6. CONCLUSION AND FUTURE OUTLOOK

The annual crop productivity of economically important plants are severely declined because of pathogenic fungi, viruses, nematodes and bacteria. This indirectly causes several socio-economic and cultural impact on human beings. Thus, a systematic and comprehensive understanding of the biochemical, physiological and molecular impacts of pathogens on plants is essential for designing an eco-friendly microbicidal agent to control plant diseases.

Phytopathogenic fungi release premeditated molecules during the invasion process that contribute to the establishment of the infection. These compounds can be suppressors or host-specific toxins for suppression of host defense responses, non-host-specific toxic metabolites that account for the development of specific symptoms or host cell wall degradation enzymes for favouring penetration and creating oxidative stress via reactive oxygen or nitrogen species (ROS/RNS).

*Alternaria* belongs to the Class Deuteromycetes, Order Moniliales and Family Dematiaceae. Its conidia are long, dark coloured produced in chains. The spores are broadest near the base and taper gradually to an elongate beak
with transverse and vertical septae. During infection, *Alternaria* produces spots with a series of concentric rings on the host.

Sesame (*Sesamum orientale* L.) is one of the ancient oil crops used by human beings from decades. The cultivated sesame derives from wild populations originated from South Asia, especially the western Indian peninsula. The present study was an attempt to unravel the ROS cycle in *Sesamum orientale* var. *thilarani* against *Alternaria sesami* and to develop an effective biocounter.

Scanning electron microscopy revealed the mode of penetration of the pathogen into the host tissue via stomata with or without appresorium. Internal mesophyll damage was also visualized. Significant levels of free radicals and ROS were confirmed specifically by quantitative analysis of superoxide (O$_2^-$) radical and H$_2$O$_2$, which in turn induce lipid peroxidation. Analytical data further supported by histochemical localization results. Transmission electron microscopic analysis of cellular organelles like chloroplast and mitochondria of infected leaves showed anomaly, compared with the healthy control (Fig. 33). Primary metabolic products such as carbohydrates, total protein, free amino acids, proline showed variability due to pathogenicity of *A. sesami*. In the case of pigmental analysis, chlorophyll a was more affected than chlorophyll b. Meanwhile, carotenoids showed an increase. Cell wall materials like cellulose, pectin decreased, while hemicellulose and lignin increased during infection.
Fig. 33 Flow diagram representing the impact of ROS in sesame due to *A. sesami* infection and its rejuvenation by plant extracts / biocontrol agent
Host cell wall damage further supported by the potassium leak. Total phenols and fractionated phenolic acids of diseased plants displayed variations from control. FT-IR finger printing of infected plants revealed low intensity peaks of functional molecules compared with the control.

H⁺-ATPase, the enzyme facilitates transportation of nutrients showed poor activity. The key enzyme of phenyl propanoid pathway - Phenylalanine ammonia lyase (PAL) increased from the 1st day to 9th day after infection and then decreased. Similarly, polyphenol oxidase (PPO) showed down regulation in the infected variety-Thilarani compared to the controls. Isozyme pattern also showed varied expressions among catalase (CAT), esterase (EST), acid phosphatase (ACP), peroxidase (POX), polyphenol oxidase (PPO) and superoxide dismutase (SOD). Endonuclease showed an increased activity during infection suggesting the nuclear DNA damage of the host cells due to oxidative stress induced by the pathogen via ROS cycle.

Activity profile of antioxidant machinery enzymes such as NADPH oxidase and superoxide dismutase substantiates the quantified data of $O_2^{\cdot-}$ and $H_2O_2$ content. $H_2O_2$ scavenging enzymes such as catalase and peroxidase were active up to 9th day after infection and then declined. Ascorbate and glutathione contents corroborate with ascorbate peroxidase and glutathione reductase activities. The enzymes monodehydro ascorbate reductase showed marginal increase to recycle the reduced ascorbate to scavenge the $H_2O_2$ via the ascorbate-glutathione cycle.
Diversity among 15 sesame varieties characterized for oil content, H$_2$O$_2$ level and DNA polymorphism by random amplified polymorphic DNA (RAPD) markers. A cluster analysis based on DNA polymorphism separated the varieties into two major groups and a single variety forms a minor group, suggesting high degree of polymorphism among the varieties. Moreover, the differential display of mRNA expression noticed in the KYM-1 and Thilarani throw sufficient insight on the detection of the marker genes responsible for the biosynthesis of the oil or resistance.

Common strategy for developing a biological control consists of selecting antagonistic microorganisms or plant extracts and studying their modes of action. In this part, aqueous extract of eight higher plants and two microbes were analyzed for biological control. Among these, *M. scandens* revealed a pool of phytochemicals in the essential oil and phenolic acids by GC-MS and RP-HPLC analysis respectively. Further, remarkable changes in the activity of phenylalanine ammonia lyase (PAL), peroxidase (POX), PPO, total phenolic content and isozyme pattern of superoxide dismutase (SOD) in extract treated sesame was noticed. Foliar application of a talc-based formulation *Pseudomonas fluorescens* isolate induced a high profile of defense-related proteins *viz.*, chitinase, β-1, 3 glucanase, POX and PPO in sesame. The native-PAGE of these enzymes substantiated the assay data.
The aquatic fern, *Salvinia molesta* extract was also employed as a fungicide. Plant extract of the fern contains 16 isoprenoid cytokinins, which induce resistance in infected sesame by activating mitogen activated protein kinase-4. Pot and field oriented studies for two consecutive years were carried out for assessment of disease after the treatment with plant extracts or biocontrol agent. In all the cases, significant reduction in disease severity and disease incidence was noticed when compared with the control and synthetic fungicide.

Thus, the present study outlines the plausible defense mechanisms of Thilarani, the variety of sesame to defend themselves against *A. sesami* by activating antioxidant enzymes to an extent and the disease management strategies are trialed by antagonistic microorganisms and plant extract based control.

**Studies planned for future perspectives include the following:**

The ligno-cellulolytic enzymes of fungus are responsible for invasion into the host tissue. So the isolation, assay, purification and characterization of cell wall degrading enzymes will provide additional information to design strategies to inhibit these enzymes, thereby regulating the invasion of pathogens in the host.

Disease resistance (DR) proteins of plants identify the disease-causing pathogens by recognizing specific pathogen effector epithets that produced during the infection process and its signal transduction. These probably evolved
to subvert diverse host processes for promotion of the pathogen multiplication. The roles of DR proteins require phosphorylation, protein degradation, specific localization within the host cell and signalling specific DR gene pathways. Genomics and proteomics tools used to investigate the role of DR proteins in plant disease resistance.

Like ROS, reactive nitrogen species (RNS) also activates mitogen-activated protein kinases (MAPK). Up and down-regulation of MAPK gene expression control localized programmed cell death is characteristic of the tolerance response in the plants. Therefore, RNS orchestrate events following pathogen challenge to be traced for effectively controlling the plant diseases. Future studies planned in this direction also.

Resistant genes confer quantitative levels of disease resistance to multiple pathogen races and another step towards effective crop protection. Pathogen associated molecular patterns (PAMPs), effector proteins, PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) approaches have great potential in host pathogen cross reactions. Approaches towards these directions are also planned.

Phytopathogen phenotypic plasticity may have transgenerational consequences in the host and their heritability probably have an epigenetic component. Epigenetic epidemiology has currently emerged as a promising area for research on diseases. Incorporation of epigenetic inheritance and epigenetic plasticity mechanisms to evolutionary models and empirical studies
of host–pathogen interactions will provide new insights into the evolution and co-evolution of plant-pathogen associations.

Chemical communication among fungi revolutionized the plant pathogen interactions. Fungi monitor their population density through the synthesis and detection of small signalling auto inducers or quorum sensing (QR). These molecules regulate cell division, production of virulence factors, motility, sporulation etc. Future study includes identification and characterization of these QR sensors by in vitro culture techniques.

Techniques for spatial analysis of metabolites such as sucrose, glucose, and ATP at the single cell dimension, application of the non-invasive method of chlorophyll fluorescence, gene expression, assimilate and secondary metabolites levels, and microbial spreading, imaging, further, non-invasive techniques like fluorescence-labelled pathogens, reporter gene expression, $^{11}$C-labelled assimilates, FRET sugar nanosensors, multicolour fluorescence imaging and spontaneous photon emission also additionally enrich stress phytopathogenic physiological studies. Works are directed in this way to get a complete spectrum of host pathogen interplay.

Similarly, functional approaches to modulate carbohydrate metabolism and signalling are required to investigate how the carbohydrate status and its regulation influence plant-pathogen interactions. Combined investigations of both host plant and pathogen using modern imaging technology and functional
approaches are outstanding to understand the molecular and physiological basis for plant-pathogen interactions. This information also supports the development of programmes to enhance pathogen resistance in plants for practical applications.

Plant associated microbes play significant functions for plant growth and development. The mode of action based on optimal nutrient acquisition and hormonal induction. Different processes are involved in the inhibition of plant pathogens, which leads to plant growth. Dependent on their way of action and effects, these microbes can be employed as biofertilizers, plant strengtheners, phytostimulators or biopesticides. Therefore, designing of potential microbial inoculants by agricultural biotechnology is also planned.

Despite the diversity of phytochemicals and its functions, the biochemical processes underlying plant-pathogen interaction are still unknown. These molecules bind to receptor regions of various pathogen proteins such as keys into locks and function as microbicidal. Studies are warranted in this direction to unravel the mechanism involved between the polyphenols and pathogen proteins.

The data from further studies related with understanding of molecular complexities of host resistance induction by bioagents will provide additional knowledge for the management of plant diseases in future.