstration of an isoparaffin-based mixture (not derived from a microorganism) could produce ISR.

In 2013, Dufour *et al.* reported that a salicylic acid analogue [Benzothiadiazole (BTH)] strengthens plant defence mechanisms against a pathogens diverse spectrum. They reported the role of BTH-pretreatment in enhancing resistance against infection with various isolates of *Plasmopara viticola* and *Erysiphe necator* causing downy and powdery mildews in grapevine leaves. The authors developed some tools for better assessment of the defence status of the plant. They reported that in compatible interactions more than 57.2% of differentiated transcripts from *P. viticola* infected-leaves (Pv-infected leaves) out of a set of 19 genes were down-regulated at 24 h post-inoculation (hpi). Under similar conditions, they also showed down regulation of about 90% of differentiated transcripts from from *E. necator* infected leaves (En-infected leaves), indicating a manipulation of host responses by the pathogens. Pathogen growth was reported to be inhibited by 61–98% (depending on the pathogen isolate) following BTH treatment that enhanced grapevine defences. Up-regulation of pathogenesis-related protein genes (PR-1, PR-2, PR-3, PR-8 and PR-10) were observed by the authors in BTH treated-Pv-infected leaves. Under similar conditions of treatment and En-infection the leaves showed up regulation of PR-3, PR-6 and PR-10 genes. They also showed that treatment with BTH led to regulation of indole pathway transcripts. According to them, particularly, anthranilate synthase was down-regulated at 24 hpi in all infected leaves but strongly up-regulated afterwards in relation to rate of pathogen development. Their quantitative studies (of polyphenols and stilbenes) showed that pterostilbene was specifically accumulated in pre-treated leaves and associated with biological efficacy.

Ghosh (2015) investigated a variety of enzymatic responses of ginger plants to *Pythium* infection after induction of SAR (systemic acquired resistance). They reported that *P. aphanidermatum* lnfected a susceptible ginger cultivar which showed increased disease intensity up to 28 days but Polyphenol oxidase (PPO), Lipoxygenase (LOX) and Phenyl alanine ammonia lyase (PAL) activities were found to be increased up to 14 days. Peroxidase (PO) activity reached their peaks on 21st day after inoculation and then decreased sharply as reported. To induce SAR, the authors

soaked rhizome seeds for 1 hour prior to sowing, separately, in salicylic acid (SA-5 mM) and *Acalypha* leaf extract (ALE – 10%). They observed significant disease reduction in both SA and ALE treated plants. SA and ALE treatment, enhanced activities of all four defence related enzymes (as studied by the authors) in ginger leaves. Untreated inoculated and treated non-inoculated plants in relation to their respective controls were tested by the authors. Treated inoculated plants exhibited maximum activity for all four enzymes they studied. SA stimulated PO and PAL more than that of ALE. According to them a correlation exists between reduction of disease intensity due to SAR induction and greater stimulation of specific enzymatic activities in ginger plants. They also suggested that all four enzymes were not equally responsive to a defence activator.

Chandra *et al*. (2007) studied phenylalanine ammonia lyase (PAL) activities leading to decline in disease formation caused by *Rhizoctonia solani* following application of salicylic acid (SA). They applied 1.4 mM SA (pH 6.5) twice. There after they inoculated the plants with *Rhizoctonia solani*. Finally, they observed quantitative change in polyphenol oxidase (PPO), peroxidase (POX) isoforms and increasing activity of PAL in Bundel-1, UPC-4200 and IFC- 902 cowpea genotypes. PAL activity was increased in *Rhizoctonia solani* inoculated UPC-4200, whereas total soluble protein were significantly increased in the same genotype after SA treatment and *Rhizoctonia* inoculation. In their isoform analysis (out of ten isoforms) isoforms 7 and 10 of polyphenol oxidase and isoform 4 of peroxidase showed increased activities when SA application were done. The disease symptoms measured by them, indicated less disease formation in SA sprayed Bundel-1 and UPC-4200 genotypes in compare to controls.

Azami-Sardooei *et al*. (2013) reported great economic losses, due to grey mould caused by *Botrytis cinerea*, in greenhouse-cultivated tomato, bean and cucumber. They also investigated the effects of foliar applications of different concentrations of BTH (a chemical analog of salicylic acid) on resistance to *B. cinerea.* According to their observation increased protection of tomato against *B. cinerea* were found in case of leaf treatments with 50 mg/l BTH in one spray. In case of bean and cucumber, only concentrations

of 250 mg/l and higher were reported to reduce susceptibility against B. cinerea. The authors also reported that BTH concentrations above 100 mg/l had a negative effect on plant height, flower and fruit numbers in bean and cucumber plants under pathogen-free conditions. But in tomato only the highest BTH dose (1000 mg/l) resulted in a significant negative effect on vegetative and generative growth.

Pye *et al.* (2013) used BTH (1,2,3-benzothiadiazole-7-thio carboxylicacid-S-methyl-ester) commercially known as 'Actigard' and TDL [N-(3-chloro-4-methylphenyl)-4-methyl-1, 2, 3 -thiadiazole-5-carboxamide,] commercially known as 'Tiadinil' for induction of defense in tomato plants. BTH and TDL were examined for their role on abscisic acid (ABA)-mediated, salt-induced disease predisposition in tomato seedlings. They showed that salt stress to roots significantly increased the severity of disease caused by *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Phytophthora capsici* relative to non-stressed plants. According to their results, root treatment with TDL induced resistance to Pst in leaves and provided protection in both non-stressed and salt-stressed seedlings in wild-type and highly susceptible NahG plants. Non-stressed and salt-stressed ABA-deficient mutants were highly resistant to *Pst*. Neither TDL nor BTH induced resistance to root infection by *Phytophthora capsici*, nor did they moderate the salt-induced increment in disease severity. Root treatment with these plant activators increased the levels of ABA in roots and shoots similar to levels observed in salt-stressed plants. From their results they indicated that SAR activators could protect tomato plants from bacterial speck disease under predisposing salt stress. They were also of the opinion that some SA-mediated defense responses function sufficiently in plants with elevated levels of ABA.

2.4. Molecular aspects of defense related genes (identification and expression analysis)

In 1996, Lawton *et al.* reported that Benzothiadiazole (BTH) was a novel chemical activator of disease resistance in tobacco, wheat and other important agricultural plants. In their report, it was shown that BTH works

by activating SAR in *Arabidopsis thaliana.* They treated plants with BTH and showed that treated plant were resistant to infection by turnip crinkle virus, *Pseudomonas syringae* pv 'tomato' DC3000 and *Peronospora parasitica.* Thus, they reported the induction of resistance against viras, bacteria and fungus. According to them chemical treatment induce accumulation of mRNAs from the SAR-associated genes, PR-1, PR-2 and PR-5. They found that BTH induced both *PR-1* mRNA accumulation and resistance against *P. parasitica* in the ethylene response mutants, *etrl* and *ein2,* and in the methyl jasmonate-insensitive mutant, *jar1.* From their results, they suggested that BTH action was independent of plant hormones, whose mutants were taken in the tests. They also reported that BTH can induce both *PR-1* mRNA accumulation and *P. parasitica* resistance in transgenic *Arabidopsis* plants expressing the *nahG* gene, suggesting that BTH action does not require salicylic acid accumulation. They were also of the opinion that BTH activates the SAR signal transduction pathway because BTH-treatment failed to induce either *PR-1* mRNA accumulation or *P. parasitica* resistance in the noninducible immunity mutant, *niml.*

Xu *et al.* (2008) isolated a full-length cDNA and genomic DNA of phenylalanine ammonia-lyase gene from *Ginkgo biloba* (*GbPAL*). From their sequenced results, they found out that both the sequences of *GbPAL* were same having a gene coding region of about 2172 bp long. The deduced protein of the genes were consists of 724 amino acids with a predicted molecular mass of 79.1 kDa and a pI of 5.96. They reported that *Gb*PAL protein was highly identical to other plant PALs. According to the workers (on the basis of southern hybridization analysis of the genomic DNA), *GbPAL* belonged to a small multi-gene family. Real-time PCR analysis of tissues (tissue expression analysis) revealed that *GbPAL* constitutively expressed in all the tested tissues but high expression were reported in leaf and stem tissues. Induction of *GbPAL* has been reported by a variety of stresses including UV-B, wounding, cold and salicylic acid. On the basis of temporal expression profiling analyses, the tanscription levels of *GbPAL* were found to be significantly correlated with flavonoid accumulation.

Thus, the authors suggested that *GbPAL* might play a regulatory role in flavonoid biosynthesis in leaves of *G. biloba* at the transcriptional level.

Huang *et al.* (2010) worked with Phenylalanine ammonia-lyase (PAL) that catalyzes the first step of the phenylpropanoid pathway that produces precursors to a variety of important secondary metabolites. According to them the Arabidopsis (*Arabidopsis thaliana*) contains four PAL genes (PAL1–PAL4). They analysed combined mutations for the four Arabidopsis PAL genes. Contrary to others the workers found that three independent *pal1 pal2* double mutants were fertile and generated yellow seeds due to the lack of condensed tannin pigments in the seed coat. The *pal1 pal2* double mutants were also deficient in anthocyanin pigments in various plant tissues, which accumulate in wild-type plants under stress conditions. The authors are of opinion that, PAL1 and PAL2 have a redundant role in flavonoid biosynthesis. Furthermore, the *pal1 pal2* double mutants were more sensitive to ultraviolet-B light but more tolerant to drought than wild type plants. They also generated two independent *pal1 pal2 pal3 pal4* quadruple knockout mutants, which were stunted and sterile. Interestingly, from their study it was evident that quadruple knockout mutants contained about 10% of the wild-type PAL activity even after knocking out of the genes. Thus, they suggested about leaky PAL mutant genes or presence of one or more other unknown PAL genes. Further, substantially reduced levels of salicylic acid accumulation were found in case of the quadruple mutants that also showed increased susceptibility to a virulent strain of the bacterial pathogen (such as *Pseudomonas syringae)*. Distinct and overlapping roles of the Arabidopsis PAL genes in plant growth, development, and responses to environmental stresses were, finally, stressed by the authors.

Ziaei *et al.* (2012) studied gene expression and activity of PAL in *Ocimum basilicum* L. at different stages of growth (such as seedling stage, beginning and middle of growth phase, budding stage and flowering). The level of gene expression was analysed by semi quantitative RT-PCR and by identification of phenylpropanoid compounds (by gas chromatography/ mass spectrometry). They indicated that the level of gene expression and

activity of enzyme (PAL) were altered during the plant development, where the highest expression and activity was achieved at budding stage. In their experiment, changes of methylchvicol content were found to be correlated to the transcription and activity of PAL enzyme.

Xu *et al*. (2012) for the first time isolated a full-length cDNA of PAL gene from *Juglans regia*, and reported as *JrPAL*. The *JrPAL* gene (full-length cDNA) contained a 1935bp open reading frame which encodes a 645-amino-acid protein with molecular weight of about 70.4 kD and isoelectric point (pI) of 6.7. The deduced *JrPAL* protein was highly identical with other plant PALs. Molecular model of *JrPAL* (3D model of *JrPAL*) showed similarity to that of PAL protein from *Petroselinum crispum* (*PcPAL*). They reported that *JrPAL* might have similar functions with *PcPAL* due to their similarity in 3D molecular model. On the basis of phylogenetic tree analysis it was reported by the authors that *JrPAL* shared the same evolutionary ancestor of other PALs and had a closer relationship with other angiosperm species. They also reported that *JrPAL* was expressed in all tested tissues including roots, stems, and leaves, but the highest transcription level was found in roots. Real-time PCR (expression profiling) analyses revealed that *JrPAL* expression was induced by a variety of abiotic and biotic stresses including UV-B, wounding, cold, abscisic acid and salicylic acid.

Three different PAL genes have been isolated from the *Epimedium sagittatum* (*EsPAL1*, *EsPAL2* and *EsPAL3*). Among these three gene isolates, the metabolic accumulation as well as expression profile was studied by the authors. They found that *EsPAL3* contain high levels of active components and highly expressed in flavonoid-rich leaves and tissues, whereas *EsPAL1* highly expressed in leaves and tissues containing high lignin content (Zeng *et al.*, 2013).

Alvarez *et al*. (2013) suggested Phenylalanine-ammonia-lyase (PAL) plays an important role in resistance against *Mycosphaerella fijiensis* causal organism of Black leaf streak disease of banana. They sequenced partial or complete PAL gene from four different cultivar of banana

('Calcutta 4', 'Grain Nain', 'Yangambi Km5' and 'Williams') and secondary structures analysis and 3D model of deduced PAL protein were also done. They reported that the PAL gene expression was dependent on the cultivar and they found highest expression of PAL in 'Calcutta 4' (resistant cultivar) in the early hours of infection in comparison to 'Williams' (susceptible cultivar).

Jin *et al*. (2013) cloned a phenylalanine ammonia-lyase (PAL) gene from *Dendrobium candidum* using homology cloning and RACE. They also found the full-length sequence and catalytic active sites that appear in PAL proteins of *Arabidopsis thaliana* and *Nicotiana tabacum.* PAL cDNA of *D. candidum* (designated as *DcPAL1*, GenBank accession No. JQ765748) contain 2,458 bps and also contains a complete open reading frame (ORF) of 2,142 bps, which encodes 713 amino acid residues. The reported amino acid sequence of *DcPAL1* showed more than 80% sequence similarity (as indicated by multiple alignments) with the PAL genes of other plants. The dominant sites and catalytic active sites, which were similar to that showing in PAL proteins of *Arabidopsis thaliana* and *Nicotiana tabacum*, were also found in *DcPAL1*.According to phylogenetic tree analysis studies *DcPAL* is more closely related to PALs present in plants of orchidaceae than to those of other plants. The differential expression patterns of PAL found in protocorm-like body, leaf, stem, and root, suggested that the PAL gene had multiple physiological functions in *Dendrobium candidum*.

Hashemitabar *et al*. (2014) cloned and characterized a full length cDNA of sugarcane (*Saccharum officinarum*) phenylalanine ammonia-lyase (*SoPAL*). They also studied the Differential tissue expression pattern of the *SoPAL* transcript as well as the enzyme activity in the tillering stage of growth. They cloned 2118 bp *SoPAL* cDNA through encoding technique which contained a protein with 706 amino acids. They found highest gene expression levels of *SoPAL* transcript in the root and stem in comparison with leaves and sheath respectively but the enzyme activity of *SoPAL* was highest in the leaves.

Kim and Hwang (2014) identified the pepper (*Capsicum annuum*) *PAL* (*CaPAL1*) gene and subsequently they induced *PAL* gene expression in

pepper leaves by avirulent strain [*Xanthomonas campestris* pv. *vesicatoria* (*Xcv*)] infection. When *CaPAL1 gene was* silenced, pepper plants exhibited increased susceptibility to virulent and avirulent *Xcv* infection. They reported that PAL activity was significantly compromised during *Xcv* infection in the *CaPAL1*-silenced pepper plants. It was also observed by the authors that SA accumulation was reduced as expression of the salicylic acid (SA)-dependent marker gene *CaPR1* was found to be reduced. Reactive oxygen species (ROS) and hypersensitive cell death was also reported to be much slower in the *CaPAL1*-silenced pepper plants. Increased resistance to *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Hyaloperonospora arabidopsidis* infection in *Arabidopsis was observed due to o*ver expression (OX) of *CaPAL1* gene. Restricted *Pst* growth, increased ROS burst and cell death, and induction of *PR1* expression and SA accumulation was observed in *CaPAL1*-OX leaves. The degree of PAL activity was higher in over expressed (*CaPAL1*-OX) plants (both healthy and *Pst*-infected) than in wild-type *Arabidopsis*. Combining the two observations, the authors suggested that CaPAL1 acts as a positive regulator of SA-dependent defence signalling to control pathogens through its enzymatic activity (in the phenylpropanoid pathway).

Wang *et al*. (2014) constructed a cDNA library to obtain detailed and general data from the flowers of *Camellia chekiangoleosa*. They explored the transcriptome of *C. chekiangoleosa* and investigated genes involved in anthocyanin biosynthesis. A 454 GS FLX Titanium platform was used to generate an EST dataset. They got about 46,279 sequences and about 24,593 (53.1%) were annotated. They used Blast search against AGRIS, and 1740 unigenes were found to be homologous to 599 Arabidopsis transcription factor genes. Based on their transcriptome dataset they found nine anthocyanin biosynthesis pathway genes (PAL, CHS1, CHS2, CHS3, CHI, F3H, DFR, ANS, and UFGT). The genes were cloned and analysed the spatio-temporal expression patterns using quantitative real-time polymerase chain reaction. Their studies provided valuable information concerning anthocyanin biosynthesis study and also enriched the gene resource for further studies.

Okorska *et al.* (2014) studied part of the *PSPAL1* gene (corresponding to the proximal promoter, exon 1 and intron) from eight pea varieties. They also compared their sequences with the published sequences of *PSPAL1* gene from Midoriusui cultivar (GenBank: D10002.1). Their sequences showed a very high level of similarity (96–99%), except in five varieties where a motif TTATTACAAAATATTA close to the Goldberg-Hogness (TATA) box was found. The motif was not detected in the other four varieties, including Midoriusui. From the pathogenicity test of plants of eight pea varieties with *Mycosphaerella pinodes* and from results, the disease index was determined by the authors that ranged from 5.2 to 42.3%. The *PSPAL1* gene motif was reported to be present in most cultivar Walor (resistant cultivar) but it was not found in cultivar Polar, the most susceptible cultivar as reported. They also reported that, the relationship was not clear in varieties with intermediate levels of resistance. According to the authors, a weak negative correlation with disease severity (R=-0.53) was observed following analysis of expression level of *PSPAL1* gene in four varieties (Walor, Ezop, Ramrod and Polar) after 1, 3, 6, 9, 12 and 15 h post inoculation. They also showed that the activation of *PSPAL1* gene occurred in infected pea leaves, stems and roots but degree of expression varied a lot (with the relative level of *PSPAL1* transcripts amounting to 0.15 in roots and 38.75 in leaves). Thus, they indicated some kind of signal transmission beyond the infected plant tissues.

Optimal growth with minimal effects of biotic and abiotic stress is essential for growth of Willow as it is an important biomass crop for the bioenergy industry (Jong *et al.* 2015). They reported that phenylpropanoid pathway is responsible for the biosynthesis lignin, flavonoids, condensed tannins, benzenoids and phenolic glycosides. All the above mentioned compounds have a role in protecting the plant against biotic and abiotic stress. It has also been reported that all products of the phenylpropanoid pathway are important for the healthy growth of short rotation cropping species such as willow. However, the phenylpropanoid pathway in willow remains largely uncharacterised (Jong *et al.* 2015). They identified and characterised five willow phenylalanine ammonia-lyase (PAL) genes such as

Willow PAL1, PAL2, PAL3, PAL4 and PAL5. Four genes (PAL1, PAL2, PAL3 and PAL4) were orthologous to the poplar genes. They did not found any orthologue of PAL5 gene. Two tandemly repeated PAL2 orthologues were identified in a single contig. Willow PALs showed similar sub-cellular localisation as in poplar genes. The gene expression and enzyme kinetics of the willow PAL genes differed slightly. Willow PAL2 has been shown to be more widely expressed than its poplar orthologues and that led the authors to suggest a wider role for PALs in the production of flavonoids, condensed tannins, benzenoids, and phenolic glycosides, in willow.

Miltiadis *et al.* (2015) observed increased phenolics in mature fresh walnut (*Juglans regia* L.) kernels under cold storage condition. Based on that observation, they investigated changes in individual soluble phenolic compounds under cold stress in relation to changes in total phenols. Specific activity of phenylalanine ammonia-lyase (PAL) was also studied with the association of particular compounds with phenylpropanoid pathway. Several experiments were performed by them such as treatments with inhibitors of phenylalanine ammonia-lyase (PAL), mRNA level studies, RNA and protein synthesis, activities of the enzymes polyphenol oxidase (PPO) and peroxidase (POX). On the basis of their results, increase in specific and total activity of PAL during cold storage was found. Among phenolics they identified 2,4-dihydroxybenzoic acid and protocatechuic acid ethyl ester by HPLC–DAD–ESI-MS. They also reported increase in acids such as 4-hydroxybenzoic, 2,4-dihydroxybenzoic, syringic and vanillic with increased PAL activity. According to their studies ellagic acid was largely independent on PAL. Increase in protocatechuic acid and decreases in protocatechuic acid ethyl ester also could not be directly related to PAL.

Plant hormones play important roles in biotic and abiotic stresses in rice throughout its entire growth period. Most of the interactions of stresses in rice are not completely understood (Zhang *et al.* 2015). Zhang *et al.* (2015) determined physiological performance of rice seedlings under a single stress and a sequential combination of various stresses (intercross stress). They found that superoxide dismutase, catalase, and peroxidase

activities and malondialdehyde were highly regulated by intercross stresses. The expression levels of pathogenesis-related genes and drought stress-related genes under various treatments were also analyzed by them. In case of drought-disease intercross stress, the expression of the *PR4*, *PAL*, and *Cht-1* genes were upregulated but in case of salt-disease intercross stress, the expression levels of the *PR1a*, *PBZ1*, *Gns1*, and *Cht-1* genes changed significantly. The expression of *LOX-RLL* was significantly found to be changed regardless of the type of intercross stress. They also showed that the expression of drought stress-related genes (*OsSKIPa*, *OsNADPH1*, *JRC0594*, and *OsGL1-2*) *to be* significantly regulated.

Liew *et al*. (1998) isolated cDNA clones encoding chalcone syntbase (CHS) (EC 2.3.1.74), a key enzyme involved in flavonoid and anthocyanin biosynthesis from flowers of the orchid, *Bromheadia* finlaysoniana (Lindl.). They determined complete nucleotide sequences of the 3 clones such as *OCHS3*, *OCHS4* and *OCHS8.* The lengths of *OCHS3*, *OCHS4* and *OCHS8* were 1445, 1382 and 1439 bp, respectively. All the cDNAs contained a single open reading frame of 1 185 bp, encoding a polypeptide of 394 amino acids with molecular weight of 42.9 kDa. A high degree of nucleotide sequence similarity (> 97 %) was observed within the three cDNAs. The deduced amino acid sequences showed 76-82 % homology, but the nucleotide sequence showed 59-68 % homology to CHS of other plants.

Pang *et al*. (2005) the genomic DNA sequence of chalcone synthase (CHS) gene was cloned from *Ginkgo biloba*. The *Gbchs* was 1295 bp long and composed of two exons and one intron, one of the typical features of chalcone synthase genes. The genomic Southern blot analysis indicated that *Gbchs* belonged to a multigene family. RT-PCR analyses revealed that *Gbchs* expressed differentially in the root, stem and leaf tissues of *G. biloba*, and the expression could be induced by UV-B and wounding treatments. The recombinant GbCHS protein was successfully expressed in Escherichia coli strain M15 [pREP4] with pQE30 vector and the result showed that the expressed GbCHS protein had molecular weight of about 42 kDa, a size matching with that of the predicted one by bioinformatic analysis.

Farzad *et al.* (2005) reported that chalcone synthase (CHS), the first committed enzyme in the flavonoid biosynthetic pathway, is commonly encoded by multi-gene families with select members of these families accounting for the majority of expression. They examined the CHS gene family in *Viola cornuta*, a plant whose flowers undergo ontogenetic color change. Using both RNA and RNA/DNA samples isolated from floral tissues at different pigment stages, they obtained 14 unique sequences from 60 total clones of a 288 bp fragment from the catalytic region of CHS. The *V. cornuta* sequences were monophyletic when compared to CHS orthologs from other taxa. According to them substitution models generally indicated unequal rates of transition and transversion. They also found significant rate variation among sites. With a Tamura-Nei correction, nucleotide divergence ranged from 0.3 to 10.6% with the vast majority as synonymous changes. The nucleotide divergence pattern suggested designation of three *V. cornuta* CHS clades. Based on divergence of CHS orthologs, the reported clades were consistent with three CHS orthologs in *V. cornuta*. Sequences from only a single clade were found to be expressed in all three floral pigment stages.

Tian *et al.* (2006) presented the expression of a full-length *chs* cDNA with 1225 bp from grape seedlings as well as they prepared antibody against the expressed protein. They introduced a full-length *chs* cDNA into an expressed plasmid pET-30a (+) vector at the *Eco*RI and *Sal*I restriction sites. pET-*chs* was found to be highly expressed in *Escherichia coli* BL21(DE3) pLysS cells with isopropyl-β-D-thiogalactopyranoside (IPTG) induction. A fusion protein with the His · tag label was purifed by Ni–NTA His · Bind Resin and then used as the antigen to immunize rabbit. The resulting antibody was purified to immuno-recognize the CHS of the crude protein extracts of different grape tissues with a molecular wt. of 43 kDa.

Since the early evolution of land plants from primitive green algae, flavonoids have played an important role as UV protective pigments in plants. Flavonoids occur in liverworts and mosses, and the first committed step in the flavonoid biosynthesis is catalyzed by chalcone synthase (CHS Jiang *et al.* (2006)). They cloned and characterized CHS from the

gametophores of *Physcomitrella patens*, a moss. *PpCHS* exhibited similar kinetic properties and substrate preference profile to those of higher plant CHS. p-Coumaroyl-CoA was the most preferred substrate, suggesting that *PpCHS* is a naringenin chalcone producing CHS. Consistent with the evolutionary position of the moss, phylogenetic analysis placed *PpCHS* at the base of the plant CHS clade, next to the microorganism CHS-like gene products. Thus, the authors were of opinion that *PpCHS* was one of the oldest CHSs that appeared on earth.

Dao *et al.* (2011) reported that CHS gene expression could be induced in plants by stress conditions such as UV light, bacterial or fungal infection. According to them CHS expression accumulates flavonoid and isoflavonoid phytoalexins and also involve in the salicylic acid defense pathway.

Roslan *et al.* (2013) isolated a cDNA encoding a chalcone synthase from the leaves of *Polygonum minus* by rapid amplification of cDNA ends (RACE) and designated as *pmCHS* (GenBank accession no. JQ801338). The full-length cDNA of *P. minus pmCHS* was 1472 bp with an 1179 bp open reading frame (ORF) that corresponded to a predicted protein of 392 amino acid deduced protein. *In silico* analysis showed that the calculated molecular weight and theoretical isoelectric point (pI) of *pmCHS* were 43.1 kDa and 5.78, respectively. Several important motifs, such as the product binding site, active site and dimer interface, were also successfully identified from the deduced amino acid sequence. Multiple sequence alignment indicated that the *pmCHS* sequence was highly conserved and shared high sequence identity (>90%) with chalcone synthases from other plants. Gene expression analysis via qRT-PCR showed that *pmCHS* was most highly expressed in the roots, showing a 10-fold increase compared to leaves and a 15-fold increase compared to stems.

2.5. Management of pathogenic microorganisms by botanicals and biocontrol agents

Ravikumar and Garampalli (2013) evaluated antifungal property of 39 plant extracts against *Alternaria solani* a pathogen of early blight of

tomato by poison food technique in Potato Dextrose Agar medium. From their results, they found that out of 39 plant extracts 13 plants extracts significantly reduced the mycelial growth of the pathogen they tested. Seven plant extracts *Crotalaria trichotoma*, *Citrus aurantifolia*, *Azadirachta indica*, *Polyalthia longifolia*, *Datura metel*, *Muntingia calabura*, and *Oxalis latifolia* showed maximum inhibition (above 20%) of the disease at 4% concentration and six extracts *Crotalaria trichotoma, Azadirachta indica, Polyalthia longifolia*, *Datura metel*, *Capsicum annum* and *Citrus aurantifolia* showed significant growth inhibition at 2% concentration.

Falade (2017) tested the *in vitro* effect of 30, 50 and 65% concentrations of six plant extracts. Plants they selected were *Tridax procumbens, Jatropha gossypifolia, Sida acuta, Blighia sapida, Ricinus communis* and *Datura stramonium* on growth, conidial germination and sporulation of *Colletotrichum lindemuthianum*. Their results showed that the extracts of all six plants did not have any inhibitory effect on conidial germination and sporulation but significantly reduced the growth rates of fungus in comparison to the control. The maximum growth inhibition rate was to be found at 65% concentrations. Out of six extracts *Datura stramonium, Ricinus communis* and *Jatropha gossypifolia* showed significant effect and reduced the growth while *Blighia sapida* caused the least inhibition of growth. The growth inhibition rate of *Datura stramonium* at 30, 50 and 65% concentrations were 10, 16 and 33% respectively whereas *Blighia sapida* showed 2, 8 and 10% respectively.

Pawar (2011) examined antifungal activity of 18 plant leaf extracts against 5 seed-borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichoderma viride* by using cup-plate method. From their results it was observed that nine plant leaf extracts showed antifungal activity. *Azadirachta indica* leaf extracts showed maximum activity; while *Holoptelia integrifolia* leaf extracts showed minimum activity against the pathogenic fungi they tested.

Parimala and Sangeetha (2016) reported the antifungal activity of *Ricinus communis* leaf extracts against fungal isolates *Aspergillus niger*,

Aspergillus flavus, *Aspergillus fumigatus*, *Aspergillus terreus, Penicillium chrysogenum, Rhizopus stolonifer, Fusarium oxysporum*, *Candida albicans, Candida krusei, Candida tropicalis* and *Candida glabrata*. Methanol extracts showed highest inhibition zone; while minimum inhibition zone was observed in aqueous extracts against the fungal isolates.

In 2005, Peraza-Sanchez *et al*. screened 7 Yucatecan plant extracts and tested for fungicidal activity against *C. gloeosporioides*. They purified root extract of one of the most active plants, *Acacia pennatula,* following bioassay. They isolated new compound 15,16-dihydroxypimar-8(14)-en-3-one. Inhibitory activity on growth, sporulation, and germination of the fungus was observed by the isolated compound in "agar dilution" bioassay *in vitro*.

Deepak *et al*. (2005) screened antisporulant activity of forty commonly growing plant species of India. They used methanolic extracts against *Sclerospora graminicola*, the causative organism of pearl millet downy mildew. Out of the plant extracts tested, they showed that the extracts of 11 species (*Agave americana, Artemisia pallens, Citrus sinensis, Dalbergia latifolia, Helianthus annus, Murraya koenigii, Ocimum basilicum, Parthenium hysterophorus, Tagetes erecta, Thuja occidentalis* and *Zingiber officianale*) exhibited antisporulant effect even after 10-fold dilution of the crude extracts.

Bioactive compounds from lipophilic leaf extracts of medicinal plants (used by Himalayan people), were screened for antifungal properties by Guleria and Kumar (2006) by direct bioautography. Two fungi (*Alternaria alternata* and *Curvularia lunata*) were used as test organisms in bioautography. They evaluated fungal growth by measurement of radial growth. Out of 12 plants tested, they showed five plant species showed antifungal activity. They used $CHCl_3$: CH_3OH (1:9, v/v) as a solvent to develop silica gel TLC plates. Lipophilic extracts of *Vitex negundo*, *Zantoxylum alatum*, *Ipomea carnea*, *Thuja orientalis* and *Cinnamomum camphora* showed clear inhibition zones on TLC plates. According to them *T. orientalis showed* best antifungal activity.

Thirty aquous plant extracts were screened *in vitro* against *Sclerotium rolfsii* by Kiran *et al.* (2006) to examine the inhibitory effect on mycelial growth and sclerotial production. Plant extracts of *Prosopis juliflora* (10% concentration) inhibited (74%) of mycelial growth. Two other plant extracts (*Agave Americana* and *Nerium indicum*) also showed antifungal activity by inhibition of growth. But, best inhibition of sclerotial production was shown by *Agave americana* and *Clerodendron inerme.* Leaf and fruit extract of *Riccinus communis* could also inhibit sclerotial production.

Antifungal efficacy of cloves against *Aspergillus* spp was reported by Reddy *et al.* (2007). They isolated, characterized and tested the components of cloves. They identified eugenol as a major component on TLC plate as dark coloured spot with R_f 0.5 along with standard. In TLC plate bioautography test, TLC plates were spray-inoculated with four species of *Aspergillus* (*A. flavus*, *A. paraciticus*, *A.niger*, *A. ochraceus*) and they reported that eugenol on TLC plates inhibited mycelia growth of all four species of *Aspergillus*.

Antibacterial activity of seven semi purified plant extracts made from flowers, leaves, fruits, stems, pods and seeds of some plants and four antimicrobial chamicals were evaluated Meena *et al.*(2007). The bacterial plant pathogens used for the purpose were *Pseudomonas solanacearum*, *Xanthomonas campestris* pv. *Campestris*, *Xaxonopodis* and *Xanthomonas* pv. *Citri.* They followed disc diffusion method to test the antibacterial activity. Product componantes from mahua flowers and Satyanashi leaves were found effective, at 1000 ppm, against *Pseudomonas solanacearum*.

Mewari *et al.* (2007) tested two mosses viz. *Entodon plicatus* C. Muell and *Rhynchostegium vagans* jaeg for their antimicrobial activity against *Bipolaris sorokiniana, Fusarium solani, Pseudomonas sclanacearum*, and *Xanthomonas oryzae.* Aquous extracts of the two mosses were ineffective. Ethanolic extracts of E. plicatus and petroleum ether extract of *R. vagans* showed inhibitory effects against *B. Sporokiniana.* Extract of *R. vagans* were more effective inhibitors of *F. solani* than those of *E. plicatus*.

Antifungal activities of leaf extracts of four plant species (*Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* and *Catunaregum spinosa*) have been studied Malabadi and Vijay kumar (2007). They evaluated the of dichloromethen, aceton, hexane, and methanol extracts of leaves against four pathogens viz. *Candida albicans*, *Kluyeromyces polysporus*, *Aspergillus niger*, *Aspergillus fumigantus*. On the basis of MIC values of methanolic extract of *Anaphylis wightiana it was reported that the plant extract was highly antifungal* against particularly *C. albicans* and *K. polysporus.*

Broad spectrum of antimicrobial activity on human pathogenic microorganisms of six bacteria and two fungal strains by ether and ethyl acetate extracts *Crotalaria madurensis* was studied by Bhakshu *et al.* (2008). The plant is an endemic medicinal plant found in the forest of Nallamallias of Eastern-ghat of India.

Antifungal activity of essential oils of some medicinal plants was studied by Bansod and Rai (2008). They screened the activity against *A. fumigatus* and *A. niger* by determination of MIC. The oil of plants they found to be antifungal were *Cymbopogon martini, Eucalyptus globules, Cinnamomum jeylenicum*, *Cymbopogon citrates.* Antifungal activity of some plants oils was similar to control by Miconazole nitrate. The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The oils *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare* and *Elettaria cardamomum* showed comparatively low activity against the two pathogens.

Salar and Suchitra (2009) evaluated antimicrobial activity of different parts (roots, stems, leaves and fruits) of *Solanum xanthocarpum* against bacteria and fungus. They extracted the antimicrobial properties of the plant parts in aqueous and organic solvents viz. ethanol, benzene, acetone and methanol. They studied the activity (antimicrobial) against Gram-positive (*Staphylococcus aureus*, *S. epidermidis*), Gram-negative

(*Escherichia coli, Pseudomonas aeruginosa*) bacteria and the fungus *Aspergillus niger*.

Thembo *et al*. (2010) screened the antifungal activity of aqueous, hexane, dichloromethane and methanol extracts of four weeds (*Tagetes minuta*, *Lippia javanica*, *Amaranthus spinosus* and *Vigna unguiculata*) against four fungi (*Fusarium verticillioides*, *F. proliferatum, Aspergillus flavus* and *A. parasiticus*). All extracts except water extracts of *Vigna unguiculata* and *Amaranthus* spinosus showed antifungal activity against *Fusarium* spp.

Johnny *et al*. (2011) studied antifungal activities of 15 selected medicinal plants such as *Alpinia galanga* (L.) Willd.,*Alstonia spatulata* Blume., *Annona muricata* L., *Blechnum orientale* L., *Blumea balsamifera* L., *Centella asiatica* L., *Dicranopteris linearis* (Burm. f.) Underw., *Dillenia suffruticosa* (Griff ex Hook.f. and Thomson) Martelli, *Litsea garciae* Vidal., *Melastoma malabathricum* L., *Momordica charantia* L., *Nephrolepis biserrata* (Sw.), *Pangium edule* Reinw., *Piper betle* L. and *Polygonum minus* Huds., against pathogenic fungus, *Colletotrichum capsici*. They used methanol, chloroform, acetone and Kocide 101 leaf extracts. *Piper betle* extracts in all the solvents have shown antifungal activities against *C. capsici*.

Aye and Matsumoto (2011) selected sixteen naturally available phytoextracts and tested *in-vitro* for their potential to control phytopathogens of rice, such as *Rhizoctonia solani, Rhizoctonia oryzae, Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum*. Four plant extracts (Clove, Neem, rosemary and pelargonium) showed significant antifungal activity against the rice pathogens mentioned above.

Naz and Bano (2012) investigated the antimicrobial activity of methanol, ethanol and aqueous leaf extracts of *Ricinus communis* against gram positive bacteria like, *Bacillus subtilis* and *Staphylococcus aureus*; gram negative bacteria like, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as well as against some fungal strain such as, *Aspergillus fumigates* and *Aspergillus flavus*. Methanol leaf extracts showed potential

activity to reduce the growth of pathogenic bacteria and fungal strain better than the ethanol and aqueous leaf extracts.

Ishnava *et al.* (2012) *In vitro* screened the antifungal activity of leaf extracts against nine different fungi by agar diffusion test and minimum inhibitory concentration (MIC). Out of 17 plants leaf extracts *Ocimum sanctum*, *Datura metel, Adhatoda vasica, Holoptelea integrifolia* and *Aegle marmelos* showed maximum antifungal activity against *Alternaria* sp., *Aspergillus parasi*, *Aspergillus nidulans*, *Aspergillus flavus* and *Trichoderma harzianum*.

Pandey *et al.* (2012) reviewed the pathogenicity of *Colletotrichum gloeosporioides* a major causal organism of mango anthracnose disease and its botanical management. They showed that phytoextracts of *Azadirachta indica* was most effective botanical to reduce the radial growth of the fungas. The activity of the plant extract was shown to be similar with that of *Trichoderma* spp (a biocontrol agent).

Crude extracts of different plants such as Neem, Tulsi, Onion, Garlic, Basak, Leucus, Ginger, Turmeric, Ashwagandha, etc. have been used as effective phytoextracts *i.e.* botanicals, against many pathogenic fungi and bacteria (Baljeet *et al.*, 2015; Jain *et al.*, 2015; Ambareen *et al.*, 2015). Other than botanicals many microorganisms like *Bacillus*, *Pseudomonus, Tricoderma* etc. have been used as biocontrol agents to control several plant pathogens. They suppress the pathogen either by producing a specific toxin or by preventing establishment of other microorganisms through competition or other modes of action (Arunachalam and Sharma 2012; Shaikh and Sahera, 2016).

Padder *et al.* (2010) evaluated the efficacy of three bioagents (*Trichoderma viride*, *T. harzianum* and *Gliocladium virens*) and five biopesticides (Achook, Neemgold, Wannis, Spictaf and Neemazal) against *Colletotrichum lindemuthianum.* All the three antagonistic fungi significantly could inhibit mycelial growth. Among the tested bioagents and biopesticides, *T. viride* and Wanis (1000 µl/ml) were reported to be most effective to reduce the seed borne infection. They also found that disease

could effectively be managed by seed-dressing either with Bavistin or biopesticide followed by foliar treatment of fungicide or biopesticide.

Pallavi *et al*. (2012) isolated three *Bacillus* strains (MB1, MB2, and MB3), and three *Pseudomonus* strains (MP1, MP2, and MP3) from various soil sample and evaluated their efficacy as biocontrol agents against *Pestalotiopsis theae*, a causal organism of grey blight disease of tea. They found that selected six strains showed highest antagonistic effect against the pathogen.

Fitsum *et al*. (2014) evaluated the efficacy of three biocontrol agents (*Trichoderma viridae, T. harzianum,* and *Pseudomonus fluoroscens*) by dual culture and double dilution microtiter method against *Colletotrichum lindemuthianum*. Anthracnose disease of *phaseolus vulgaris is* caused by *Colletotrichum lindemuthianum*. They found that *T. viride* showed highest effect to inhibit mycelia growth followed by *T. harzianum* and *P. fluorescens.*

Koley *et al*. (2015) studied the efficacy of six bio-control agents (BCAs) and 12 botanicals against fungus *Alternaria solani* causing early leaf blight of tomato by *in-vitro* growth inhibition technique. Among the BCAs *Bacillus subtilis* and among the botanicals *Datura stramonium* showed significant control of the pathogen in in vitro studies.

Mardanova *et al*. (2017) isolated two *Bacillus* strains (GM5 and GM2) from the rhizosphere soil of potato roots and evaluated their antagonistic activity against phytopathogenic fungi *Fusarium solani* and F. *oxysporium*. According to them GM5 strain was more effective than the GM2 strain to inhibit the growth of fungus *in vitro*. Thus they reported the efficacy of the two strains as biocontrol agents.