SUMMARY & CONCLUSIONS
The main objectives of the present investigation were to understand the metabolism of the bacterial pathogen, *Xanthomonas axonopodis pv vesicatoria*, the initial leaf spot of chilli and the host-pathogen interaction of the chilli plant and *X a pv vesicatoria*. The study broadly covers three main aspects of this disease:

1. **Diagnosis of the leaf spot of chilli, Isolation and characterization of *Xanthomonas axonopodis pv vesicatoria* and Host-range studies of the bacterium**

2. **Physiological and Biochemical analysis of *X a pv vesicatoria* and the host plant during pathogenesis [using virulent isolate of the pathogen (XavS) and two chilli cultivars (a highly susceptible, LCA-304 and a highly resistant Pusa jwala)]**

3. **Management of leaf spot of chillies.**

The pathogen was isolated and characterized by morphological and biochemical tests for species identity as *X a pv vesicatoria* The bacterium grew luxuriantly in a medium supplemented with 1% sucrose and 0.1% casein hydrolysate as carbon and nitrogen sources. Among the inorganic amendments tested, calcium alone proved to be the best; however, a mixture of elements along with 0.1% yeast extract supported excellent growth. *In vitro* nutritional studies of the pathogen indicated maximum growth, when the medium was supplemented
with sucrose, mannose and glucose as carbon sources, glutamic acid, aspartic acid and asparagine as organic nitrogen sources and ammonium chloride as inorganic nitrogen source. Though the bacterium had no specific requirement for any vitamin-B sources, thiamine followed by riboflavin and niacin were somewhat the best stimulatory. An amino acid mixture, in combination with the above three vitamin-B sources, supported excellent growth.

Host-range studies on solanaceous and non-solanaceous members showed the survival of the pathogen on *Lycopersicon esculentum*, *Solanum nigrum*, *Physalis minima*, *Datura innoxia*, *Argemone mexicana* and *Tinospora cordifolia*. *Lycopersicon esculentum* followed by *Solanum nigrum* showed high disease intensity, although less intense than *Capsicum frutescens*. Under natural conditions, each of these host plants is susceptible to infection by *X. a pv. vesicatoria*. It is concluded that this pathogen on non-host plants may provide a continuous inoculum for chilies grown near on with them.

Of the six chilli cultivars tested *Pusa jwala* was resistant (small lesions) and LCA-304 was susceptible to *X. a pv. vesicatoria*. These two varieties were used for further physiological and biochemical comparison at five different stages of disease development. The disease progressed through five distinct stages when 45 day old plants were inoculated. The early stage was characterized by small, round to irregular water-soaked lesions, which later enlarged as chlorotic spots, turned into necrotic and dark brown. These later turned to angular lesions, coalesced to become brittle, rapidly dry out and leaves may fall off or disintegrate.
Bacterial leaf spot caused by \( X. a. vescatoria \) in chilli is ideal for biochemical studies because it is confined to a small area of diseased tissue and symptoms are produced rapidly. The biochemical analysis was carried out quantitatively both in healthy and infected tissues. Infection resulted in the depletion of chlorophylls, starch, nitrogen and lipid fractions and ascorbic acid oxidase activity, and accumulation of carotenoids, reducing and non-reducing sugars, total amino acids, total proteins, nucleic acids, total phenols and activity of active-oxygen producing and scavenging enzymes (peroxidases and catalase) and defense related enzymes (polyphenoloxidases and phenylalanine ammonia lyases) in inoculated plants compared to healthy ones.

The reduction in chlorophyll may be due to higher chlorophyllase activity. The reduction in starch content may be due to reduced synthesis of starch or to enhanced hydrolysis of the same as a result of increased activity of the enzyme amylase. The decrease in nitrogen fractions may be due to utilization by the bacterium. The gradual decrease in phospholipid content in inoculated leaves also might be due to oxidation of phospholipids for pathogen utilization.

An increase in carotenoid content in susceptible inoculated leaves could induce susceptibility by suppressing production of an active oxygen species without which, induction of phenolics, phytoalexin synthesis and lignification in host plants could not take place. An observed increase in total amino acids in
infected leaves could be due to degradation of host proteins into free amino acids by pathogen proteases or due to marked accumulation of a few amino acids like phenylalanine or tyrosine suggesting the inhibition of protein synthesis in infected tissue.

Proteins synthesized by the pathogen might be responsible for the observed increase in total protein in susceptible infected tissues. Electrophoretic studies revealed that β-1, 3-glucanases (PR-2 group) and chitinases (PR-3 group) as the pathogenesis-related proteins co-ordinately induced in infected leaf tissues of susceptible plants compared to healthy plant.

The increase in nucleic acid content in susceptible inoculated plants may be due to pathogen nucleic acids that accumulated in tissues or enhanced activation of host nucleic acid synthesis as a result of infection, or both. Protease and RNase activities were higher in inoculated susceptible plants than in resistant inoculated plants. High protease activity was observed in the bacterial cell mass than in culture filtrate.

Permeability of infected leaves was greater than healthy plants and indicates infection may damage cell membranes to increase requisite nutrients for the pathogen's growth and multiplication. This resulted in the development of water soaked lesions, the initial symptom of the disease. Along with the electrolyte leakage, leakage of phenols also takes place.
The increase of total phenols in inoculated leaves showed that phenol synthesis occurred not only with aging of plants, but also a stimulation as a result of infection. This may be due to a prolonged reduction potential in oxygen infected tissues and inhibition of peroxidase and phenylalanine ammonia lyase in vivo for a prolonged period, thus favouring the development of the leaf spot symptoms.

Alterations in enzyme activity due to infection were quite evident. Increased peroxidase activity in susceptible plants may enhance membrane permeability, but probably are not involved in resistance. Higher amounts of catalase in healthy resistant plants than in healthy susceptible plants, indicate a potential role of catalase in resistance. It might be stated or concluded that chilli plants respond to leaf spot infection by adjustment of its metabolic process. Increased levels of peroxidase and PPO would be needed to neutralize the peroxide radical formed during pathogenesis. Similarly it also responds by increasing levels of phenols. Such biochemical changes manifested by the plant as a stress response would, ultimately affect the crop yield of chilli.

Peroxidases and PPO and phenolic compounds are involved in the browning reaction. PPO released in the cells commence oxidising polyphenols increased in advance and form quinines. These oxidised substances easily react on amino group containing proteins and amino acids to produce melanin like coagulated substance. These substances result in the formation of black or dark.
brown lesions or spots on leaves. Beta-amylase is the enzyme responsible for the degradation of starch to sugars, the activation of which will result in reduction in starch content. In the present study, the increased activity of beta-amylase in infected tissues is responsible for the observed decrease in starch content and increase in sugars in infected tissues during various stages of disease development.

**Schematic representation of various stages during mechanism of symptom expression in leaf spot disease of chillies**

Use of chemical and biological inducers of resistance showed that aspirin, followed by copper sulphate and mercuric chloride sprays at 1000 ppm reduced lesion number and size. The highest chemical concentration (1000 ppm) was more effective than 500 and 200 ppm. Of the five antibiotics tested, penicillin, followed by streptomycin, tetracycline, and chloramphenicol, were comparatively more effective at concentrations of 1000 ppm than lower concentrations (aspirin,

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CuSO₄ etc.) Inhibitory effects of plant extracts against the pathogen showed *Bixa orellana*, *Melia azadirachta*, *Clerodendrum inerme*, *Azadirachta indica*, *Murraya koenigii* and *Parthenium hysterophorus* were the best control agents of the pathogen. The above plants extracts provide an effective formulation of chemotherapeutant against bacterial leaf spot of chillies.

The greatest current interest in future research is in the application of modern molecular techniques and their integration with conventional procedures to understand and modify host-pathogen interactions. Integration of biological and chemical control systems will probably continue to receive much attention in disease management. The complex interactions that take place between the host and indigenous pathogenic microbiota needs to be continually considered during the development of commercial microbial products. Interest in natural products as sources of plant protection compounds for bacterial, fungal and viral diseases should recognise the need for isolation, characterization and structural identification of active antimicrobial compounds.