INTRODUCTION

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Tomato (*Lycopersicon esculentum*) is one of the most popular vegetables in the world. It is the second most important vegetable crop next to potato (Ojha and Chatterjee, 2012). The continued importance of tomato as a vegetable and salad commodity is reflected by the large volume of research on virtually all aspects of the crop. Salad tomatoes must have a flavour, colour and texture that satisfy the consumer's preference (Passam *et al.*, 2007). Tomato belongs to the family Solanaceae and is a major summer vegetable crop that has achieved tremendous popularity over the last century. It is considered as the 'pillar of kitchen gardening'. It forms the companion of salads, various foodstuff compositions and sauces (Noureen *et al.*, 2010).

Tomatoes are commonly classified as 'Determinate' (bush type bearing fruits all at once and topping off at a specific height) or 'Indeterminate' (developing into vine that never tops off and continues producing fruits until killed by frost). 'Determinate' tomatoes are preferred by commercial growers as they can harvest the whole field at once. Indeterminate tomatoes are preferred by home growers and small farmers who like to sell ripened fruits in the market throughout the season (Khan *et al.*, 2011). It has great nutritional importance such as vitamins A, C, thiamine, niacin, riboflavin and minerals like iron and calcium. The need to increase nutrient production has suffered some set back due to infection of the roots and fruits by microorganisms in the field and on storage (Suleiman, 2011).

Plants in their natural habitats are exposed to a number of organisms and they respond to pathogen attack by activating a wide variety of protective mechanisms (Ojha and Chatterjee 2012). Soil- borne pathogens such as *Pythium aphanidermatum* are a major threat for vegetable production in the humid tropics. *Pythium aphanidermatum*, one of the causes of root rot disease of tomato is a destructive soil-borne pathogen (Suleiman, 2011).

Among the fungal diseases, the damping-off disease caused by *Pythium* species is responsible for more than 60 per cent mortality of seedlings both in the nursery and in the main field and is widely distributed throughout the world. The main causal agent of the damping-off in tomato is *P.aphanidermatum* (Muthukumar *et al.*, 2011). It occurs in two phases – the pre-emergence damping off phase and the post-emergence damping-off phase (Dar *et al.*, 2012).

Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man (Doornbos *et al.*, 2012).

Fungal disease control is achieved through the use of fungicides which is hazardous and toxic to both people and domestic animals. This leads to environmental pollution. Therefore, a more balanced, cost effective and eco-friendly approach must be implemented and adopted by farmers. Use of natural enemies to control disease is termed biological control. It is an alternative to the use of chemical pesticides. Biological fungicides may act to suppress the population of the pathogenic organisms through competition with pathogenic organisms. Stimulated plant growth, may allow plants to quickly outgrow any pathogenic effects or damage the pathogen by means of toxins produced. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms (Kader *et al.*, 2012).

Bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plant's physiology to a greater extent, especially considering their competitiveness in root colonization. Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas others trigger beneficial effects (Saharan and Nehra, 2011).

Plant Growth-Promoting Rhizobacteria (PGPR), a group of root associated bacteria intimately interacting with plant roots and consequently influencing plant health and soil fertility. They offer an excellent combination

of traits useful in disease control and plant growth promotion. This group can produce bioactive substances to promote plant growth and protect them against pathogens (Almoneafy *et al.*, 2012).

A large number of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Rhizobium* and *Serratia* have been reported to enhance plant growth (Kumar *et al.,* 2012).

The successful application of *Trichoderma* species for the management of damping-off caused by *Pythium* species in chilli and tomato has been previously reported (Muthukumar *et al.*, 2011). *Pseudomonas cepacia* or *Pseudomonas fluorescens* applied to pea seeds acts as biological control agent against *Pythium* damping-off and Aphanomyces root rot which were able to reduce the disease incidence (Kader *et al.*, 2012).

Majority of the antagonistic bacteria perform efficiently in controlled environmental conditions such as the laboratory and greenhouse, but fail to do so in the field due to many reasons including the impact of environmental factors. One of the most important reasons for the failure of bacterial antagonists in the field may be related to the lack of proper formulations. Farmers will be able to use such formulations as a seed treatment particularly for controlling seed and root diseases. Studies have shown that the efficacy of bacterial antagonists in biological control of some plant diseases have increased if they are mixed with some organic and inorganic carriers (Jorjani *et al.,* 2012).

Pseudomonas spp. commonly inhabits soil and has been used for biocontrol, promoting plant growth and bioremediation. 2, 4-diacetylphloroglucinol (DAPG) - producing strains are the major groups in biocontrol microorganisms, because of their easy colonization, good competition and broad antimicrobial spectrum (Gao *et al.,* 2012).

With Induced Systemic Resistance (ISR) a beneficial bacterium, *Pseudomonas corrugata*, stimulates what can be loosely considered the plant's 'immune system' so that in can protect itself from *Pythium aphanidermatum* (Chen *et al.*, 2000). This is distinct from Systemic Acquired Resistance (SAR) which can occur when a plant is exposed to a low level of a specific pathogen and then acquires resistance to that same pathogen in the future. ISR and SAR function through different phloem mobile chemical signals produced in plant tissues and are entirely distinct pathways (Jack and Nelson, 2010).

Besides the phylogenetic diversity, microorganisms can act as natural antagonists of various plant pathogens. A well developed and diverse rhizosphere community is thought to be critical in the suppression of pathogens. Knowledge of the structure and diversity of the fungal community in the plant rhizosphere will lead to a better understanding of pathogen-antagonist interactions (Gao *et al.,* 2012).

PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones i.e. Indole-3- acetic acid (IAA), gibberellic acid, cytokinins, and ethylene. Indirect mechanisms involves the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and siderophore are through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield (Kumar *et al.*, 2012).

Existence of various types of hormones in tomato plant provides a regulatory control through which different forms of growth in the plant are achieved. Auxin plays an important role in cell elongation, cell division, initiation of cambium and early differentiation of xylem and phloem tissues in tomato. Indole-3-Acetic Acid (IAA) at the lowest concentration stimulates stem

elongation as well as root numbers. IAA is an essential factor for growth of floral stem; tomato shoot cuttings treated with IAA formed more roots than control (Khan *et al.*, 2011).

Gibberellic Acid (GA), is a naturally occurring growth hormone, is a member of a type of plant hormone called gibberellins, which regulates the growth and development of plants. The GA are associated with various plant growth and development processes such as seed germination, stem and hypocotyls elongation, leaf expansion, floral initiation, uniform flowering, floral organ development, reduced time to flower, increased flowering number and size and induction of some hydrolytic enzymes in the aleurone of cereal grains (Kavina *et al.*, 2011).

Plant enzymes are involved in defense reactions against plant pathogens. These include oxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO), which catalyse the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure. Other enzymes such as phenylalanine ammonia-lyase (PAL) are involved in phytoalexin or phenolic compound. Biosynthesis of such enzymes have been correlated with defense against pathogens in several plants, including tobacco, tomato, cucumber and rice. These plant enzymes have long been thought to play an important role in the plant defense (Liang *et al.*, 2011).

Biological seed treatments may provide an alternative to chemical control of many soil and seed-borne pathogens. Bio-priming, a seed treatment system that integrates the biological and physiological aspects of disease control, involves coating the seed with fungal or bacterial biocontrol agents (Kader *et al.*, 2012).

With the above supporting studies the following objectives were designed under the topic, "Induction of defense related enzymes and plant growth promoting traits in tomato plant (*Lycopersicon esculantum*)" are

1. To screen the *Pseudomonas fluorescens* against *Pythium aphanidermatum* under *in vitro* condition.
2. To study the activity of the defense enzymes in the tomato plant affected with *Pythium aphanidermatum*
3. To study the growth promoting traits such as root length, shoot length and growth hormones of the tomato plant.

Exhaustive literatures for the study are discussed in the following chapter.