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Effect of Actinobacteria on Defence Enzymes Production in Color Capsicum

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Abstract

The main aim of this study was to know the effect of seed bacterization in color capsicum for the production of defence enzymes. The efficient actinobacterial isolates were used as both single inoculants and consortia for priming the seeds. The presence of enzymes was estimated at 3, 5 and 7 days after initiation for phytopathogens. The treatment T_9 showed the highest activity of PAL in green capsicum at 7 DAI (148.22, µmol of cinnamic acid min^{-1} mg^{-1} of protein) which was on par with T_1 (138.71 µmol of cinnamic acid min^{-1} mg^{-1} of protein). In red capsicum treatment T_8 showed the highest activity of PPO at 7 DAI (9.97, change in OD min^{-1} g of leaf $tissue^{-1}$) which was on par with T_6 (9.34 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_9 (9.09 change in OD min^{-1} g of leaf $tissue^{-1}$) and in yellow capsicum treatment T_8 showed the highest activity of PPO at 7 DAI (16.72, change in OD min^{-1} g of leaf $tissue^{-1}$). The treatment T_9 showed the highest β -1, 3-glucanase activity at 7 DAI (430.58, 365.33 and 410.06 nmol glucose released min^{-1} g^{-1} leaf tissue) followed by T_8 (412.32, 333.40 and 389.22 nmol glucose released min^{-1} g^{-1} leaf tissue). The highest activity of the enzymes was reported in the treatment which was inoculated with consortia of efficient actinobacterial isolates.

Keywords : Actinobacteria, Peroxidase, Phenols, Color capsicum

Vegetables belonging to the family of *Solanaceae* are more concerned due to their high nutritional and economic value (Sneha and Brahmaprakash, 2017). They are cultivated under protected conditions to meet the standards of different markets and as an enterprise in urban and peri-urban areas (Ganesh *et al*., 2022 and Sanjeev *et al*., 2018). Among the *Solanaceae* vegetables, the Capsicum is mostly cultivated in protected conditions. Color capsicum is one of the important vegetable crops grown in India for home consumption and export. The major bell pepper producing countries in the world are China, Indonesia, Sri Lanka, Pakistan, Turkey, Korea, Hungary, Spain, Bulgaria, Romania, Italy, Yugoslavia, Nigeria, Ghana, Tunisia, Mexico, USA, Central America, Argentina and Peru. Globally, Capsicum is grown in an area of 1.93 million hectares with a production of 31 million tonnes (Anonymous, 2019). Capsicum is also known as bell pepper or sweet pepper and it does not contain capsaicin, so the group has become an important green vegetable crop globally. It is a temperate crop and prefers relatively low temperatures for fruit set and fruit development and fruit colour development (yellow/ red). It grows well in the summer season also especially in hills and cooler regions. In India, bell pepper is grown for its mature green, yellow and red fruits, which are eaten raw, baking, stuffing, pizzas, soup preparation, salads, canning and stews for imparting favour. Sweet Pepper is a rich source of vitamin-C and has twice the amount of vitamin-C of citrus fruits and is also a good source of β-carotene (Anonymous, 2019). Actinobacteria represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and of extracellular enzymes (Doumbou *et al*., 2001). Among the enzymes produced

by actinobacterial isolates, chitinases are of great importance and many *Streptomyces* spp. are observed to inhibit both fungal pathogens and insect pests (Tahmasebpour *et al*., 2014).

The early stage of the crop cycle often determine the success of the entire year as it is important to go into the production cycle with strong, well established plants. Seed treatments are useful for many vegetable crops to prevent root diseases, as well as certain diseases carried on or within the seed. In order to improve seedling establishment, seeds can be primed before planting (Halmer, 2004). Seed priming is used commercially in many horticultural crops, ensures that the entire seed batch is at the same point in the germination process, so that once the seed is planted, there is a rapid and more uniform emergence, particularly under adverse environmental conditions. Various priming techniques exist, including hydropriming, osmopriming, solid matrix priming, steeping priming and drum priming (Caseiro *et al.,* 2004). Priming process helps to accelerate germination and improve seedling establishment in many crop and ornamental plants, especially under unfavorable soil conditions and in soils infested with plant pathogens. However, if seeds are infected or contaminated with pathogenic or saprophytic fungi, fungal growth can be enhanced during priming (Tylkowska and Van den Bulk, 2001), thus resulting in undesirable effects on plant health. Fungicides have been applied for elimination of microbial growth during priming processes, but have limited use as stated previously. Therefore, application of antagonistic microorganisms during priming (biopriming/microbial seed priming) could represent an environmental friendly strategy for control of seed borne pathogens. Inoculation of seeds with biocontrol agents (BCAs) in combination with priming has, in several cases, been reported to enhance and stabilize the efficacy of BCAs, but such approaches have been used mainly for control of soil borne diseases (Halmer, 2000). Much study has not been done on the effect of microbial seed priming with plant growth promoting rhizobacteria (PGPR) on color capsicum. Keeping the above facts in view, an attempt was made in this study to know the effect of microbial seed priming for defence enzyme production in color capsicum under green house condition.

Material and Methods

Experimental Details

The pot experiment was conducted at Department of Agricultural Microbiology, UAS, GKVK, Bengaluru with color capsicum as test crop (Arka Mohini, Arka Gaurav and Arka Basanth) using CRD design with 10 treatments replicated thrice. The treatment details are as follows.

Treatment Details

T_1 - Control (sterilized pot mixture)

T_2 - Negative control (fungus only)

T_3 - FA 15

T_4 - FA 13

T_5 - SA 12

T_6 - CA 18

T_7 - CA 4

T_8 - Potential actinobacteria isolates (FA 15 + FA 13 + SA 12 + CA 18 + CA 4)

T_9 - Potential actinobacteria isolates + fungal spore suspension

T_{10} - Reference (*Streptomyces luridus*)

Note : FA 15 (*Micromonospora maritima*), FA 13 (*Streptomyces luteogriseus*), SA 12 (*Streptomyces pratensis*), CA 18 (*Cellulomonas* sp.) and CA 4 (*Streptomyces chartreusis*).

Isolation and Purification of Actinobacteria

Isolation of actinobacteria from samples was done by a tenfold serial dilution pour plate method. A 10g of sample was suspended in to 90 ml sterile water blank and shaken on a mechanical shaker for 15 minutes. Further, a ten-fold dilution series was prepared by transferring 10 ml of aliquots of suspension each time to 90 ml sterile water blanks till 10^{-5} dilutions were obtained. The contents in the flasks were shaken between each transfer to ensure uniform suspension. One ml aliquot from the desired dilution was transferred to sterile petri-plates and starch

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casein agar (SCA) was poured into plates and was incubated for seven to ten days. The isolated colonies of the actinobacteria in dilutions of 10^{-3} to 10^{-4} were picked and streaked on freshly prepared starch casein agar medium and then individual colonies were sub-cultured on SCA slants and stored at 4 °C. Cultures were maintained by sub- culturing after every 15 days (Tian *et al*., 2004).

Seed Bacterization

Seeds were surface-sterilized with 0.1 per cent mercuric chloride for 30 seconds and washed 5-6 times with sterile distilled water and dried under a stream of sterile air. Ten ml of bacterial inoculum containing 3×10^8 cfu mL^{-1} was added to Petri plates. Seeds were soaked in 10 mL of bacterial suspension for 3 to 4 h. Then, the bacterial suspension was drained off and the seeds were dried in sterile petri plates and then seeds used for sowing the seeds imbibed in sterile water without any further treatment served as control. For challenge inoculation with the pathogen, micro-conidial suspension (1000 conidia mL^{-1}) of fungal cultures were mixed with sterilized soil at 50 ml/kg before filling the bags serves as a negative control.

Bioassay to Study the Defense-Related Enzymes Phenylalanine Ammonia-Lyase (PAL)

Leaf sample of 1 g was homogenized in 1 ml of cold 25 mM borate HCl buffer (pH 8.8) containing 0.4 ml of 5 mM mercaptoethanol. The homogenate was centrifuged at 15,000 x g for 15 min and the supernatant was used as the enzyme source. The assay mixture consisted of enzyme extract (0.2 ml), water (1.3 ml) and borate buffer (0.5 ml), (pH 8.8). The reaction was initiated by the addition of 1 ml of 0.1 ml phenylalanine and the reaction mixture was incubated for 1 hr at 32 °C. The reaction was stopped by the addition of 0.5 ml of 2 N HCl. A blank was run in which phenylalanine was added after adding 2 N HCl. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. The enzyme activity was expressed as μmol of cinnamic acid min^{-1} mg^{-1} of protein (Dickerson *et al*., 1984).

Estimation of Polyphenol Oxidase Activity (Lobo and Cano, 1998)

Leaf sample of 0.5 g was weighed and macerated with 10 ml of 0.2 M potassium phosphate buffer and centrifuged at 5,000 rpm for 10-15 min. One ml of 0.05 M catechol was taken in a test tube and to that, one ml of potassium phosphate buffer and one ml of enzyme extract were added. The readings were taken at 490 nm in 30-sec intervals up to 120 sec. Solution without enzyme extract served as blank. Enzyme activity was calculated and expressed as a change in OD min^{-1} g^{-1} of leaf tissue.

Estimation of Peroxidase Activity (Sadasivam and Manickam, 1992)

Leaf sample of 0.5 gm was weighed and macerated with 5 ml of phosphate buffer and centrifuged at 5000 rpm for 10-15 min. To one ml of supernatant solution, 3 ml of pyrogallol and 0.5 ml of hydrogen peroxide were added. Changes in OD were read at 425 nm with 30sec intervals up to 120sec. Solution without enzyme extract served as blank. Enzyme activity was calculated and expressed as a change in OD min^{-1} g of leaf tissue.

Assay of β-1, 3-glucanase

β-1, 3-glucanase activity was assayed by the laminar in the dinitro salicylic acid method (Pan *et al*., 1991). Leaf samples (1 g) were extracted with 2 ml of 0.05 M sodium acetate buffer (pH 5.0) and centrifuged at 16000 g for 15 min at 4 °C. The supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5 μl of 4 per cent laminarin and 62.5 μl of enzyme extract. The reaction was carried out at 40 °C for 10 min. Their action was then stopped by adding 375 μl of dinitro salicylic acid and heating for 5 min on boiling water, vortexed and its absorbance was measured at 500 nm. The enzyme activity was expressed as nmol glucose released min^{-1} g^{-1} leaf tissue.

Estimation of Total Phenols (Zieslin and Ben-Zaken 1993)

One gram of plant material (leaf) was homogenized in 10 ml of 80 per cent methanol and agitated for 15 min at 70°C. One ml of the methanolic extract was

added to 5 ml of distilled water and 250 µl of Folin-Ciocaleau reagent and the solution was kept at 25°C. The absorbance of the developed blue colour was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenol was expressed as µg catechol g^{-1} fresh weight tissue.

Results and Discussion

Isolation of Actinobacteria

Actinobacteria was isolated from three different samples like rhizosperic soil, forest soil and compost. The five efficient isolates like SA12 from rhizospheric soil, FA13 and FA15 from forest soil and CA4 and CA18 from compost were used for seed bacterization. The reference strain *Streptomyces luridus* 8586 was procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Standard strains of the pathogenic fungus, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Sclerotium rolfsi*, *Alternaria alternata* and pathogenic bacteria *Xanthomonas campestris* and *Ralstonia solanacearum* were procured from the Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru.

Defense Enzyme Production

The effect of actinobacterial isolates in color capsicum were evaluated for the increased synthesis of enzymes phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and peroxidase (PO), chemical constituent total phenol and (Pathogenesis Related) PR proteins which were responsible for improved plant defense mechanism, challenged with fungal plant pathogens. These compounds were estimated at 3, 5 and 7 DAI for phytopathogens. The results are presented in Table 1 to 5.

Phenylalanine Ammonialyase (PAL)

PAL activity was expressed as ìmol of cinnamic acid min^{-1} mg^{-1} of protein. In general irrespective of the treatments, the enzyme activity was less at 3 DAI and increased at 5 DAI and 7 DAI. The treatment T_9 showed the highest activity of PAL in green capsicum at 7 DAI (148.22, µmol of cinnamic acid min^{-1} mg^{-1} of protein) which was on par with T_1 (138.71 µmol of cinnamic acid min^{-1} mg^{-1} of protein) followed by T_5-reference strain (132.51 µmol of cinnamic acid min^{-1} mg^{-1} of protein). In red capsicum treatment T_8 showed the highest activity of PAL in green capsicum at 7 DAI (148.22, µmol of

Table 1

Effect of microbial seed bacterization on the activity of phenylalanine ammonialyase (PAL) in pathogen challenged plants

Treatments	Phenylalanine ammonialyase (PAL) (µmol of cinnamic acid min^{-1} mg^{-1} of protein)								
	Green capsicum			Red capsicum			Yellow capsicum		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	40.81 ^{i}	45.55 ^{i}	90.04 ^{f}	38.26 ^{h}	55.43 ^{g}	89.22 ^{g}	44.24 ^{g}	60.39 ^{g}	100.22 ^{c}
T_2	20.08 ^{j}	34.55 ^{j}	59.22 ^{i}	32.55 ^{i}	41.62 ^{h}	60.08 ^{h}	21.42 ^{h}	30.96	49.22 ^{i}
T_3	52.12 ^{e}	60.22 ^{g}	90.01 ^{f}	45.51 ^{f}	69.06 ^{f}	90.35 ^{d}	49.12 ^{e}	60.04 ^{h}	88.84 ^{h}
T_4	50.22 ^{f}	61.34 ^{f}	89.56 ^{g}	49.12 ^{g}	60.04 ^{f}	88.84 ^{f}	50.22 ^{d}	61.34 ^{e}	89.56 ^{g}
T_5	44.51 ^{h}	69.25 ^{e}	90.91 ^{e}	50.87 ^{e}	60.34 ^{d}	89.92 ^{e}	44.51 ^{g}	69.25 ^{c}	90.91 ^{d}
T_6	60.08 ^{d}	70.13 ^{d}	100.55 ^{d}	61.33 ^{c}	70.48 ^{c}	99.27 ^{c}	52.12 ^{c}	60.22 ^{e}	90.01 ^{f}
T_7	49.12 ^{g}	60.04 ^{h}	88.84 ^{h}	52.66 ^{d}	60.88 ^{d}	90.51 ^{d}	45.51 ^{f}	69.06 ^{d}	90.35 ^{e}
T_8	76.55 ^{c}	94.56 ^{c}	138.71 ^{b}	72.44 ^{a}	92.16 ^{a}	120.85 ^{a}	84.22 ^{a}	120.81 ^{a}	152.67 ^{a}
T_9	79.22 ^{b}	100.34 ^{b}	148.22 ^{a}	70.44 ^{b}	85.16 ^{b}	100.92 ^{b}	81.28 ^{b}	108.95 ^{b}	132.57 ^{b}
T_{10}	83.28 ^{a}	108.95 ^{a}	132.51 ^{c}	49.82 ^{f}	60.19 ^{e}	90.84 ^{d}	52.66 ^{c}	60.88 ^{f}	90.51 ^{e}

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

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TABLE 2

Effect of microbial seed bacterization on the activity of polyphenol oxidase (PPO) in pathogen challenged plants

Treatments	Polyphenol oxidase (change in OD min^{-1} g of leaf $tissue^{-1}$)								
	Green capsicum			Red capsicum			Yellow capsicum		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	2.31 ^{h}	3.24 ^{g}	3.99 ^{i}	2.06 ^{h}	3.12 ^{g}	4.01 ^{i}	2.15 ^{h}	3.11 ^{i}	3.35 ^{i}
T_2	1.22 ^{i}	1.85 ^{h}	2.04 ^{j}	1.08 ^{i}	1.66 ^{h}	2.08 ^{j}	1.08 ^{i}	1.68 ^{j}	2.01 ^{j}
T_3	3.71 ^{g}	4.30 ^{f}	6.33 ^{g}	3.58 ^{g}	4.20 ^{f}	6.88 ^{f}	5.76 ^{b}	6.22 ^{f}	8.39 ^{e}
T_4	5.12 ^{c}	7.22 ^{c}	9.34 ^{c}	5.06 ^{c}	6.42 ^{d}	8.29 ^{e}	5.16 ^{d}	6.42 ^{e}	8.26 ^{f}
T_5	4.31 ^{e}	6.62 ^{d}	8.85 ^{d}	3.88 ^{e}	4.20 ^{f}	5.65 ^{h}	4.78 ^{f}	5.23 ^{g}	6.33 ^{g}
T_6	4.03 ^{f}	6.29 ^{e}	7.28 ^{f}	5.12 ^{b}	7.22 ^{b}	9.34 ^{b}	3.58 ^{g}	4.20 ^{h}	6.08 ^{h}
T_7	3.71 ^{g}	4.20 ^{f}	6.04 ^{h}	3.71 ^{f}	4.30 ^{f}	6.33 ^{g}	5.12 ^{d}	7.22 ^{c}	8.85 ^{d}
T_8	5.71 ^{b}	11.24 ^{a}	13.86 ^{a}	4.31 ^{d}	8.61 ^{a}	9.97 ^{a}	5.52 ^{c}	10.16 ^{a}	16.72 ^{a}
T_9	7.06 ^{a}	9.28 ^{b}	12.06 ^{b}	5.29 ^{a}	7.13 ^{c}	9.09 ^{c}	7.12 ^{a}	8.99 ^{b}	11.34 ^{b}
T_{10}	5.09 ^{d}	6.42 ^{d}	8.26 ^{e}	4.31 ^{d}	6.62 ^{e}	8.85 ^{d}	5.02 ^{e}	6.62 ^{d}	9.35 ^{c}

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

cinnamic acid min^{-1} mg^{-1} of protein) which was on par with T_9 (120.85 µmol of cinnamic acid min^{-1} mg^{-1} of protein) followed by T_5 (100.92 µmol of cinnamic acid min^{-1} mg^{-1} of protein). Similar trend was followed in yellow capsicum T_8 (152.67 µmol of cinnamic acid min^{-1} mg^{-1} of protein) and T_9 (132.57 µmol of cinnamic acid min^{-1} mg^{-1} of protein). Lowest enzyme activity observed in T_2 (pathogen only) 49.22 µmol of cinnamic acid min^{-1} mg^{-1} of protein (Table 1)

Polyphenol Oxidase (PPO)

Polyphenol oxidase (PPO) activity was expressed as change in OD min^{-1} g of leaf $tissue^{-1}$. In general irrespective of the treatments, the enzyme activity was less at 3 DAI and increased at 5 DAI and 7 DAI. Initially at 3 DAI, T_8 showed the highest activity followed by T_9 for all the crops (Table 2). The treatment T_8 showed the highest activity of PPO in green capsicum at 7 DAI (13.86, change in OD min^{-1} g of leaf $tissue^{-1}$) which was on par with T_9 (12.06 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_4- *Streptomyces luteogriseus* (9.34 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_5-reference strain (8.26 change in OD min^{-1} g of leaf $tissue^{-1}$). In red capsicum treatment T_8 showed the highest activity of PPO at 7 DAI (9.97, change in OD min^{-1} g of leaf $tissue^{-1}$) which was on par with T_6 (9.34 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_9 (9.09 change in OD min^{-1} g of leaf $tissue^{-1}$) and in yellow capsicum treatment T_8 showed the highest activity of PPO at 7 DAI (16.72, change in OD min^{-1} g of leaf $tissue^{-1}$) which was on par with T_9 (11.34 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_{10}- *Streptomyces luridus* (9.35 change in OD min^{-1} g of leaf $tissue^{-1}$) followed by T_7- *Streptomyces chartreusis* (8.85 change in OD min^{-1} g of leaf $tissue^{-1}$). Lowest enzyme activity observed in T_2 (pathogen only) 2.04, 2.08 and 2.01 change in OD min^{-1} g of leaf $tissue^{-1}$ in green, red and yellow capsicum due to absence of Actinobacterial isolates.

Peroxidase (PO)

Peroxidase (PO) activity was expressed as change in OD min^{-1} g of leaf $tissue^{-1}$. In general irrespective of the treatments, the enzyme activity was less at 3 DAI and increased at 5 DAI and 7 DAI (Table 3). The treatment T_9 showed the highest activity of PO at 7 DAI (16.72, 14.76 and 13.88 change in OD min^{1} g of leaf $tissue^{-1}$) followed by T_8 (15.24, 14.74 and 12.48 change in OD min^{1} g of leaf $tissue^{-1}$).

TABLE 3

Effect of microbial seed bacterization on the activity of peroxidase (PO) in pathogen challenged plants

Treatments	Peroxidase (change in OD min^{-1} g of leaf $tissue^{-1}$)								
	Green capsicum			Red capsicum			Yellow capsicum		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	1.94 ^{g}	3.44 ^{i}	6.25 ^{h}	1.12 ^{g}	1.89 ^{i}	4.22 ^{h}	3.51 ^{h}	2.77 ^{f}	4.90 ^{i}
T_2	0.04 ^{h}	1.05 ^{j}	1.14 ^{i}	0.08 ^{h}	1.02 ^{j}	1.69 ^{i}	0.11 ^{i}	0.89 ^{g}	1.49 ^{j}
T_3	4.23 ^{c}	8.39 ^{d}	11.22 ^{d}	2.78 ^{f}	3.33 ^{h}	5.21 ^{g}	3.62 ^{g}	4.22 ^{d}	6.05 ^{f}
T_4	2.42 e	5.15 ^{h}	7.81 ^{g}	3.55 ^{e}	4.09 ^{g}	7.22 ^{d}	4.03 ^{e}	5.55 ^{b}	7.82 ^{c}
T_5	4.71 ^{b}	8.22 ^{e}	9.25 ^{f}	4.22 ^{c}	6.07 ^{d}	10.23 ^{b}	3.92 ^{f}	2.99 ^{e}	4.16 ^{h}
T_6	2.33 ^{f}	6.80 ^{g}	11.04 ^{e}	3.91 ^{d}	6.22 ^{c}	9.75 ^{c}	4.12 ^{d}	5.23 ^{c}	7.15 ^{d}
T_7	3.43 ^{d}	9.24 ^{b}	12.68 ^{c}	2.76 ^{f}	4.35 ^{f}	5.59 ^{f}	4.01 ^{e}	3.71 ^{f}	6.33 ^{e}
T_8	4.78 ^{b}	8.46 ^{c}	15.24 ^{b}	5.72 ^{a}	8.73 ^{a}	14.74 ^{a}	4.99 ^{b}	5.24 ^{c}	12.48 ^{b}
T_9	5.52 ^{a}	10.16 ^{a}	16.72 ^{a}	5.21 ^{b}	7.86 ^{b}	14.76 ^{a}	5.13 ^{a}	7.43 ^{a}	13.88 ^{a}
T_{10}	3.46 ^{d}	7.23 ^{f}	10.33 ^{e}	3.92 ^{d}	5.23 ^{e}	6.99 ^{e}	4.21 ^{c}	3.99 ^{e}	5.61 ^{g}

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

These treatments were the combined inoculation of actinobacterial isolates along with pathogens and significantly differ from controls T_1 (sterile soil) (6.25, 4.22 and 4.90 change in OD min^1 g of leaf $tissue^{-1}$) and T_2 (pathogen only) (1.14, 1.69 and 1.49 change in OD min^1 g of leaf $tissue^{-1}$). Similar results were reported for effect of seed priming in melon (*Cucumis melo*) by Nascimento and De Aragao, 2004. Improved seed priming techniques are used to reduce emergence time, accomplish uniform

TABLE 4

Effect of microbial seed bacterization on the activity of β-1, 3-glucanase in pathogen challenged plants

Treatments	β-1, 3-glucanase (nmol glucose released $min^{"1}$ $g^{"1}$ leaf tissue)								
	Green capsicum			Red capsicum			Yellow capsicum		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	48.95 ^{h}	49.22 ^{i}	78.23 ^{i}	50.31 ^{h}	80.88 ^{i}	130.24 ^{i}	50.88 ^{h}	90.78 ^{i}	99.52 ^{i}
T_2	21.46 ^{i}	26.08 ^{j}	30.25 ^{j}	18.92 ^{i}	23.42 ^{j}	36.23 ^{j}	24.21 ^{i}	40.81 ^{j}	92.33 ^{j}
T_3	102.44 ^{e}	138.92 ^{f}	183.37 ^{g}	96.72 ^{f}	111.46 ^{f}	180.53 ^{h}	86.27 ^{f}	120.22 ^{f}	200.34 ^{e}
T_4	120.22 ^{c}	211.68 ^{c}	310.82 ^{c}	87.56 ^{g}	100.23 ^{h}	220.45 ^{d}	102.58 ^{d}	131.92 ^{e}	188.37 ^{f}
T_5	108.22 ^{d}	178.90 ^{d}	200.85 ^{e}	100.23 ^{d}	121.57 ^{e}	224.88 ^{c}	120.22 ^{c}	211.68 ^{c}	310.82 ^{c}
T_6	102.58 ^{e}	131.92 ^{g}	188.37 ^{f}	121.42 ^{a}	150.22 ^{c}	210.33 ^{e}	102.44 ^{d}	138.92 ^{d}	183.37 ^{g}
T_7	87.44 ^{g}	160.57 ^{e}	263.71 ^{d}	98.88 ^{e}	104.32 ^{g}	188.44 ^{f}	98.25 ^{e}	102.32 ^{g}	180.44 ^{h}
T_8	142.86 ^{a}	250.16 ^{a}	412.32 ^{b}	105.68 ^{b}	250.33 ^{b}	333.40 ^{b}	138.62 ^{b}	289.44 ^{a}	389.22 ^{b}
T_9	137.78 ^{b}	228.41 ^{b}	430.58 ^{a}	100.67 ^{d}	271.56 ^{a}	365.33 ^{a}	140.21 ^{a}	272.34 ^{b}	410.06 ^{a}
T_{10}	98.25 ^{f}	102.32 ^{h}	180.44 ^{h}	102.44 ^{c}	138.92 ^{d}	183.37 ^{g}	87.56 ^{g}	100.23 ^{h}	220.45 ^{d}

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

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emergence, better allometric (changes in growth of plant parts over time) attributes and requisite stand in many horticultural and field crops (Ashraf and Foolad, 2005 and Farooq *et al.,* 2005). These include hydropriming, osmoconditioning, osmohardening, hardening and hormonal priming or soaking prior to sowing (Basra *et al.,* 2005 and Ashraf and Foolad, 2005).

β-1, 3-Glucanase

β-1,3-glucanase activity was expressed as nmol glucose released min^{-1} g^{-1} leaf tissue. In general irrespective of the treatments, the enzyme activity was less at 3 DAI and increased at 5 DAI and 7 DAI (Table 4). The treatment T_9 showed the highest β -1, 3-glucanase activity at 7 DAI (430.58, 365.33 and 410.06 nmol glucose released min^{-1} g^{-1} leaf tissue) followed by T_8 (412.32, 333.40 and 389.22 nmol glucose released min^{-1} g^{-1} leaf tissue). These treatments were the combined inoculation of actinobacterial isolates along with pathogens and significantly differ from controls T_1 (sterile soil) (78.23, 130.24 and 99.52 nmol glucose released min^{-1} g^{-1} leaf tissue) and T_2 (pathogen only) (30.25, 36.23 and 92.33 nmol glucose released min^{-1} g^{-1} leaf tissue). Priming also enhances the activities of anti-oxidative enzymes in treated seeds (Hsu *et al.,* 2003). Moreover, priming increases the activities of glyoxysome enzymes in primed bitter gourd seeds (Lin and Sung, 2001).

Total Phenol

Total phenol was expressed as μg catechol g of plant $tissue^{-1}$. In general irrespective of the treatments, phenol content was less at 3 DAI and increased at 5 DAI and DAI (Table 5). The treatment T_8 showed highest phenol content (360.44 μg catechol g of plant $tissue^{-1}$) in green capsicum. Whereas in red and yellow capsicum T_9 showed highest phenol content (402.22 and 372.06 44 μg catechol g of plant $tissue^{-1}$ respectively) followed by T_{10}-reference strain (*Streptomyces luridus*) with 290.33 and 290.33 μg catechol g of plant $tissue^{-1}$ respectively.

The treatment with pathogen suspension alone showed lowest phenol content in color capsicum (121.25, 120.24 and 115.34 μg catechol g of plant $tissue^{-1}$) at 7 DAI. Siddiqui and Meon (2009) found that the enhanced growth performance of chilli seedlings may possibly due to synergistic physiological effect of seeds and bacterial isolates during seed bacterization. They reported that maximum activities of these four

TABLE 5

Effect of microbial seed bacterization on the total phenol content in pathogen challenged plants

Treatments	Total phenol (μg catechol g of plant $tissue^{-1}$)								
	Green capsicum			Red capsicum			Yellow capsicum		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T_1	166.23 ^{h}	120.36 ^{g}	132.88 ^{i}	200.42 ^{c}	175.33 ^{g}	199.29 ^{g}	190.42 ^{d}	180.56 ^{d}	192.55 ^{e}
T_2	109.33 ^{j}	101.45 ^{h}	121.25 ^{j}	102.46 ^{i}	100.47 ^{i}	120.24 ^{h}	110.65 ^{h}	100.22 ^{h}	115.34 ^{i}
T_3	198.34 ^{b}	210.42 ^{d}	230.73 ^{f}	166.23 ^{h}	150.36 ^{h}	211.88 ^{e}	198.34 ^{c}	210.42 ^{c}	230.73 ^{d}
T_4	200.33 ^{a}	220.21 ^{c}	298.72 ^{c}	180.24 ^{g}	185.45 ^{e}	190.96 ^{f}	114.55 ^{g}	134.89 ^{f}	156.10 ^{g}
T_5	196.42 ^{c}	200.44 ^{e}	250.53 ^{e}	221.08 ^{b}	199.31 ^{d}	230.44 ^{d}	180.24 ^{e}	180.45 ^{d}	190.45 ^{f}
T_6	190.35 ^{e}	210.44 ^{d}	290.33 ^{d}	190.35 ^{f}	210.44 ^{c}	230.73 ^{d}	190.55 ^{d}	156.79 ^{e}	190.54 ^{f}
T_7	114.55 ^{i}	134.89 ^{f}	156.10 ^{h}	180.24 ^{g}	180.45 ^{f}	190.45 ^{f}	166.23 ^{f}	120.36 ^{g}	142.88 ^{h}
T_8	186.22 ^{f}	260.34 ^{a}	360.44 ^{a}	199.42 ^{d}	220.81 ^{b}	390.55 ^{b}	214.92 ^{a}	271.32 ^{a}	365.48 ^{b}
T_9	192.63 ^{d}	228.12 ^{b}	352.37 ^{b}	224.52 ^{a}	250.21 ^{a}	402.22 ^{a}	208.52 ^{b}	245.71 ^{b}	372.06 ^{a}
T_{10}	180.24 ^{g}	180.45 ^{e}	190.45 ^{g}	198.34 ^{e}	210.42 ^{c}	290.33 ^{c}	190.35 ^{d}	210.44 ^{c}	290.33 ^{c}

Note : Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

enzymes in tomato treated with AR12 strain occurred in different stages: activity of PAL, PPO, POD and SOD increased to the top level at 48, 48, 12 and 12 h, respectively, after pathogen inoculation and kept high level for some time. Six actinobacterial isolates with the highest antagonistic activity were evaluated for antagonistic activity by Sakineh *et al*. (2019). The ability of the superior biocontrol strains to induce antioxidant enzymes activity and systemic resistance (ISR) was investigated. Increased activity of catalase (CAT) in plant treated with strains as well as an increase in peroxidase (POX) and Polyphenol oxidase (PPO) activity in plants treated with Y28 pointed to a strain specific-induced systemic resistance (ss-ISR) in tomato against *Fusarium oxysporum.* Biopriming/ microbial seed priming is a technique of seed treatment that integrates biological and physiological aspects of disease control was recently used as an alternative method for controlling many seed and soil borne plant pathogens (EL-Mohamedy, 2008). Applying beneficial microorganisms to seed during the priming process is commercially realistic, as microbial suspensions can easily be incorporated into the water used for seed priming.

Actinobacteria are the large group of Gram-positive bacteria, secrete a wide range of extracellular enzymes involved in the degradation of organic compounds and biopolymers. They were also able to induce certain defence enzymes like phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and peroxidase (PO), chemical constituent total phenol and (Pathogenesis Related) PR proteins which were responsible for improved plant defense mechanism, challenged with fungal plant pathogens. Compared to single isolate, the treatment with consortium of isolates induced more enzymatic activity.

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