DOI Link: https://doi.org/10.61586/XKTNICF
Vol.32, Issue.9, Part.1, Sep 2025, PP. 2-18

Efficacy of biostimulants against *Alternaria solani* (Ell. and Mart.) Jones and Grout causing early blight of tomato and their impact on tomato seedling growth parameters

Diksha Sinha*, DK Banyal, Amar Singh, Sonali Parwan

Department of Plant Pathology, CSK HPKV, Palampur – 176 062 (H.P.), India
*email-sinhadiksha34@gmail.com

Received Feb 2025 Accepted Aug 2025 Published Sep 2025

Abstract

Early blight caused by *Alternaria solani* has been documented as a serious threat to tomato plants which leads to significant loss in yield in India and across the world. This study aimed to evaluate the efficacy of four biostimulants, *i.e*, salicylic acid, gallic acid, chitosan and Cusilano (gelatin enriched with colliadal silver and copper) against plant pathogenic fungi *Alternaria solani* causing early blight of tomato. Cusilano (5 µg/ml) was found most effective against *A. solani* under both *in vitro* and *in vivo* conditions, with maximum mycelial inhibition (60.00%) and maximum disease control (52.48%) over check. The effect of application of biostimulants was evaluated on different vegetative growth parameters of tomato after 20 days of foliar application. Seedlings treated with Cusilano and chitosan were found to have the maximum root length, *i.e.* 6.00 and 5.00 cm which were statistically at par with each other. The shoot length recorded for Cusilano (10.00 cm) and gallic acid (9.00 cm) were higher than salicylic acid and chitosan. Non-significant differences with respect to number of branching and stem diameter were observed among the treatments including the untreated control. Seedling fresh weight was significantly high in Cusilano treated seedlings (0.50g) followed by gallic acid (0.40g). Similar trend was observed in case of dry seedling weight. The activity of defense enzyme peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine lyase (PAL) was measured in tomato leaves (cv. Avtar) challenged with *A. solani*. The results demonstrate a stimulatory effect of biostimulants in tomato plants against early blight and reveal that gelatin enriched with colliadal silver and copper resulted in most enhanced production of plant defense enzymes during interaction with the *A. solani*.

Keywords: Tomato, *Alternaria solani*, early blight, biostimulants, plant defense enzymes

1. Introduction

Tomato (*Solanum lycopersicon* L.) is one of the most important vegetable crops popularly grown worldwide under both field and protected conditions (Blanca et al., 2012). It is a nutritious vegetable which is either used fresh, in culinary preparations or processed. India ranks second in the area under cultivation as well as in production of tomato after China. The total cultivated area of tomato in India is about 0.81 million ha with total 21.19 million metric tons production (NHB, 2022). India contributes about 11 per cent in the world tomato production annually (Kushwaha et al., 2018). Tomato is one the most profitable crops for small to marginal farmers nevertheless numerous biotic and abiotic factors hamper the productivity. Among the biotic factors, early blight caused by *Alternaria solani* is one of the most important diseases which results in crop failure and heavy economic losses (Panno et al., 2021; Kumar and Chandra, 2024). The disease is widespread in the tropical and subtropical tomato growing regions recording significant yield loss of 10 to 70 per cent (Abada et al., 2008; Adhikari et al., 2017; Shinde et al., 2018; Bektas, 2022). Therefore, to support the year-round demand of tomato fruits, it is essential to explore effective strategies to combat the disease stress. Due to lack of suitable resistant cultivars, presently, cultural practices and extensive fungicide applications are employed for the management of early blight in tomato (Adhikari et al., 2017). However, the excessive cost involved and possible environmental hazards necessitate the exploration of sustainable disease management practices (Senthilnathan, 2015; Wezel et al., 2020; Chanthini et al., 2023; Ponsankar et al., 2023; Khalil and Youssef, 2024).

In the recent years, plant disease management using biostimulants has received attention because of their natural origin, effectiveness and low to non-existent toxicity. Plant biostimulants are formulations of biological origin that stimulate or enhance natural processes such as nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality. They have been applied to modify physiological processes, mitigate stress-induced limitations and to improve plant growth and yield (Ricci et al., 2019; Gebashe et al., 2021). Farouk et al. (2012) tested several natural compounds on tomato plants grown under field conditions and natural infestation to evaluate the potential of biostimulants on improving tomato plant growth, yield and to induce protection from early blight infection. Foliar application of chitosan, humic acid, seaweed extract and thiamine has been proved to be effective in reducing disease incidence in tomato plants. Recently, application of nanoparticles has emerged as an attractive tool to enhance plant protection against early blight (Ammar et al., 2019; Khan et al., 2023). Selenium, copper, silica and silver are the popular candidates for this method, which enhance the activity of antioxidant enzymes like superoxide dismutase, catalase, peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine lyase (PAL) in the plant tissues (Jindo et al., 2021). Biopolymers of sodium alginate, exogenous hexanoic acid and chitosan were tested and found effective to protect tomato plants from infection by *A. solani* (Sathiyabama et al., 2014; Dey et al., 2019; Esfahani, 2022; Bektas, 2022; Rabiei et al., 2022; Shalaby et al., 2022). The

induction of antifungal enzymes and regulation of defense gene expression are the underpinning factors for plant disease reduction by biostimulants.

In chloroplast, peroxidase scavenges H_2O_2 (hydrogen peroxide) for the protection of plants under stress conditions. Peroxidase activity is known to play a significant role in lignin biosynthesis and formation of structural protein molecules for reinforcement of cell wall, in addition to antioxidative activity (Shalaby et al., 2022). It is suggested that induction of peroxidase is usually increased in the cells surrounding the infection area and there is close correlation between peroxidase activity and induced resistance in host (Garcıia-Limones et al., 2002). Within the host cell, polyphenol oxidase (PPO) constitutes one of the first line of defense against reactive oxygen species. It primarily helps in the lignification of cell wall and protects the nucleus against fatal mutations caused by O_2 molecules. PPO are essential for preventing activities like cell membrane depolarization, increased membrane permeability, induced lipid peroxidation which cause cellular disruption and cell death (Li et al., 1998; García-Limones et al., 2002). Phenylalanine lyase (PAL) is the key enzyme of phenylpropanoid pathway in plants which catalyzes the conversion of phenylalanine to trans-cinnamic acid which act as a precursor of flavonoids, lignin and phytoalexins that are crucial for plant defense against the pathogen attack by blocking the entry pathway of the pathogen (Halbrock and Scheel, 1989). Studies have shown that PAL activity responds to manifold stresses, such as wounding, drought, salinity, heavy metals and infection by different pathogens. Keeping these in view, this study was conducted with three objectives. First, to evaluate the efficacy of salicylic acid, gallic acid, chitosan and Cusilano (gelatin enriched with colloidal silver and copper) against early blight of tomato. Second, to determine the influence of these biostimulants on tomato seedling growth parameters and to assess the influence on plant defense enzymes.

2. Materials and methods

2.1. In vitro antifungal assay

Antifungal assay of salicylic acid, gallic acid, chitosan and Cusilano (gelatin with colloidal silver and colloidal copper) against *A. solani* was evaluated by the agar diffusion technique (Grover and Moore, 1962). Three concentrations of each biostimulant were evaluated. Salicylic acid (SA), gallic acid (GA) and Cusilano were evaluated at 1, 3 and 5 µg/ml concentrations while sterile chitosan solution was evaluated at concentrations 0.5, 1 and 5 mg/ml. The biostimulants were added to 100 ml sterilized PDA to prepare the test concentrations and aseptically poured in sterilized Petri dishes. Mycelial plug of 5 mm diameter from 7 days old active colony of *A. solani* was placed in the centre of Petri plates and incubated at 25±1°C. The experiment was laid in factorial design conducted as completely randomized design with three replications along with negative control (PDA medium only). Seven days after inoculation, growth of the fungus was measured

in each Petri plate and growth inhibition was expressed as the percentage of inhibition of radial growth relative to control. The inhibition percentage of the mycelial growth was determined using equation:

Inhibition (%) = [(C – T)/ C] × 100

where "C" is the mycelial growth in negative control and "T" is the mycelial growth in different treatments.

2.2 In vivo assay

Experiment was laid out in a completely randomized design with three replications for each treatment on tomato (cv. Avtar) at department of plant pathology, CSKHPKV, Palampur. Each replication comprised of one pot with five plants. Seeds were sown in surface sterilized plug trays filled with potting mixture (sterilized soil, compost, perlite and cocopeat in the ratio 1:1:1:2). Seedlings of tomato plants that had developed 2 to 4 mature leaves were transplanted to pots filled with sand and potting medium in the ratio 1:3, respectively. The potting medium was composed of FYM and soil in the proportion 1:2, respectively. Before transplanting, salicylic acid, gallic acid and Cusilano @ 3µg/ml each while chitosan @ 5mg/ml were applied by seedling dip method. After 7 days, seedlings were subjected to foliar spray treatments using distilled water as a control. Ten ml solution was sprayed on each pot (Pradhanang et al., 2005). After 5 days of foliar application, the plants were spray inoculated with homogenized conidial suspension of *A. solani* ($1x10^5$ spores/ml). The inoculated plants were placed in net house by maintaining RH> 90 per cent for 48 hours at temperature 25-30°C.

Data pertaining to seedling growth parameters, *i.e.* root length, shoot length, number of branches, stem diameter, fresh and dry seedling weight were recorded 10, 20 and 30 days after foliar application. The observation on per cent disease index was recorded periodically using rating scale given by Pandey et al. (2003). The PDI values were expressed using the formula given by McKinney (1923). Disease progression was measured by calculating AUDPC and apparent rate of infection (r) as per logistic equation given by Vander plank (1963).

$$\text{PDI} = \sum \frac{Severity\ grade \times Number\ of\ leaves}{Maximum\ grade \times Total\ number\ of\ leaves\ scored} \times 100$$

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where, y_i = Disease severity at the i^{th} observation

t_i = time (in days) at the i^{th} observation

n = total number of observations.

$$r = \frac{2.303}{t2-t1} \log 10 \frac{X2\,(1-X1)}{X1\,(1-X2)}$$

Where,
r = apparent infection rate per day
t2-t1 = time interval between first and last observation
X1 & X2 = proportion of leaf area covered by lesion at t1 and t2 time intervals, respectively
(1-X1) & (1-X2) = proportion of healthy leaf area at t1 and t2 time intervals, respectively

2.3. Biochemical analysis of host defense enzymes

2.3.1. Preparation of enzyme extracts

About 0.5 g fresh tomato leaf tissue at 0, 24-, 48-, 72- and 96-hours post inoculation (hpi) with the test pathogen, were collected from each treatment and processed in liquid nitrogen before preserving at -80°C until used for biochemical assay to prevent any metabolic alteration. The leaf sample were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was used as source of enzyme.

2.3.2. *Peroxidase (POD) activity*

Peroxidase (POD) activity was determined using the method described by Tatiana et al. (1999) with minor modifications. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 5.5), 2 per cent H_2O_2, 0.05 M guaiacol and 0.1 ml enzyme extract in a final volume of 5 ml. The reaction was started after adding enzyme extract. The formation of tetra guaiacol was measured at 470 nm. One unit of enzyme was defined as the amount of enzyme to decompose 1 µmol of H_2O_2 per min at 25°C.

2.3.3. *Polyphenol oxidase (PPO) activity*

Polyphenol oxidase (PPO) activity was determined using the method described by Chance and Maheli (1995). The reaction mixture comprised of 200µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer of pH 6.5 was added to 200 µl of 0.01M catechol to start the reaction and the activity was expressed as change in absorbance at 495 nm per min/mg.

2.3.4. *Phenylalanine ammonia lyase (PAL)*

Phenylalanine ammonia lyase activity was determined using the method described by Edward and Kessmann (1992). Homogenization buffer consisted of

25 mM tris buffer (pH 8.8). Reaction mixture was prepared by adding 0.1 ml of enzyme extract and 0.4 ml of 0.05 M tris buffer (pH 8.8) containing 0.2 mM phenylalanine and was incubated in water bath at 37°C for 60 min. Reaction was stopped by adding 0.1 ml of 0.5 N HCL. The transcinnamic acid was extracted by adding 2 ml toluene. The absorbance was taken at 412nm and the enzyme activity was expressed in µmol trans-cinnamic acid min/g fresh weight.

3. Results

3.1. In vitro assay

The *in vitro* assay, using 3 different concentrations of each biostimulant indicated their antifungal activity and significant inhibition of mycelial growth of *A. solani* (Table 1 and figure 1). The mycelial growth significantly reduced as compared to the control at all concentrations of each biostimulant. The highest concentration of Cusilano (5 µg/ml) recorded maximum inhibition of mycelial growth (60.00%) over control which was at par with gallic acid @5µg/ml with 54.81 per cent mycelial inhibition. This was followed by Cusilano @3µg/ml and Gallic acid @3µg/ml with 53.33 and 48.89 per cent mycelial inhibition, respectively. The results showed that salicylic acid @1µg/ml (20.37%) and chitosan @0.5 mg/ml (23.70%) showed least antagonistic effect against *A. solani* among all the biostimulants.

Table 1 *In vitro* evaluation of biostimulants against *Alternaria solani*

Biostimulant	Conc.	Mycelial growth (mm)	Mycelial growth inhibition (%)
Gallic acid	1µg/ml	52.33^{d}	41.85
	3µg/ml	46.00^{c}	48.89
	5µg/ml	40.67^{ab}	54.81
Salicylic acid	1µg/ml	71.67^{g}	20.37
	3µg/ml	62.33^{f}	30.74
	5µg/ml	56.67^{de}	37.04
Cusilano	1µg/ml	52.33^{d}	41.85
	3µg/ml	42.00^{bc}	53.33
	5µg/ml	36.00^{a}	60.00
Chitosan	0.5mg/ml	68.67^{g}	23.70
	1mg/ml	62.33^{f}	30.74
	5mg/ml	57.67^{e}	35.93
Control	-	90.00^{h}	-
CD (p=0.05)	-	4.87	-

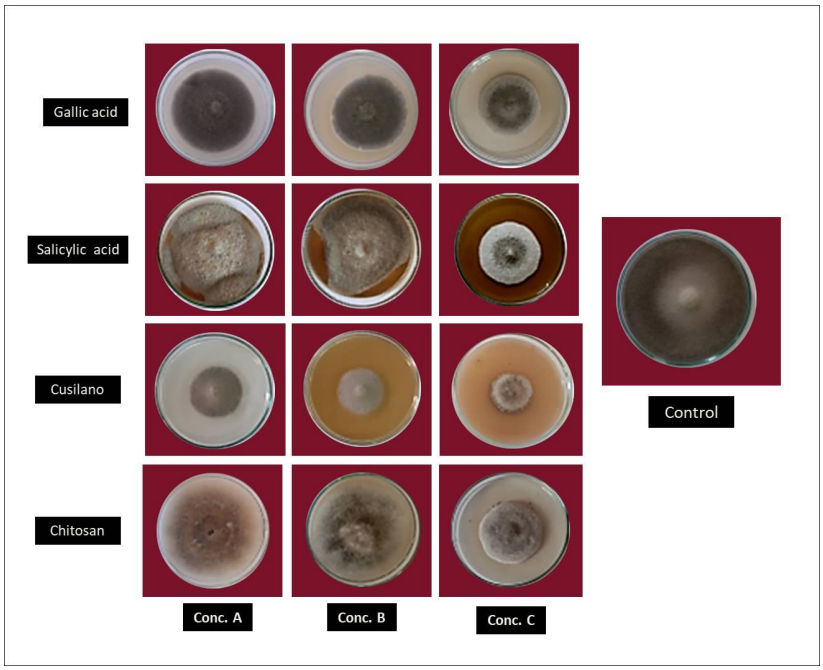

Figure 1. Inhibition of mycelial growth of *Alternaria solani* by biostimulants (gallic acid @1, 3, 5µg/ml, b. salicylic acid @1, 3, 5µg/ml, c. Cusilano @1, 3, 5µg/ml, d. chitosan @0.5, 1, 5mg/ml)

3.2. In vivo assay

3.2.1. Effect of biostimulants on early blight disease severity

Biostimulants *i.e.* salicylic acid, gallic acid and Cusilano @3µg/ml each while chitosan @5mg/ml were applied by seedling dip method followed by one foliar application (7 days after transplanting) at 4 leaf stage (cv. Avtar) of tomato. The plants were inoculated with the pathogen ($1x10^5$spores/ml) after 5 days of foliar application of biostimulants.

The exogenous application of biostimulants significantly reduced the severity of early blight on tomato leaves in comparison to the control (Table 2 and figure 2).

Table 2. Effect of biostimulants on early blight disease severity under *in vivo* conditions

Biostimulant	Dose	Disease severity days after disease appearance						AUDPC
		7		14		21		
		Severity (%)	Disease Control (%)	Severity (%)	Disease Control (%)	Severity (%)	Disease Control (%)	
Gallic acid	**3µg/ml**	22.33	46.62	28.67	41.09	30.45	49.53	385.42
Salicylic acid	**3µg/ml**	36.33	13.15	41.67	14.38	47.50	21.27	585.10
Cusilano	**3µg/ml**	20.85	50.16	25.10	48.43	28.67	52.48	349.02
Chitosan	**5mg/ml**	32.67	21.90	38.45	21.00	48.67	19.33	553.84
Control	-	41.83	-	48.67	-	60.33	-	698.25
CD (p=0.05)		3.88	-	3.05	-	4.17	-	20.67

Cusilano (3 µg/ml) recorded maximum disease control (52.48%) over control, which was at par with gallic acid @3 µg/ml (49.53%) 21 days after disease appearance. The results showed that chitosan @5 mg/ml (19.33%) and salicylic acid @3 µg/ml (21.27%) showed least disease control with 48.67 and 47.50 per cent disease severity as compared to 60.33 per cent in the control. Similar trend of disease severity was observed at 7 and 14 days after disease appearance. Maximum AUDPC was recorded in salicylic acid (585.10) followed by chitosan (553.84), while it was minimum for Cusilano (349.02) indicating effective control of disease progress as compared to 698.25 in control. These results were in congruence with the *in vitro* mycelial inhibition of *A. solani.*

3.2.2. Effect of biostimulants on seedling growth parameters

The effect of application of biostimulants was evaluated on different vegetative growth parameters of tomato after 20 days of foliar application and the data is presented (Table 3 and figure 2). The seedlings treated with Cusilano and chitosan were found to have the maximum root length, *i.e.* 6.00 and 5.00 cm which were statistically at par with each other. Minimum root length (3.00 cm) was recorded in the untreated control. The shoot length recorded for Cusilano (10.00 cm) and gallic acid (9.00 cm) were higher than salicylic acid and chitosan which recorded 8 cm shoot length each. Non-significant differences with respect to number of branching and stem diameter were observed among the treatments including the untreated control.

Table 3. Effect of biostimulants on tomato seedling growth parameters

Biostimulant	Dose	Seedling growth parameter					
		Root length (cm)	Shoot length (cm)	No. of branching	Stem diameter (mm)	Fresh Seedling Weight (g)	Dry Seedling Weight (g)
Gallic acid	**3µg/ml**	4.00	9.00	4.00	1.00	0.40	0.29
Salicylic acid	**3µg/ml**	4.50	8.00	4.00	1.50	0.30	0.18
Cusilano	**3µg/ml**	6.00	10.00	4.00	2.00	0.50	0.37
Chitosan	**5mg/ml**	5.00	8.00	4.00	1.00	0.25	0.15
Control	-	3.00	6.00	4.00	1.00	0.10	0.05
CD (p=0.05)		1.22	1.04	NS	NS	0.05	0.04

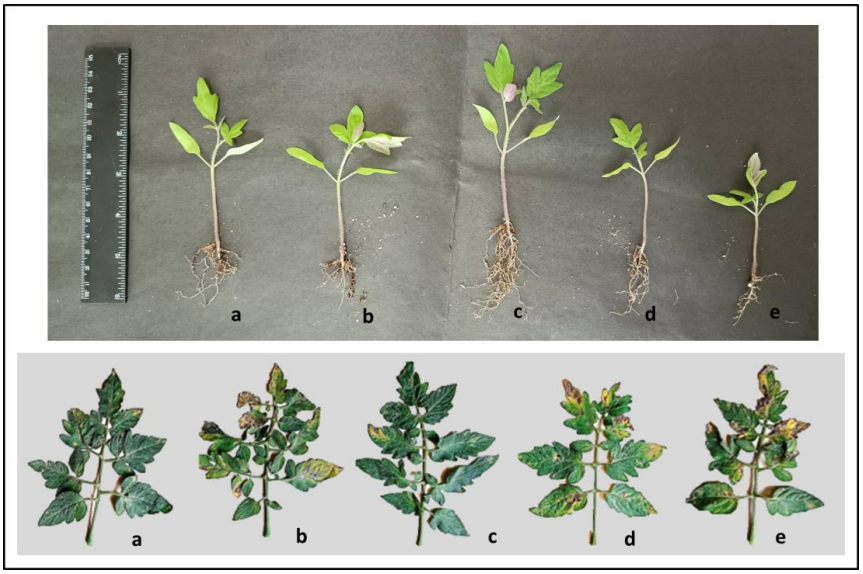

Figure 2. Effect of biostimulants on tomato seedling growth and early blight of tomato (a. gallic acid @3µg/ml, b. salicylic acid @3µg/ml, c. Cusilano @3µg/ml, d. chitosan @5mg/ml, e. control)

The seedling fresh weight was significantly influenced by the biostimulants. After 20 days, the highest values were shown by Cusilano treated

seedlings (0.50g) followed by gallic acid (0.40g). Seedling fresh weight in salicylic acid (0.30g) and chitosan (0.25g) treated plants were found to be at par with each other. Minimum fresh seedling weight was recorded in control (0.10g). Similar trend was observed in case of dry seedling weight. The highest value was shown by Cusilano treated seedlings (0.37g) followed by gallic acid (0.29g), whereas salicylic acid (0.18g) and chitosan (0.15g) were found to be at par with each other. Minimum dry seedling weight was recorded in control (0.05g).

3.3. Biochemical assay of host defense enzymes

Biochemical analysis was conducted to elucidate the plant defense triggered by gallic acid (3µg/ml), salicylic acid (3µg/ml), Cusilano (3µg/ml) and chitosan (5mg/ml) as compared to untreated control. The activity of defense enzyme peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine lyase (PAL) was measured in tomato leaves (cv. Avtar) challenged with *A. solani* (Table 4 and figure 3).

Table 4 Effect of bio stimulants on plant defense enzymes in tomato against *Alternaria solani*

Bio stimulant			Gallic acid	Salicylic acid	Cusilano	Chitosan	Control	Healthy control	Tukey's HSD (p=0.05)
Dose			3µg/ml	3µg/ml	3µg/ml	5mg/ml	-	-	
Host defense enzyme	PO (µmol/min/gm protein)	0 hr	7.50	7.14	7.92	7.66	5.76	6.00	0.51
		24 hrs	10.87	9.12	14.64	8.44	7.40	6.02	0.32
		48 hrs	13.98	9.17	20.28	9.42	8.02	6.03	0.28
		72 hrs	29.08	10.32	23.95	10.16	9.08	6.03	0.31
		96 hrs	8.17	9.62	26.64	7.72	7.11	6.04	0.25
	PPO (µmol/min/gm protein)	0 hr	0.97	0.92	0.92	0.99	0.79	0.76	0.11
		24 hrs	1.32	0.99	1.99	1.12	0.82	0.74	0.47
		48 hrs	1.82	1.82	2.45	1.42	0.93	0.78	0.33
		72 hrs	2.62	1.68	3.44	1.57	0.99	0.82	0.67
		96 hrs	3.03	1.22	2.46	1.27	0.9	0.80	0.51
	PAL (µg of t-cinnamic acid formed $h^{-1}g^{-1}$ fw)	0 hr	0.49	0.47	0.45	0.48	0.49	0.40	0.01
		24 hrs	0.53	0.49	0.64	0.54	0.51	0.40	0.01
		48 hrs	0.60	0.52	0.84	0.60	0.57	0.41	0.02
		72 hrs	0.85	0.68	0.89	0.83	0.62	0.40	0.03
		96 hrs	0.83	0.66	1.07	0.81	0.54	0.40	0.02

3.3.1. Peroxidase (POD) activity

Significant increase in peroxidase activity was recorded in tomato plants treated with biostimulants as compared to control. At 0 hr, no substantial difference in peroxidase activity was observed in any of the treatments, however at 24 hrs after treatment a significant increase in peroxidase activity (µmol/min/mg protein) was observed in the pathogen inoculated plants. POD activity was recorded at 24 hrs interval and it reached its peak at 72 hrs after inoculation of *A. solani* in all treatments except Cusilano. After 72 hrs, POD activity decreased significantly in case of gallic acid, salicylic acid and chitosan,

whereas in case of Cusilano higher value of POD was recorded at 96 hrs (26.64). At 72 hours after inoculation, among the treatments highest POD activity (23.95) was recorded in Cusilano and least was recorded in chitosan (10.16) which was at par with salicylic acid (10.32). POD activity was observed lower as compared to all treatments in the control.

3.3.2. Polyphenol oxidase (PPO) activity

Significant increase in PPO activity was recorded in tomato plants treated with biostimulants as compared to control. At 0 hr, no substantial difference in PPO activity was observed in any of the treatments, however, at 24 hrs after treatment, a significant increase in PPO activity (µmol/min/mg protein) was recorded. PPO activity was recorded at 24 hr interval and it reached its peak at 72 hours after inoculation of *A. solani* in all treatments except gallic acid. After 72 hrs, PPO activity decreased significantly in case of salicylic acid, Cusilano and chitosan, whereas in case of gallic acid higher value of PPO activity was recorded at 96 hrs (3.03). At 72 hours after inoculation, among the treatments highest PPO activity (3.44) was recorded in Cusilano and least was recorded in chitosan (1.57) which was at par with salicylic acid (1.68). PPO activity was observed lower as compared to all treatments in the control.

3.3.3. Phenylalanine Ammonia Lyase (PAL) activity

Significant increase in PAL activity was recorded in tomato plants treated with biostimulants as compared to control. At 0 hr, no substantial difference in PAL activity (µg of t-cinnamic acid formed $h^{-1}g^{-1}$ fw) was observed in any of the treatments, however at 24 hr after treatment a significant increase in PAL activity was recorded in plants. PAL activity was recorded at 24 hr interval and it reached its peak at 72 hours after inoculation of *A. solani* in all treatments except Cusilano. After 72 hrs, PAL activity decreased significantly in case of gallic acid, salicylic acid and chitosan, whereas in case of Cusilano higher PAL activity (1.07) was recorded at 96 hrs. At 72 hours after inoculation, among the treatments highest PAL activity (0.89) was recorded in Cusilano. This was followed by gallic acid (0.85) and chitosan (0.83) while least PAL activity (0.68) was recorded in salicylic acid. PPO activity was observed lower as compared to all treatments in the control.

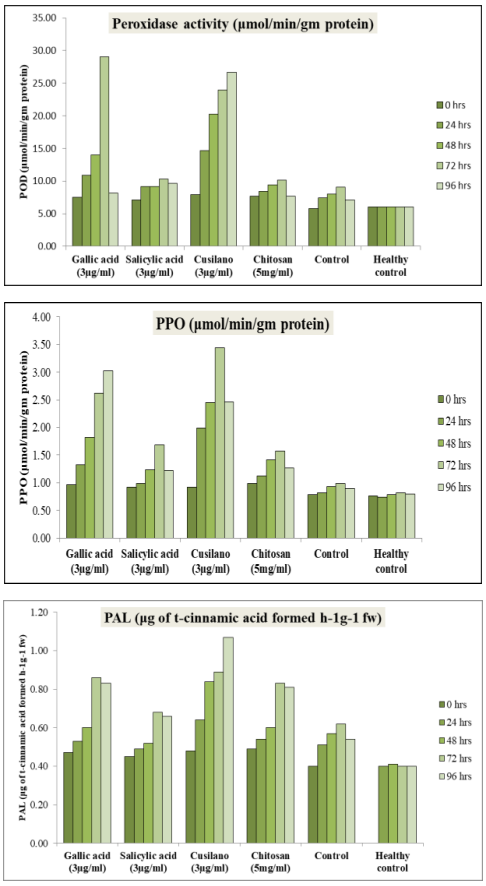

Figure 3. Effect of biostimulants on plant defense enzymes in tomato against *Alternaria solani*

4. Discussion

Early blight disease of tomato caused by *A. solani* severely impacts the fruit quality and productivity. Hence, exploring sustainable disease management strategies is a vital requirement for reducing the hazardous fungicide compounds that are harmful to human beings and animals. The efficacy of biostimulants in plant protection against pathogens has been demonstrated by many authors (Du, 2015; Malik et al., 2020; Patkowska, 2021). Our results showed that the tested biostimulants have potential antifungal activity against *A. solani* causing early

blight of tomato. Similar observations have been recorded by other workers. Nagar et al. (2020) observed that syringic acid (a derivative of gallic acid) at 100 ppm concentration recorded 89.48 per cent inhibition of mycelial growth. Gallic acid and its derivatives were found to negatively affect the conidial germination and appressorium formation of *Magnaporthe grisea* (Ahn et al., 2005). However, Shalaby et al. (2022) reported that salicylic acid amendment of the culture media up to 200 µM did not significantly affect the mycelial growth of *A. solani*. Inhibition of growth of *A. solani* in chitosan-amended medium at a concentration of 1 and 5 mg/ml was observed by Sathiyabama et al. (2014) and the growth inhibition was recorded as 62.3 and 68.6 per cent, respectively over the control. In this study Cusilano i.e., gelatin enriched with colloidal silver and copper was found most effective against pathogenic fungi *A. solani*. Our results are in concurrence with other workers who reported that colloidal Cu and nanoparticles possess significant antifungal activity against different pathogenic fungi adversely affecting the sporulation and structure of fungal mycelia (Abd-El-Aty and Ammar, 2016; Seku et al., 2018). It has been reported that copper and silver in colloidal form can readily enter the fungal cell wall, disrupt protein synthesis and cause cell deformation. As a result of strong van der Waals interactions, colloidal particles tend to aggregate during storage. Therefore, in order to ensure their long-term stability, coating with different polymers like gelatin, tri-sodium citrate and SDS is recommended. In several studies, gelatin has been found to be the best natural capping and stabilizing agent that prevents rapid oxidation used during the preparation of colloids and nanoparticles (Zhang and Yang, 2013; Mao et al., 2015). It is speculated that the efficacy of Cusilano in mycelial inhibition and disease control may be due to the facilitated penetration of gelatin-enriched colloidal paericles through the fungal cell wall, disruptting the DNA amplification and thus inhibiting the mycelial growth and sporulation.

The biostimulants had significant positive impact on the tomato seedling growth parameters. These findings are supported by Ramadan et al. (2013) who found that exogenous application of salicylic acid (0.5 and 1.0 mM) enhanced the growth and plant yield of tomato in addition to the reduction of infection with *A. tenuissima*. Wafaa and Ghafar (2004) observed that application of salicylic acid against tomato bacterial spot disease, under artificial conditions decreased the disease severity.

Several workers have reported the role of different biochemical application in conferring direct or indirect defense in host against various pathogens (Meena et al., 2001; Chandra et al., 2001; Tripathi et al., 2019). Spraying salicylic acid triggers the SAR pathway and stimulates defence mechanism against future attacks (Achuo et al.; 2004; Satija et al.; 2005). In wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), both pathogen infection and SAR treatment can induce broad-spectrum resistance to various diseases, including powdery mildew, leaf rust and Fusarium head blight (Wang et al., 2018). According to Sathiyabama et al. (2014) delayed symptoms appearance and reduced disease severity (75% reduction) was observed in chitosan-treated plants when compared with the control. This was attributed to the induced level of chitinase activity and new

isoforms of chitinase, resulting in the reduction of early blight disease severity in tomato leaves. Sahlata and Tal (1998) reported increased POD activity in salt tolerant and sensitive species of tomato. Salim et al. (2011) also observed that Fusarium wilt resistant tomato genotypes expressed enhanced enzymatic activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase than susceptible cultivar. Patel et al. (2011) reported that early blight tolerant tomato variety NDT-6 exhibited rapid induction of defense-related enzymes, *viz*., catalase, peroxidase, β-1,3 glucanase, phenylalanine ammonia lyase (PAL), chitinase and polyphenoloxidase on treatment with *A. solani* culture filtrate. PAL act as a defense enzyme and hinder the pathogen development in the plant cells (MacDonald and Dcunha 2007; Gao et al. 2008). The present findings are in conformity with the results obtained by Hura et al. (2007) who observed an increase in the activity of PAL in the cases of winter triticale and a drought resistant maize genotype.

The results of this study are supported by Bigirimana and Hofte (2002) who tested benzothiadiazole for ablity to induce resistance against *Colletotrichum lindemuthianum* in susceptible and moderately resistance variety of bean plants. Mosa (2002) reported the potential of salicylic acid and benzothiadiazole to induce systemic resistance in rice blast disease (*Magnaporthe grisea*). Similarly, Saikia et al. (2003) recorded the effect of salicylic acid in growth promotion and induced systemic resistance against Fusarium wilt of chickpea (*F. oxysporum* sp. *ciceri*) and observed a significantly increased activity of peroxidase, polyphenol oxidase and total phenol in treated plants. Chandra et al. (2015) and Zhao (2007) have reported that exogenous application of chitosan nanoparticles on tea leaves elevate the defense response.

The biostimulants exhibited good antifungal activity against *A. solani*, positive impact on the tomato seedling growth parameters and synergistic effect on plant defence enzymes. It was also concluded that gelatin-enriched with colloidal silver and copper was found most effective for management of early blight of tomato. These results facilitate the future use of gelatin-colloidal particles in plant disease management.

Acknowledgement This work was supported by ICAR and World Bank jointly funded project on NAHEP-CAAST (Protected Agriculture and Natural Farming).

Conflict of interest: The authors declare no conflict of interest.

References

Abada, K.A., Mostafa, S.H., Hillal, M.R., 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. Egyptian Journal of Applied Sciences 23(20): 47–58.

Adhikari, P., Oh, Y., Panthee, D.R., 2017. Current Status of Early Blight Resistance in Tomato: An Update. Int J Mol Sci. 18(10): 2019. doi: 10.3390/ijms18102019.
Ammar, H.A., Rabie, G.H., Mohamed, E., 2019. Novel fabrication of gelatin-encapsulated copper nanoparticles using *Aspergillus versicolor* and their application in controlling of rotting plant pathogens. Bioprocess and Biosystems Engineering, 42(12): 1947–1961. doi:10.1007/s00449-019-02188-5
Bektas, Y., 2022. FytoSol, a Promising Plant Defense Elicitor, Controls Early Blight (*Alternaria solani*) Disease in the Tomato by Inducing Host Resistance Associated Gene Expression. Horticulturae 8(6): 484.
Blanca, J., Canizares, J., Cordero, L., Pascual, L., Diez, M.J., Nuez, F., 2012. Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. PLoS One 7(10): e48198.
Chance, B., Maheli, A.C., 1995. Assay of catalases and peroxidases. Methods Enzymol 2: 764–775.
Chanthini, K.M.P., Senthil-Nathan, S., Sivanesh, H., Ramasubramanian, R., Stanley-Raja, V., Karthi, S., Sundar, N.S., Ali, M.A., Alwahibi, M.S., 2023. Inhibition of pathogen and induction of resistance against early blight: *Alternaria solani* Sorauer in tomato plants (*Solanum lycopersicum* L.) through external application of compounds from seaweed *Chaetomorpha antennina*. Bory. Chem. Biol. Technol. Agric. 10: 120. https://doi.org/10.1186/s40538-023-00484-7
Dey, P., Ramanujam, R., Venkatesan, G., Nagarathnam, R., 2019. Sodium alginate potentiates antioxidant defense and PR proteins against early blight disease caused by *Alternaria solani* in *Solanum lycopersicum* Linn. *PLoS One* 14(9): e0223216.
Du, J.P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 196: 3–14.
Edward, R., Kessmann, H., 1992. Isoflavonoid phytoalexins and their biosynthetic enzymes. In Molecular Plant Pathology: A Practical Approach, SJ Gurr, MJ McPherson and DJ Bowles, eds (Oxford: IRL Press). pp. 45–62.
Esfahani, M.N., 2022. An IPM plan for early blight disease of potato *Alternaria solani* sorauer and *A. alternata* (Fries.) Keissler. Archives of Phytopathology and Plant Protection 55(7): 785–796. https://doi.org/10.1080/03235408.2018.1489600.
FAO, 2022. FAOSTAT Statistical Database 2020. Food and Agricultural Organization of the United Nations, Rome. http://www.fao.org/faostat/en/#data.
Farouk, S., Youssef, S.A., Ali, A.A., 2012. Exploitation of Biostimulatants and Vitamins as an alternative strategy to control early blight of tomato plants. Asian Journal of Plant Sciences 11(1): 36–43.
Gebashe, F., Gupta, S., Staden, J.V., 2021. Biostimulants for crops from seed germination to plant development. Chapter 18 - Disease management using biostimulants. Academic Press, P 411–425.
Grover, R.K., Moore, J.D., 1962. Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. Phytopathology 52: 876–880.
Khalil, M.I.I., Youssef, S.A., 2024. Physiological and biochemical responses of *Alternaria alternata* infected tomato to *Trichoderma harzianum* and *Chaetomium*

globosum application. South African Journal of Botany 166: 116-125. https://doi.org/10.1016/j.sajb.2024.01.020.
Khan, S., Zahoor, M., Khan, M.S., Ikram, M., Islam, N.U, 2023. The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon 9: 6. https://doi.org/10.1016/j.heliyon. e16928.
Kumar, S., Chandra, R., 2024. Integrated field application of *T. viride*, botanicals and fungicides for managing early blight (*Alternaria solani*) and enhancement of plant growth, tuber nutritional quality, and potato yield. Journal of Natural Pesticide Research, 8: 100070
Kushwaha, R.K., Sharma, N.P., Baldodiya, V.K., 2018. Profitability of Tomato (*Lycopersicon esculentum*) Production in some Selected Areas in Panna District of Madhya Pradesh Int.J.Curr.Microbiol.App.Sci. Special Issue 6: 2117-2124
Malik, A., Mor, V.S., Tokas, J., Punia, H., Malik, S., Malik, K., Sangwan, S., Tomar, S., Singh, P., Singh, N., 2020. Biostimulant-Treated Seedlings under Sustainable Agriculture: A Global Perspective Facing Climate Change. Agronomy 11: 14.
Mckinney, H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthos poriumsativum*. Journal of Agricultural Research 26: 195–217.
NHB, 2022. 2nd Advance Estimate, National Horticultural Board, 2021–22. Government of India. nhb.gov.in/
Pal, G., Roy, S., Singh, N., Singh, P.M., Yerasu, S.R., Yadava, R.B., Behera, T.K., 2023. A study on economic impact assessment of tomato var. Kashi Aman using the economic surplus model. Vegetable Science 50(1): 46-51 doi: 10.61180/vegsci.2023.v50.i1.06
Pandey, K.K., Pandey, P.K., Kalloo, G., Banerjee, M.K., 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. Journal of General Plant Pathology 69: 364–371.
Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandic, A., Noris, E., Matic, S., 2021. A Review of the Most Common and Economically Important Diseases That Undermine the Cultivation of Tomato Crop in the Mediterranean Basin. Agronomy 11(11): 2188. https://doi.org/10.3390/agronomy11112188
Patkowska, E., 2021. Biostimulants managed fungal phytopathogens and enhanced activity of beneficial microorganisms in rhizosphere of Scorzonera (*Scorzonera hispanica* L.). Agriculture 11(4):347. https://doi.org/10.3390/agriculture11040347.
Ponsankar, A., Senthilnathan, S., Vasanthasrinivasan, P., Pandiyan, R., Karthi, S., Kalaivani, K., 2023. Systematic induced resistance in *Solanum lycopersicum* (L.) against vascular wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) by *Citrullus colocynthis* and *Trichoderma viride*. PLoS One 18(5): e0278616.
Pradhanang, P.M., Ji, P., Momol, M.T., Olson, S.M., Mayfield, J.L., Jones, J.B., 2005. Application of acibenzolar–S–methyl enhances host resistance in tomato against *Ralstonia solanacearum*. Plant Disease 89(9): 989–993.
Rabiei, Z., Hosseini, S., Dehestani, A., Pirdashti, H., Beiki, F., 2022. Exogenous hexanoic acid induced primary defense responses in tomato (*Solanum*

lycopersicum L.) plants infected with *Alternaria solani*. Scientia Horticulturae 295: 110841.
Ricci, M., Tilbury, L., Daridon, B., Sukalac, K., 2019. General Principles to Justify Plant Biostimulant Claims. Front. Plant Sci. 10: 494.
Sathiyabama, M., Akila, G., Charles, R.E., 2014. Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer. Archives of Phytopathology and Plant Protection 47(16): 1963–1973.
Senthilnathan, S., 2015. A review of biopesticides and their mode of action against insect pests. In: Thangavel P, Sridevi G, editors. Environmental sustainability. New Delhi: Springer India. p. 49–63.
Shalaby, T.A.; Taha, N.A.; Taher, D.I.; Metwaly, M.M.; El-Beltagi, H.S.; Rezk, A.A.; El-Ganainy, S.M.; Shehata, W.F.; El-Ramady, H.R.; Bayoumi, Y.A., 2022. Paclobutrazol improves the quality of tomato seedlings to be resistant to *Alternaria solani* Blight disease: Biochemical and Histological Perspectives. Plants 11: 425.
Shinde, B.A., Dholakia, B., Hussain, K.H., Aharoni, A., Giri, A.P., Kamble, A.C., 2018. WRKY1 acts as a key component improving resistance against *Alternaria solani* in wild tomato, *Solanum arcanum* Peralta. Plant Biotechnology Journal 16: 1502–1513.
Tatiana, Z., Yamashita, K., Matsumoto, H., 1999. Iron deficiency Induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber root. Plant Cell Physiology 40: 273–280.
Vander Plank, J.E., 1963. Plant Diseases: Epidemics and Control. Academic Press, New York and London. p 349.
Wezel, A., Herren, B.G., Kerr, R.B., Barrios, E., Gonçalves, A.L., Sinclair, F., 2020. Agroeco- logical principles and elements and their implications for transitioning to sustainable food systems. a review. Agron Sustain Dev. 40(6): 1–3.