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Original Article

Positive role of *Bacillus* exopolysaccharides (EPS) on the enhancement of induced systemic resistance (ISR) against *Fusarium oxysporum* f.sp. *lycopersici* in tomato

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ABSTRACT

The positive role of *Bacillus* exopolysaccharides (EPS) on the enhancement of induced systemic resistance (ISR) in tomato – *F. oxysporum* f.sp. *lycopersici* pathosystem was studied under pot culture condition by employing the *Bacillus* EPS wild and EPS negative mutant strains of Bacillus together with challenge inoculation of *F. oxysporum* f.sp. *lycopersici.*

It was observed that the application of *Bacillus* EPS wild and EPS negative mutant altered the biochemical and physiological characteristics *viz.*, level of reducing and non-reducing sugars, total phenol content and defense enzyme activities, such as, peroxidase (PO), polyphenol oxidase (PPO), of tomato plant to a higher level when compared to control.

However, the application of *Bacillus* wild cells was found to augment total phenol content and defense enzyme activities, such as, peroxidase (PO), polyphenol oxidase (PPO) content of tomato plant to a higher level whereas, a reduction in reducing and non-reducing sugar level when compared to the application of EPS negative mutant cells and suggested the positive role of Bacillus EPS for the maximization of ISR in Tomato - *F. oxysporum* f.sp. *lycopersici* pathosystem.

This is the first comprehensive report on the positive role of *Bacillus* EPS, as a determinant of ISR, in tomato – *F. oxysporum* f.sp. *lycopersici* pathosystem and the subject needs further elabourate research at molecular level.

Keywords:
Bacillus EPS negative mutant, ISR, Tomato, *Fusarium oxysporum* f.sp. *lycopersici.*

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important and widely grown vegetable crop both in tropical and sub-tropical regions. The cultivation has become increasingly popular since mid-nineteenth century because of its varied climatic tolerance and high nutritive values. The fruits are rich source of vitamins, minerals and organic acids. Tomato can be used fresh and in processed forms such as salad, processed foods like ketchup, paste, soup etc. Of the several biotic and abiotic constraints, low soil fertility and incidence of diseases are considered to be the major constraints that eventually lead to the low productivity in tomato and the same must be greatly increased by providing additional nutrient inputs and through effective control of phytopathogens (Asha et al., 2011). The tomato wilt disease incidence of tomato caused by *F. oxysporum* f.sp. *lycopersici*, is one of the most destructive fungal disease of tomato crop, causing an yield loss upto 30-40 per cent (Kirankumar et al., 2008).

Now-a-days, tomato production management strategies mainly focus on the use of synthetic chemical pesticides to control the phytopathogen and enhance the per hectare yield of the crop. The persistant, injudicious use of these chemicals has toxic effects on non-target microorganisms of the soil and can cause undesirable changes in the environment. Moreover, the possible emergence of pesticide resistant phytopathogens can also occur due to the usage of the same (Lal and Soil, 2004).

Recently, a biological approach of using plant growth promoting rhizobacteria (PGPR) was attempted to reduce the drastic effects caused by consistant use of synthetic chemical pesticides to improve the productivity of tomato. Moreover, the biological approach has a great potential in supplying 'P' nutrition and biocontrol of phytopathogens which eventually leads to sustainable production in tomato. PGPR mediated Induced systemic resistance (ISR) against tomato wilt pathogen seems to be a promising approach in the reduction of biological and environmental hazards posed by the application of synthetic chemical pesticides.

Rhizobacteria that favourably, affect the plant growth and yield of commercially important crops are denominated as "Plant growth promoting rhizobacteria (PGPR)" (Kloepper et al., 1980). Several mechanisms of plant microbe interaction may participate in the association and affect plant growth, including P-solubilization, hormonal interaction, improvement in root growth and biological control against phytopathogens. Thus, the PGPR enhance the plant growth directly by producing and secreting plant growth promoting substances or by eliciting root metabolic activities by supplying phosphorous and indirectly by acting against phytopathogenic microorganisms (Kloepper et al., 1989). The nutrient well known PGPR includes bacteria belonging to the genera, namely, *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Arthrobacter, Enterobacter, Serratia* and *Rhizobium* on non-legumes.

Bacillus sp. has emerged as the largest, potentially most promising group of PGPR involved in the biocontrol of phytopathogens due to the production of secondary metabolites, such as, siderophore, antibiotics and through the enhancement of ISR (Kloepper *et al.*, 2004). The occurrence and activities of *Bacillus* sp. from the rhizosphere of tomato has been reported frequently (Solanki *et al.*, 2012). The positive role of bacterial EPS on the enhancement of ISR has been reported in *Colletotrichum orbiculare* pathosystem. As an enormous producer of EPS, the positive role of *Bacillus* EPS on the enhancement of ISR in Tomato - *F. oxysporum f.sp. lycopersici* pathosystem has not been studied, so far.

Hence, the present study has been undertaken with an aim to exploit the positive role of *Bacillus* EPS on the enhancement of ISR in tomato – *F. oxysporu*m f.sp. *lycopersici* pathosystem by employing EPS positive and EPS negative mutant strains of Bacillus.

Materials and Methods

A pot culture experiment was conducted to study the application effect of *Bacillus* cells *viz.*, Bacillus EPS wild and Bacillus EPS negative mutant together with challenge inoculation of *F. oxysporum* f.sp. *lycopersici* on the maximization of disease resistance in tomato with special emphasis to ISR mediated biocontrol against wilt disease (F. *oxysporum f.sp. lycopersici*). The study was conducted during December 2013 to Febraury 2014 with tomato cultivar PKM-1 at the polyhouse of Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, India.

Rectangular cement pots with 18" × 12" × 12" size were filled with 45 kg of tomato field soil flooded with water for 2 days and brought into fine puddle condition. Seeds of the tomato variety PKM-1 were soaked for 30 min in the different formulations of *Bacillus* cells and the experiment was arranged in randomized block design (RBD) with three replications and the following were the treatments. T1-Control, T2-BS-4W and T3-BS-4M.

During the experimental period, the annual mean minimum and the maximum temperature of experimental area is 25°C and 39°C, respectively and the mean highest and lowest humidity were 96 and 78 per cent respectively. The mean annual rainfall of this area is 1100 mm.

Tomato plants were challenge inoculated by spraying the *F. oxysporum* f.sp. *lycopersici* spore suspension at (50,000 spore/mL inoculation level) on 10th DAS with an atomizer and the control plants were sprayed with sterile water high humidity was created by sprinkling the water frequently in the polyhouse (Dinakar, 2010).

The crop was given a hand weeding on 30th DAS and well protected against pests. The pots containing plants were watered regularly. Five representative samples of plant hills in each pot were pegmarked for periodical observations.

The reducing and non-reducing sugar was estimated according to Mahadevan and

Sridhar (1986) whereas, the total phenol content was assayed according to Malik *et al.* (1997). The defense enzyme activities, such as, peroxidase (PO) and polyphenol oxidase (PPO) was assayed according to Putter (1974) and Ester-Bauer (1977), respectively.

Results and Discussion

The studies on the effect of *Bacillus* EPS on the maximization of ISR mediated biocontrol against *F. oxysporum* f.sp. *lycopersici* with special emphasis to biochemical and physiological parameters, revealed the highest performance of *Bacillus* EPS wild cells in augmenting the phenol content viz., total phenol content and orthodihydroxy (OD) phenol, reduction of reducing and non-reducing sugar level and augmentation of defense enzyme activities *viz.*, Peroxidase (PO) and polyphenoloxidase (PPO) of tomato plant when compared to EPS negative mutant cells of *Bacillus* application (Fig-1 to Fig-7). Farkas and Kiraley (1962) correlated the increasing levels of phenol contents of host plant with resistance to phytopathogens. It is well known that OD phenols are the more active forms of phenol. The oxidation of the same mediated by the enzyme PO and PPO and the resulting quinines are effective inhibitors of SH group of enzymes which may be inhibiting to the phytopathogens (Goodman et al., 1967). Usharani (2005) reported the enhancement of phenolic content of rice plant due to Pseudomonas inoculation and challenge inoculation of *Pyricularia oryzae*. Mishra et al. (2006) reported the *Rhizobium* mediated induction of phenolics in rice plant during the challenge inoculation of *Pyricularia oryzae*. Nanthakumar (1998) correlated the ISR with two fold increase in peroxidase activity against rice sheath pathosystem (Rhizoctonia solani) in rice plant. As a major source of energy, the level of carbohydrates of host plant has great influence on the incidence and development of disease. Plant tissue containing greater reserves of oxidizable carbohydrates are often more prone to pathogenic invasion than tissues containing low reserves. Altered carbohydrate metabolism of host plant in response to pathosystem infection was studied by several workers (Bhaskaran and Prasad, 1971; Kalyanasundaram, 1986). The sugar content in healthy and pathogen inoculated plants was very often correlated with resistance mechanism (Horsfal and Diamond, 1957). Kyungseok et al. (2008) reported the positive role of *Burkholderia gladioli* IN 26 EPS, as determinant of ISR in cucumber crop. The positive role of Bacterial EPS, as an elicitor of ISR in host plants has been reported by many authors (Haggag, 2007 and Kyungseok *et al.*, 2008). In the present study also, the reducing and non-reducing sugar levels were found to decrease with Bacillus EPS wild cells application together with challenge inoculation of *F. oxysporum* f.sp. *lycopersici*. The higher rate of reduction in the native level of reducing sugars may be one among the vital phenomena contributing resistance to plant. In the present study, the application of BS-4W reduced the tomato wilt disease incidence to the highest level than compared to control and BS-4M treatments. The results of the present study clearly envisaged the positive role of Bacillus EPS in augmenting the ISR against *F. oxysporum* f.sp. *lycopersici* in tomato crop. However, the mechanism of EPS mediated ISR against *F. oxysporum* f.sp. *lycopersici* in tomato plant is still unclear and the subject needs further elaborate research.

Fig - 1: Changes in reducing sugar content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

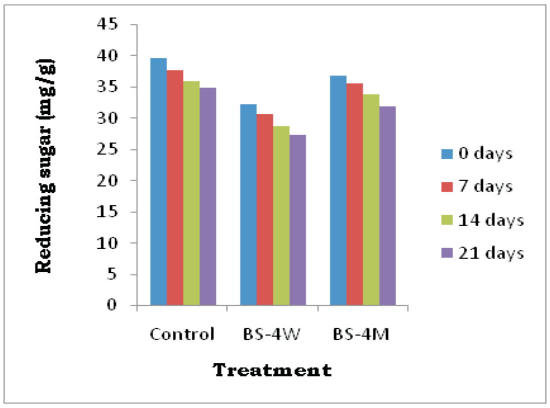


Fig - 2: Changes in non-reducing sugar content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

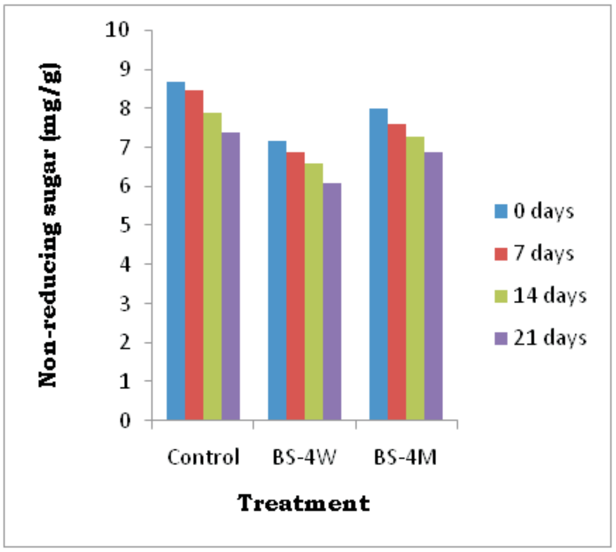


Fig - 3: Changes in starch content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

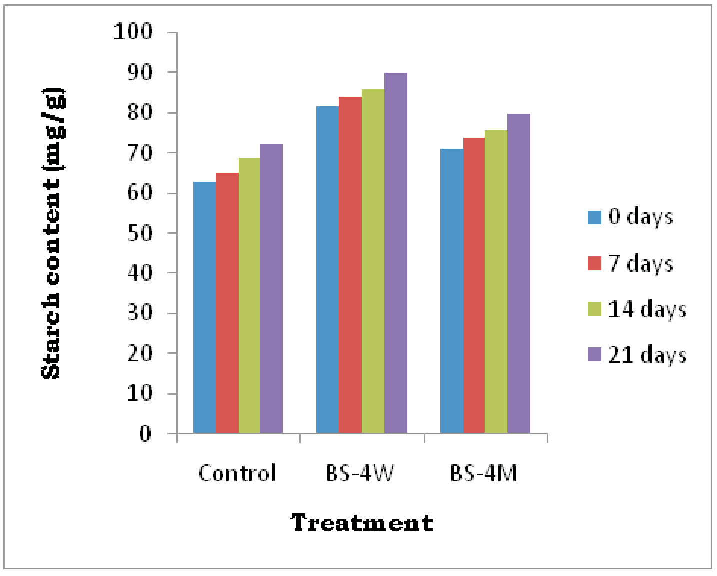


Fig - 4: Changes in total phenol content of tomato as influenced by the application of EPS positive and EPS negative Bacillus cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

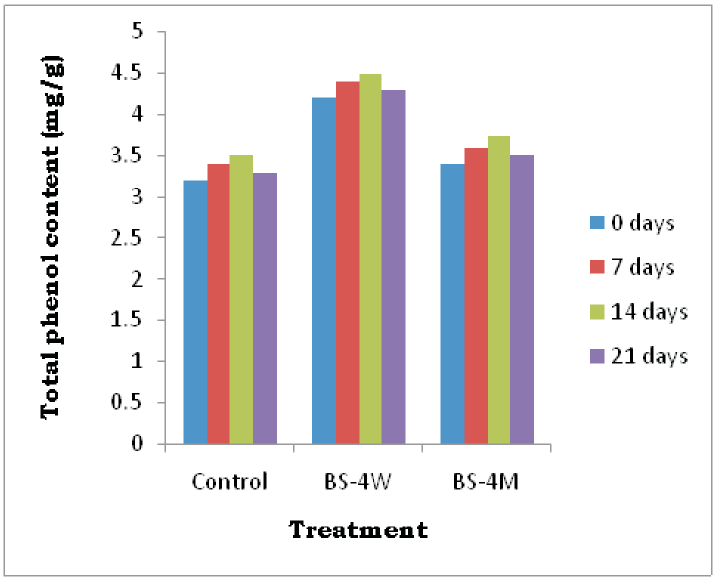


Fig - 5: Changes in ortho-dihydroxy phenol (OD-phenol) content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

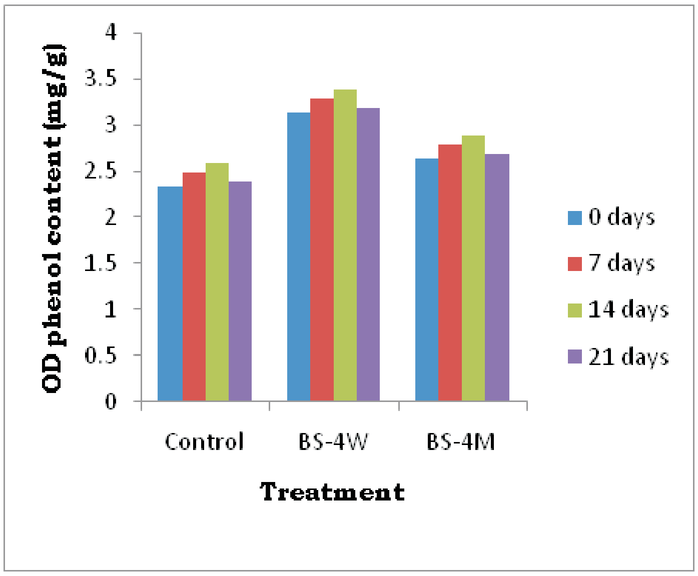


Fig - 6: Changes in peroxidase content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*

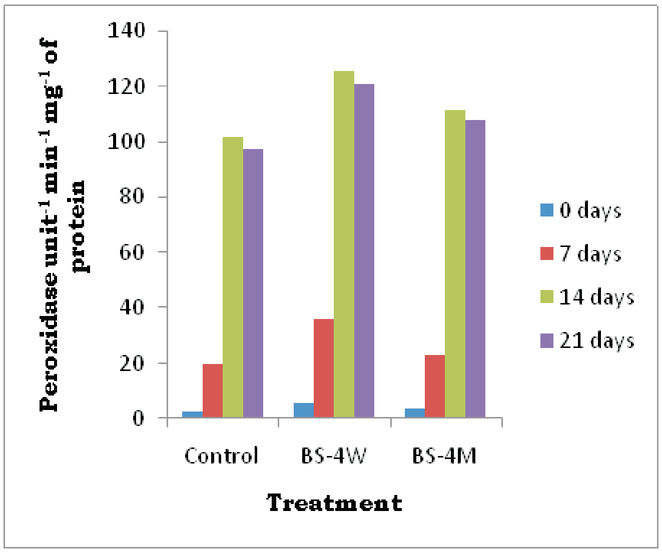
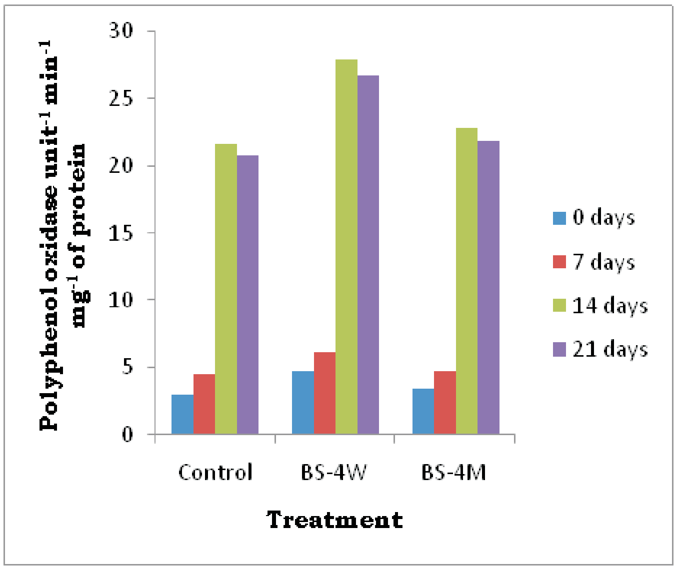


Fig - 7: Changes in polyphenol oxidase content of tomato as influenced by the application of EPS positive and EPS negative *Bacillus* cells and challenge inoculation of *F. oxysporum* f.sp. *lycopersici*



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