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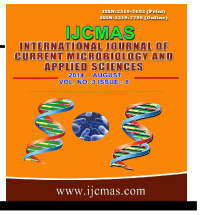

Original Research Article

Exopolysaccharides (EPS) mediated Induction of systemic resistance (ISR) in *Bacillus* - *Fusarium oxysporum* f.sp. *lycopersici* pathosystem in tomato (var. PKM-1)

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A B S T R A C T

The positive role of *Bacillus* exopolysaccharides (EPS), obtained from different bioformulations *viz*., vegetative cells, natural biofloc and artificial biofloc, on the maximization of plant growth and bio-control against the wilt disease (*Fusarium oxysporum* f.sp. *lycopersici*) in tomato was studied under potculture condition.Among the different bioformulations of *Bacillus* cells, the EPS obtained from natural biofloc of *Bacillus* reduced the wilt disease (*Fusarium oxysporum* f.sp. *lycopersici*) incidence to a higher level followed by the application of EPS, obtained from artificial biofloc and vegetative cells. Regarding the optimization of different concentrations of purified *Bacillus* EPS *viz*., 100, 200, 300 ppm on the wilt disease incidence of tomato revealed that the application of the purified EPS, obtained from natural biofloc of *Bacillus*, at 200 ppm concentration level could effectively reduced the wilt disease incidence of tomato to a higher level when compared to other EPS concentrations, obtained from other bioformulations of *Bacillus*. The results of the present study clearly revealed the dual role of *Bacillus* exopolysaccharides (EPS) on the maximization of plant growth as well as the bio-control against *F. oxysporum* f.sp. *lycopersici* in tomato whereas, the application of ISR inducing chemicals resulted with reduction in tomato wilt disease incidence alone. Moreover, the *Bacillus* EPS, obtained from natural biofloc, at a concentration of 200 ppm level could be optimized as effective one for the control of ISR mediated wilt disease incidence in tomato.

Keywords

Bacillus biofloc, EPS, ISR inducing chemicals, plant growth, biocontrol

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important and widely grown vegetable crops both in tropics and sub-tropics. The cultivation of the same 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, syrup, juice etc. Now-a-days, cultivation of tomato is the focus of horticultural industry in the world and takes a distinct place in the realm of vegetable crops (Asha *et al*., 2011). In India, it is grown in an area of 0.5 million ha with an annual production of 7.4 million tones and

productivity of 14.3 tones ha^{-1} (Shanmugasundaram, 2004; Latha *et al*., 2009). In Tamilnadu, tomato is cultivated in about 21,055 ha in the districts of Theni, Madurai, Coimbatore, Dharmapuri and Erode (Anonymous, 1997).

The wilt disease, caused by *F. oxysporum* f.sp. *lycopersici*, is one of the most destructive fungal disease of tomato, causing an yield loss upto 30-40 per cent (Asha *et al*., 2011). *Fusarium oxysporum* is one of the soil-borne plant pathogen, and it is widely distributed in various soil types, worldwide (Fravel *et al*., 2003).

Now-a-days, tomato production management strategies mainly focus on the use of synthetic chemical fertilizers and pesticides to 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, also. Moreover, these sysnthetic chemicals are too expensive for the resource poor farmers. 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 wilt pathogen seems to be a promising approach in the reduction of biological and environmental hazards posed by the application of synthetic chemical pesticides.

The Gram positive, spore forming bacteria *viz*., *Bacillus* sp. has been described as an effective PGPR (Kundu and Gaur, 1980; Petersen *et al*., 1996) and the ubiquitous occurrence of same from the rhizosphere of tomato has already been reported (Guemouri-Athmani *et al*., 2000; Von der Weid *et al*., 2000; Faria da Mota *et al*., 2002). Moreover, the antifungal activity of *Bacillus* sp. against *F. oxysporum* has been well documented (Islam *et al*., 2012). Hence, the development and deployment of this organism, as an agricultural bioinoculant, will be the suitable biological approach for the maximization of growth and yield in tomato.

The positive role of bacterial EPS, as an elicitor, for the induction of systemic resistance (ISR) against various phytopathogens of different crop species has been already reported (Haggag, 2007 and Kyungseok *et al*., 2008). The role of EPS from a PGPR, *Burkholderia gladioli* IN 26, on the elicitation of ISR against *Colletotrichum orbiculare* was investigated in cucumber (Kyungseok *et al*., 2008). A purified EPS induced the expression of PR1a GUS in tobacco and elicited ISR against *Colletotrichum orbiculare* on cucumber. The maximum level of disease protection was noted when seeds were soaked in EPS at 200 ppm concentration. Eventhough, many reports suggested the positive role of *Bacillus* inoculation in tomato, the positive role of *Bacillus* EPS application on the induction of systemic resistance (ISR) against *F. oxysporum* f.sp. *lycopersici* in tomato has not been reported, so far. The present investigation has been undertaken with an aim to elucidate the positive role of *Bacillus* EPS, obtained from different bioformulations, on the induction of systemic resistance (ISR) against *F. oxysporum* f.sp. *lycopersici* in tomato.

Materials and Methods

Preparation of inoculum

The efficient isolate of *Bacillus viz*., BS-4 collected from the rhizosphere of tomato var. PKM-1 grown at Rasipuram, was used in the present study. The *Bacillus* isolate was grown in nutrient broth in a shaking bath at 30 ± 2ºC for 24 hr (Chen *et al*., 2010).

Then, the medium was centrifuged at 5000 × g for 10 min to harvest the log phase cells and the pellets washed three times with 0.1 M phosphate buffer (pH 6.8). Finally, the cells were suspended in the same buffer to a cell concentration of 1×10^7 CFU/mL by measuring the OD at 420 nm and used as inoculum.

Preparation of *Bacillus* biofloc

The different bioformulations of *Bacillus* cells *viz*., natural biofloc and artificial biofloc was prepared according to Dinakar, 2010.

Preparation of ISR inducing chemicals

ISR inducing chemicals *viz*., salicylic acid, jasmonic acid and azibenzolar (Hi media, India) at a level of 0.01 per cent concentration were used.

Preparation of plant seed extract

The following plant seed materials namely, *Moringa oleifera*, *Strychnos potatorum*, *Allium cepa*, *Sappindus emaginatus* and *Aestracantha longifolia* were collected, crushed and sieved (0.8 mm mesh).

The seed powder is mixed with sterile water to form paste and then diluted to required strength (5% level concentration).

Quantitative estimation of bioflocculation (Madi and Henis, 1989)

The natural and artificial *Bacillus* biofloc was prepared according to Dinakar, 2010. After the incubation period, the aggregates settled at the bottom of the flask while most of the free cells remained in suspension. The supernatant was sampled and its turbidity measured in spectronic-20 colorimeter at 420 nm. The flocs were then mechanically dispersed by treatment in a tissue homogeniser for 1 min and the total OD was measured. Per cent aggregation was calculated as follows:

$$\text{Per cent aggregation} = \frac{OD_t OD_s}{OD_t} \times 100$$

Where OD_t = total optical density after mechanical dispersion and OD_s = OD of supernatant after aggregate had settled.

Effect of different bioformulations of *Bacillus* cells on *F. oxysporum* f.sp. *lycopersici* incidence

A pot culture experiment was conducted to study the effect of *Bacillus* EPS, obtained from different biofomulations viz., vegetative cells, natural biofloc and artificial biofloc together with challenge inoculation of *F. oxysporum* f.sp. *lycopersici* on the maximization of 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, 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 *Bacillus* EPS, obtained from

different bioformulations and the experiment was arranged in randomized block design (RBD) with three replications and the following were the treatments: T1-Control, T2-EPS obtained from vegetative cells, T3-EPS obtained from natural biofloc and T4-EPS obtained from artificial biofloc.

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

Challenge inoculation of tomato plant with *F. oxysporum* f.sp. *lycopersici*

F. oxysporum f.sp. *lycopersici* (provided by Dept. of Plant Pathology, Annamalai University) was maintained in Potato Dextrose Agar (PDA) medium and used for the challenge inoculation purpose. Thick spore suspension of the same was prepared with sterile water from 10 day old culture maintained in Potato Dextrose Agar (PDA) medium and strained through double layer muslin cloth so as to get a free of conidia. The population was adjusted with the help of Haemocytometer and a spore suspension with optimum spore concentration (50,000 spore's mL^{-1}) was prepared.

Then, the spore suspension was added with few drops of Tween-80 which increased the adherence capacity of the spores and acts as a sticker. The spraying of spore suspension was done under proper humid condition. Control plants were also sprayed with sterile distilled water.

After one week of challenge inoculation, the wilt disease incidence was recorded in each treatment following the score chart 0-9 scale (0: healthy; 1: 1-5%; 3: 6-10%; 5: 11-25%; 7: 26-50% and 9: >51% leaf area infected) proposed by Ramakrishnan *et al*. (1971). The statistical analysis was carried out according to Gomez and Gomez (1984).

Results and Discussion

The inoculation effect of *Bacillus* exopolysaccharides (EPS), obtained from different bioformulations *viz*., vegetative cells, natural biofloc and artificial biofloc on *F. oxysporum* f.sp. *lycopersici* disease incidence in tomato was studied under *in vitro* condition (Table-1). Among the different treatments, the EPS obtained from natural biofloc of *Bacillus* reduced the wilt disease incidence of tomato to a higher level followed by the EPS application obtained from artificial biofloc and vegetative cells. The *Bacillus* biofloc (natural) was found to record the lowest incidence of *F. oxysporum* f.sp. *lycopersici* (17.45%) followed by the artificial biofloc and vegetative cells.

The study on the optimization of different concentrations of purified EPS, obtained from natural biofloc *viz*., 100, 200, 300 ppm on the wilt disease incidence in tomato was studied under *in vitro* condition (Table-2). The results of the present study clearly revealed that the application of EPS, obtained from natural biofloc at 200 ppm concentration, could effectively reduced the wilt disease incidence to a higher level followed by other concentrations *viz*., 300 and 100 ppm.

Kyungseok *et al*. (2008) studied the different concentrations of purified EPS of *Burkholderria gladioli* IN-26 against *Colletotrichum orbiculare* in cucumber and optimized that 200 ppm of the same as effective concentration for the biocontrol of *Colletotrichum orbiculare* in cucumber. However, there was no earlier reports regarding the elicitor effect of *Bacillus* EPS,

at different concentrations, available against *F. oxysporum* f.sp. *lycopersici* in tomato. The results of the present study clearly revealed that the application of *Bacillus* EPS obtained from natural biofloc at 200 ppm could be optimized for the effective biocontrol of *F. oxysporum* f.sp. *lycopersici* incidence in tomato and the subject needs further elaborate research.

Table.1 Effect of *Bacillus* EPS, obtained from different bioformulations on *F. oxysporum* f.sp. *lycopersici*** incidence in tomato

Treatment	Percentage of disease incidence[ab]
Control	82.33 ± 0.34
Bacillus EPS (Vegetative cells)*	34.23 ± 0.23
Bacillus EPS (natural biofloc)*	17.45 ± 0.29
Bacillus EPS (artificial biofloc)*	19.86 ± 0.27

** - Wilt disease incidence in tomato was estimated according to Ramakrishnan *et al*. (1971).
* - *Bacillus* EPS collected from different bioformulations as described by Kyungseok *et al*. (2008).
a - Values are mean of three replications ± SD.
b - Values followed by different letters are significantly differed at 5% level according to student 't' test.

Table.2 Application effect of *Bacillus* EPS*, obtained from natural biofloc at different concentrations, on wilt disease** incidence in tomato

Treatment	Concentration of EPS (ppm)	Disease incidence[ab] (%)	Statistics
Control	-	82.33 ± 0.34	-
Bacillus EPS (natural biofloc)	100	12.84 ± 0.12	c
	200	11.04 ± 0.11	a
	300	11.90 ± 0.13	b

** - Wilt disease incidence in tomato was estimated according to Ramakrishnan *et al*. (1971).
* - *Bacillus* EPS collected from different bioformulations as described by Kyungseok *et al*. (2008).
a - Values are mean of three replications ± SD.
b - Values followed by different letters are significantly differed at 5% level according to student 't' test.

Table.3 Response of *Bacillus* EPS*** and ISR inducing chemicals on the maximization of growth and wilt disease incidence (*F. oxysporum* f.sp. *lycopersici*) in tomato

Treatment	Plant Height (cm)**	Disease Incidence (%)[ab]
Control	9.05 ± 1.01	72.35 ± 1.50
ISR inducing chemical*		
Salicylic acid	9.15 ± 0.11	21.00 ± 1.32
Jasmonic acid	9.10 ± 0.14	25.00 ± 1.15
Azibenzolar	9.09 ± 0.45	25.33 ± 1.18
Bacillus EPS (natural biofloc)	16.50 ± 0.21	15.66 ± 0.45

* - ISR inducing chemicals at 0.075% concentration as per Maurhofer *et al*., 1994.
** - 10^{th} DAS.
*** - *Bacillus* EPS collected from natural biofloc bioformulations as described by Kyungseok *et al*. (2008) and used at 200 ppm concentration.
a - Values are mean of three replications ± SD.
b - Values followed by different letters are significantly differed at 5% level according to student 't' test.

The inoculation effect of the purified EPS of *Bacillus*, obtained from natural bioflocs at 200 ppm level and ISR inducing chemicals, *viz*., salicylic acid, jasmonic acid and azibenzolar on the growth and *F. oxysporum* f.sp. *lycopersici* disease incidence in tomato was studied under pot culture condition (Table-3). It was observed that the EPS application of *Bacillus* was found to maximize the plant height and reduced the wilt disease incidence in tomato var. PKM-1 to a higher level when compared to the application of ISR inducing chemicals alone. Eventhough, the application of ISR inducing chemicals was also found to reduce wilt disease incidence, as in the case of purified EPS of *Bacillus*, but did not augment the growth of the tomato plant. The results of the present study clearly suggested the dual role of *Bacillus* EPS on the augmentation of growth of the host plant as well as the reduction in wilt disease incidence whereas the application of ISR inducing chemicals confined with reduction in wilt disease incidence alone. Usharani (2005) reported the phytostimulatory and biocontrol effect of *Pseudomonas fluorescens* against *Pyricularia oryzae* in lowland rice. Bahat-Samet *et al*. (2004) reported the phytostimulatory effect of *Azospirillum* EPS on wheat. The results of the present study clearly revealed the dual role (Phytostimulatory and biocontrol) of *Bacillus* EPS on tomato and in conformity with the earlier findings of Bahat-Samet *et al*. (2004) and Usharani (2005).

References

Anonymous, 1997. Hort. Stat., Directorate of Horticultural and Plantation crops. Chennai. Pp:126.

Asha, B.B., S. Chandra Nayaka, A.C. Udaya Shankar, C. Srinivas, S.R. Niranjana, 2011. Biological control of *F. oxysporum* f.sp. lycopersici causing wilt of tomato by *Pseudomonas* flourescens, International Journal of Microbiology Research, 2: pp- 79-84.

Bahat-Samet, E., S. Castro-Sowinski, and Y. Okon, 2004. Arabinose content of extra cellular polysaccharide plays a role in cell aggregation of *Azospirillum brasilense*. FEMS Microbiol. Lett., 237: 195-203.

Chen, F., M. Wang, Y. Zheng, J. Luo, X. Yang, X. Wang, 2010. Quantitative changes of plant defense enzyme and phytohormone in biocontrol of Cucumber *Fusarium* wilt by *Bacillus subtilis* B579. World J. Mirobiol. Biotechnol., 26: 675-684.

Dinakar, S. 2010. Studies on the functional components of *Azospirillum* bioflocculation and the significance of bioproduct in augmenting rice-*Azospirillum* rhizobiocoenosis under upland condition. Ph.D thesis. Annamalai University.

Faria da Mota, F., A. Nobrega, I.E. Marriel, E. Paiva, L. Seldin, 2002. Genetic diversity of *Paenibacillus polymyxa* population isolated from the rhizosphere of four cultivars of maize (*Zea mays*) planted in Cerrado soil. Appl. Soil. Ecol., 20: 119-132.

Fravel, D., C. Olivain, and C. Alabouvette, 2003. *Fusarium oxysporum* and it's biocontrol, New Phytology, 157: 493-502.

Gomez, K.A and A.A. Gomez, 1984. Statistical procedures for agricultural research, John Wiley and Sons, New York, USA. p, 20-29.

Guemouri – Athmani, S., O. Berge, M. Bourrain, P. Mavingui. J.M. Thiery, T. Bhatnagar and T. Heulin, 2000. Diversity of *Penibacillus polymyxa* in the rhizosphere of Wheat (*Triticum durum*) in Algerian Soils. Eur. J. Soil. Biol., 36: 149-159.

Haggag, W.M. 2007. Colonization of exopolysaccharide-producing *Paenibacilus polymyxa* on peanut roots for enhancing resistance against crown rot disease. Afr. J. Biotechnol., 6: 961-969.

Islam, M.R., Y.T. Jeong, Y.S. Lee and C.H. Song, 2012. Isolation and identification of antifungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. Mycobiology, 40(1): 59-66.

Kundu, B.S., and A.C. Gaur, 1980. Effects of phosphobacteria on yield and phosphate uptake by potato crop. Curr. Sci., 48: 159.

Kyungseok, P., J.W. Kloepper, and C.M. Ryu, 2008. Rhizobacterial exopolysaccharides elicit induced resistance on cucumber. J. Microbiol. Biotechnol., 18: 1095-1100.

Lal, R and C. Soil, 2004. Sequestration impacts on global climatic change and food security, Science, 304: 1623-1627.

Latha, P., T. Anand, N. Ragupathi, V. Prakasam, R. Samiyappan, 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biological

Control, 50: 85–93.

Madi, L. and Y. Henis, 1989. Aggregation in *Azospirillum brisilense* cd.: Conditions and factors involved in cell-to-cell adhesion. Plant and soil, 115: 89-98.

Maurhofer, M. C. Hase, P. Meuwly, J.P. Metraux and G. Defago, 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root- colonizing *Pseudomonas flourescens* strain P_3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology, 88: 678-684.

Petersen, D.J., M. Srinivasan, C.P. Chanway, 1996. *Bacillus polymyxa* stimulates increased *Rhizobium etli* populations and nodulation when co-resident in the rhizosphere of *Phaseolus vulgaris*. FEMS Microbiol. Lett., 142 (2-3): 271-275.

Ramakrishnan, L., S. Kamalnathan, C.S. Krishnamurthy, 1971. Studies on Alternaria leaf spot of tomato. Madras Agricultural Journal, 58(A): 275-280.

Shanmugasundaram, S. 2004. The Hindu Survey of Indian Agriculture, p. 127.

Usharani, G. 2005. Studies on the development of *Pseudomonas flourescens* as plant growth promoting rhizobacteria (PGPR) and biocontrol agent of rice (*Oryza sativa* L.) in Cuddalore district, Tamilnadu, Ph.D. Thesis, Annamalai University, India.

Von der Weid, I.A., E. Paiva, A. Nobrega, J.D. Van Elsas and L. Seldin, 2000. Diversity of *Paenibacillus polymyxa* strains isolated from the rhizosphere of maize planted in Cerrado soil. Res. Microbiol., 151: 369-381.