



Elicitation of innate immunity in tomato by salicylic acid and *Amomum nilgircum* against *Ralstonia solanacearum*

K. Narasimhamurthy^a, K. Soumya^b, A.C. Udayashankar^{a,*}, C. Srinivas^c, S.R. Niranjana^a

^a Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, 570 006, Karnataka, India

^b Department of Microbiology, Field Marshal K. M. Cariappa College, A Constituent College of Mangalore University, Madikeri, 571 201, Karnataka, India

^c Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore, 560 056, Karnataka, India

ARTICLE INFO

Keywords:

Induction of resistance
Seed treatment
Combination seed treatment *Amomum nilgircum*
Salicylic acid
Ralstonia solanacearum
Tomato
Defense enzymes

ABSTRACT

Ralstonia solanacearum, the dreaded bacterial wilt pathogen is one of the most devastating plant pathogenic bacteria reported worldwide with vast economic importance. In the present study, seed treatment with different concentrations of salicylic acid and *Amomum nilgircum* leaf powder was studied to offer protection against bacterial wilt in tomato. Among treatments, highest germination percent was recorded in *A. nilgircum* leaf powder seed treatment at 5 g kg⁻¹ seeds and with salicylic acid seed treatment at 1 mM. The combination seed treatment with salicylic acid and *A. nilgircum* leaf powder further improved germination percent and seedling vigor. In greenhouse studies, seeds treatment with *A. nilgircum* leaf powder at 5 g kg⁻¹ seeds and salicylic acid seed treatment at 1.0 mM offered 48% and 50% disease protection respectively as compared to other treatments. The combined seed treatment (salicylic acid + leaf powder) at 1.0 mM + 2.5 g kg⁻¹ seeds reduced wilt incidence by 63% in greenhouse studies. Under field experiments, combined treatment (salicylic acid + leaf powder) at 1 mM + 2.5 g kg⁻¹ seeds reduced wilt incidence by 61% as compared to control (95%). Tomato seeds treated with salicylic acid and leaf powder followed by seedlings challenged with *R. solanacearum* recorded maximum peroxidase and polyphenol oxidase enzyme activities as compared with controls. The peroxidase and polyphenol oxidase enzyme activities were higher in combined treated with 1.0 mM + 5 g kg⁻¹ seeds challenged with *R. solanacearum*, whereas no change was observed in control.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is ranked 2nd most essential crop in the world (FAO, 2013) and is a major component in the daily diet. It serves as an important source of nutrients including an excellent source of natural colours and antioxidant compounds, such as vitamin C, polyphenols and carotenoids (Hu et al., 2014). Phytopathogens like bacteria, fungus, virus and nematodes cause several diseases in tomato. Currently, about 200 tomato diseases are identified worldwide among them *Ralstonia solanacearum* causing bacterial wilt is the most damaging. *Ralstonia solanacearum* has a varied host range of over 500 crop species representing more than 54 families (Yuliar and Toyota, 2015). It is known to cause bacterial wilt across tropical and subtropical areas of the world in many important crops like tomato, eggplant, banana, peanut, olive, tobacco, potato, pepper, groundnut, ginger, etc., (James and Mathew, 2017).

Ralstonia solanacearum is a soil-borne; vascular plant pathogen that

first colonizes the root surface and then invades the roots through small natural wounds or root tips (Chen et al., 2017). It produces extracellular polysaccharides, which in turn blocks the water flow causing chlorosis, wilting of plants and finally death of the plant (Jiang et al., 2017). The bacterial wilt is challenging to control completely and highly difficult to manage by any single control method, due to which, the pathogen can persist in the soil for a long time (Genin and Denny, 2012). Synthetic pesticides are non-biodegradable, cause environmental pollution and harmful to humans and animals (Srijita, 2015). Presently the use of plant-derived alternatives seems to play an important role in the management of phytopathogens (Singh and Jagtap, 2017). About 10% of the total number of plant species worldwide has been studied for their pesticidal activity (Suffredini et al., 2004). Many reports have indicated that plant extracts have been efficiently used in the control of phytopathogens under *in vivo* and field conditions (Sales et al., 2016).

Salicylic acid (SA) is a natural hormone in plants and it is known to be involved in natural host plant defense reactions to pathogen attack in

* Corresponding author. Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, 570 006, Karnataka, India.

E-mail address: acudayashankar@gmail.com (A.C. Udayashankar).

several host-pathogen interactions (Angulo et al., 2015). It is a phenolic compound that intricates in plant processes that contain fruit maturity and senescence (Khan et al., 2015). Plant hormones like jasmonic acid (JA), ethylene, SA and abscisic acid are significant molecules of numerous signaling pathways in plant defense. SA plays a key role in regulation of various physiological and biochemical mechanisms in plants including seed germination, plant growth promotion, thermogenesis, flowering, fruit yield, root growth, nutrient transport, ethylene biosynthesis, plant water relations, photosynthesis and defense responses (Hayat et al., 2010; Hashempour et al., 2014; Miura and Tada, 2014; Zhang et al., 2017). Some of these above mechanisms are induced by SA in a dose-dependent manner, triggered by application with a low concentration of SA (Dempsey and Klessig, 2017).

Application of SA exogenously may influence the physiological reactions and plant growth promoters or bio-regulators such as transpiration rate increases plant growth and photosynthetic reactions (Bulgari et al., 2014). Most of the reports on SA have focused on mediating local and systemic defense responses against abiotic and biotic stresses in the plant (Atkinson and Urwin, 2012). Also, proteomic reports have discovered that the application of SA exogenously can up or down-regulation of certain proteins and enzymes intricate in plant energy metabolism (Tarchevsky et al., 2010).

Amomum nilgircum plant belonging to the *Zingiberaceae* family is relatively a new species from the Western Ghats, India (Thomas et al., 2012). The phytochemicals and antimicrobial activity of its leaf extracts were recently reported by Narasimhamurthy et al. (2017). Induction of systemic resistance (ISR) in plants is one of the approaches and is an emerging potential in plant protection against range of phytopathogens (Vallad and Goodman, 2004). ISR is a phenomenon in which the prior treatments of biological or chemical inducers stimulate the plant defense system against the succeeding pathogen attack (Percival, 2001). There are few defense enzymes like, peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD) which have extensive implications in elucidating defense in host plants against pathogens (Bhuvaneshwari and Paul, 2012; Sudisha et al., 2013). POX is a haem-containing glycoprotein which oxidizes a varied range of compounds in the presence of H_2O_2 (Hiraga et al., 2001). POX is involved in various mechanisms in defense responses such as redox reactions in plasma membranes, auxin and ethylene metabolism and cell wall modifications. The PPO enzyme is copper-containing, catalyzes the O_2 dependent oxidation of mono and o-diphenols to o-diquinones, and is responsible for the oxidative browning and increases the antimicrobial activity (Newman et al., 2011). The components of plant materials with different bioactive molecules are known to show antimicrobial effect and which probably is intricate in the plant defense mechanisms (Anitha et al., 2016). Therefore, in the present study, seed treatment with different concentrations of salicylic acid and *A. nilgircum* leaf powder was studied to offer protection against bacterial wilt in tomato.

2. Material and methods

2.1. Collection and preparation of plant material

Fresh *A. nilgircum* leaves were collected from the Western Ghats, India, at $11^{\circ}03'15.46''$ N, $076^{\circ}32'23.58''$ E at an elevation of 1150 m above sea level. The collected leaf samples were first washed in running tap water, followed by successive washing in distilled water, placed on blotter sheets. Clean leaves were surface sterilized with 0.1% mercuric chloride for 20 s; washed with distilled water. Washed leaves were cut into small pieces and shade dried at room temperature ($25 \pm 2^{\circ}C$) for three weeks. Dried leaves were ground to a fine powder using a blender and stored in plastic containers at room temperature for further studies.

2.2. Mode of salicylic acid seed treatment

The plant resistance activator salicylic acid solutions (0.05 mM,

0.1 mM, 0.5 mM, 1 mM, 1.5 mM and 2 mM) was prepared by dissolving in chilled distilled water. Tomato seeds (cv. Arka Meghali) were surface sterilized with 1% sodium hypochlorite for 2 min, rinsed in distilled water thrice and dried on blotter sheets. Four hundred tomato seeds were soaked for 2 h in SA solution by dissolving in chilled distilled water. Polyethylene glycol (PEG-4000) (10% w/v) was added as a carrier for seed treatment. Tomato seeds soaked in distilled water served as controls.

2.3. Mode of *A. nilgircum* leaf powder seed treatment

Surface sterilized tomato seeds were soaked in 0.2% sterilized carboxymethyl cellulose (CMC) as adhesive before treating with *A. nilgircum* leaf powder. Seeds were treated with *A. nilgircum* leaf powder at 0.5, 1, 2, 2.5 and 5 g kg^{-1} seeds. The seeds treated with distilled water alone served as control.

2.4. Effect of seed treatment with salicylic acid and *A. nilgircum* leaf powder on seed germination and seedling vigour of tomato under laboratory conditions

Effect of *A. nilgircum* leaf powder on seed germination and seedling vigor index was assessed at different concentrations viz., 0.5, 1, 2, 2.5 and 5 g kg^{-1} seeds and different salicylic acid solutions 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 1.5 mM and 2 mM were used. The germination test was conducted according to paper towel method (ISTA, 2005). One hundred treated seeds were placed equidistantly on germination paper presoaked in distilled water, rolled with another presoaked germination paper and wrapped with polythene to avoid drying of papers. The rolled papers were incubated at $24 \pm 2^{\circ}C$ for ten days. Seeds treated with distilled water served as control. Post incubation, the numbers of germinated seeds were counted and seedling vigor index was calculated. The vigor index (VI) was calculated by the following formula: vigor index (VI) = (mean root length + mean shoot length) x germination percentage (Baki and Anderson, 1973). The experiment consisted of four replicates with 100 seeds each and was repeated thrice.

2.5. Screening of salicylic acid and *A. nilgircum* leaf powder for their potential to protect against *R. solanacearum* under greenhouse conditions

The virulent strain of *R. solanacearum* isolated from bacterial wilt infected tomato plant was used in the present study (NCBI accession number: KF924743; Narasimhamurthy and Srinivas, 2012). Treatments were same as described above. The treated tomato seeds were sown in plastic trays filled with sterilized potting soil (sand, soil and manure at a ratio of 1:2:1 respectively) and watered frequently. Twenty days old seedlings from trays were transplanted to 30 cm diameter earthen pots at a depth of approximately 1.5 cm with 3 kg of sterilized mixture filled with 50% field soil +50% sand. Seedlings were challenge inoculated by trimming the lower roots with sterilized scissors dipped in fresh *R. solanacearum* suspension of 1×10^8 CFU mL^{-1} (Hindi, 2013). Post-treatment, seedlings were maintained in greenhouse. Untreated tomato seedlings served as control. Each experiment comprised of 25 seedlings per treatment with four replications (100 seedlings). The disease incidence was recorded one-week post pathogen inoculation.

2.6. Effect of combination seed treatment of salicylic acid and *A. nilgircum* on seed germination and seedling vigour of tomato

Based on *in vitro* studies, combination seed treatment contained 1 mM SA combined with *A. nilgircum* leaf powder. The SA treated seeds were shade dried, soaked in 0.2% sterilized carboxymethyl cellulose (CMC) for 1 min and mixed with 5 g kg^{-1} seeds *A. nilgircum* leaf powder. The treated seeds were subjected to germination test according to paper towel method (Baki and Anderson, 1973).

2.7. Effect of combination seed treatment of salicylic acid and *A. nilgircum* on bacterial wilt in tomato under greenhouse conditions

Seed treatment was performed as described above. Seedlings were challenge inoculated by trimming the lower roots with sterilized scissors dipped in fresh *R. solanacearum* suspension of 1×10^8 CFU mL⁻¹ (Hindi, 2013). Post treatment, seedlings were maintained in greenhouse. Untreated seedlings served as control. Each experiment included 25 seedlings per treatment with four replications (100 plants) and was set up in a randomized block design. The wilt incidence was recorded one-week post pathogen inoculation.

2.8. Effect of combination seed treatment of salicylic acid and *A. nilgircum* on bacterial wilt incidence in tomato under field conditions

Treatments were the same as described above. Treated tomato seedlings were raised in protrays for 3 weeks before transplanting to the experimental field. The field experiments were conducted at farmer's agricultural plot in Bhoomishettihalli (13°28'05.7"N 78°04'57.7"E) longitude with an altitude of 865 m elevation in Karnataka, India, during tomato growing period of March–June 2018. The three-weeks-old treated tomato seedlings were transplanted to experimental plot. Seedlings were challenged with *R. solanacearum* by root dip inoculation method (Singh et al., 2018). The experimental plot area consisted of 25 m² covering 50 seedlings per each row and the distance between rows was 50 cm. The percent (%) bacterial wilt incidence was calculated by following formula: percentage (%) of wilt incidence = Number of wilted plants in a plot/Total number of plants in a plot \times 100 (Narasimhamurthy et al., 2016, 2018).

2.9. Enzyme extraction and assay

To study the role of defence enzymes POX and PPO, tomato leaf samples were collected from post-challenge inoculated seedlings raised from 1 mM SA, *A. nilgircum* leaf powder at 5 g kg⁻¹ seeds and combined seed treatment of salicylic acid and *A. nilgircum* leaf powder. Leaf samples were collected from different time intervals; 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 h post pathogen inoculation and kept at -80 °C for enzyme studies. Distilled water treated samples served as control. One gram of leaf sample was macerated in a pre-chilled mortar and pestle with 2 mL of ice-cold 50 mM sodium phosphate buffer (pH 6.0). The homogenate was centrifuged at 12,000 rpm at 4 °C for 20 min and the supernatant was used for POX and PPO enzyme activity. All procedures were performed at 4 °C. Protein concentration was measured according to Lowry et al. (1951).

2.9.1. Estimation of peroxidase activity

Peroxidase activity was analysed by taking 100 μ L of homogenate in 3 mL of 10 mM sodium phosphate buffer (pH 6.0) (having 19 μ L of 30% hydrogen peroxide and 38 μ L guaiacol as hydrogen donor/50 mL buffer). The oxidation of guaiacol was measured by UV-Vis spectrophotometer at 470 nm. The change in absorbance was recorded at 30 s intermissions for 3 min. The peroxidase enzyme activity was expressed as a change in absorbance min⁻¹ g⁻¹ of fresh leaves (Hammerschmidt et al., 1982).

2.9.2. Estimation of polyphenol oxidase activity

The PPO activity was determined according to Mayer et al. (1965). The reaction mixture contained 200 μ L of homogenate, 200 μ L of substrate solution containing 10 mM catechol and 1.5 mL of 0.1 M sodium phosphate buffer (pH 7.4). The rate of oxidation of catechol was measured by UV-Vis spectrophotometer at 420 nm for 1 min at 25 °C. The PPO activity was expressed as a change in absorbance min⁻¹ g⁻¹ of fresh leaves.

3. Results

3.1. Effect of seed treatment with salicylic acid and *A. nilgircum* leaf powder on seed germination and seedling vigour of tomato under laboratory conditions

Seed treatment with salicylic acid and *A. nilgircum* leaf powder improved mean root length, shoot length and vigour index of germinated seedlings in comparison to control. Among *A. nilgircum* leaf powder seed treatment, maximum germination percent was recorded with 5.0 g kg⁻¹ seeds followed by 2.5, 2.0, 1.0 and 0.5 g kg⁻¹ seeds (Fig. 1). Among SA seed treatment, maximum germination was recorded at 1.0 mM seed treatment followed by 0.05 mM, 0.1 mM, 0.05 mM, 1.5 mM and 2.0 mM treatments respectively. The seed treatment with *A. nilgircum* leaf powder at 0.5, 1.0, 2.0, 2.5 and 5.0 g kg⁻¹ seeds improved seed germination percent to 87%, 88%, 88%, 89% and 90% respectively in comparison to control (85%) followed by improved vigour index of tomato seedlings to 1554, 1596, 1678, 1753 and 1833 respectively as compared to control (1232). Seed treatment with SA at 0.05 mM, 0.1 mM, 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM recorded seed germination percent of 89%, 90%, 92%, 95%, 91% and 90% as compared to control followed by improved vigour index of tomato seedlings by 1598, 1694, 1749, 1837, 1467 and 1346 respectively (Fig. 1).

3.2. Screening of salicylic acid and *A. nilgircum* leaf powder for their potential to protect against *R. solanacearum* under greenhouse conditions

Among SA and *A. nilgircum* leaf powder treatment, varied percent protection was recorded under greenhouse conditions. The *A. nilgircum* leaf powder seed treatment at 5.0 g kg⁻¹ seeds recorded maximum disease protection followed by 2.5, 2.0, 1.0 and 0.5 g kg⁻¹ seeds. The SA seed treatment exhibited maximum bacterial wilt protection at 1.0 mM concentration followed by 0.5 mM, 0.1 mM, 1.5 mM, 0.05 mM and 2.0 mM in comparison to control (97%) (Table 1). Seed treatment with *A. nilgircum* leaf powder at 0.5, 1.0, 2.0, 2.5 and 5 g kg⁻¹ seeds reduced bacterial wilt incidence by 35%, 42%, 44%, 47% and 48% respectively as compared to control (97% wilt incidence) (Table 1). Seed treatment with SA at 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 1.5 mM and 2 mM reduced wilt incidence by 33%, 39%, 42%, 50%, 38% and 32% respectively in comparison to control (Fig. 2).

3.3. Effect of combination seed treatment of salicylic acid and *A. nilgircum* on seed germination and seedling vigour of tomato

Combination seed treatment of SA and *A. nilgircum* leaf powder recorded improved seed germination and seedling vigour in comparison to individual seed treatment and control. Seed treatment with 1.0 mM SA + 5.0 g kg⁻¹ seeds *A. nilgircum* leaf powder resulted seed germination of 97% followed by 1.0 mM SA + 2.0 g kg⁻¹ seeds *A. nilgircum* leaf powder, 1.0 mM SA + 2.5 g kg⁻¹ seeds *A. nilgircum* leaf powder, 1.0 mM SA + 1 g kg⁻¹ seeds *A. nilgircum* leaf powder and 1.0 mM SA + 0.5 g kg⁻¹ seeds *A. nilgircum* leaf powder recording germination of 93%, 92%, 91% and 89% in comparison to 86% in control (Fig. 3).

3.4. Effect of combination seed treatment of salicylic acid and *A. nilgircum* on bacterial wilt in tomato under greenhouse conditions

The combination seed treatment with SA and *A. nilgircum* leaf powder reduced bacterial wilt incidence under greenhouse conditions in comparison to control (Table 2). The combined treatments (SA + *A. nilgircum* leaf powder) at 1.0 mM + 0.5 g kg⁻¹, 1.0 mM + 1 g kg⁻¹, 1.0 mM + 2 g kg⁻¹, 1.0 mM + 2.5 g kg⁻¹ and 1.0 mM + 5 g kg⁻¹ seeds reduced wilt incidence by 44%, 46%, 49%, 63% and 58% respectively as compared to control (95%) (Table 2).

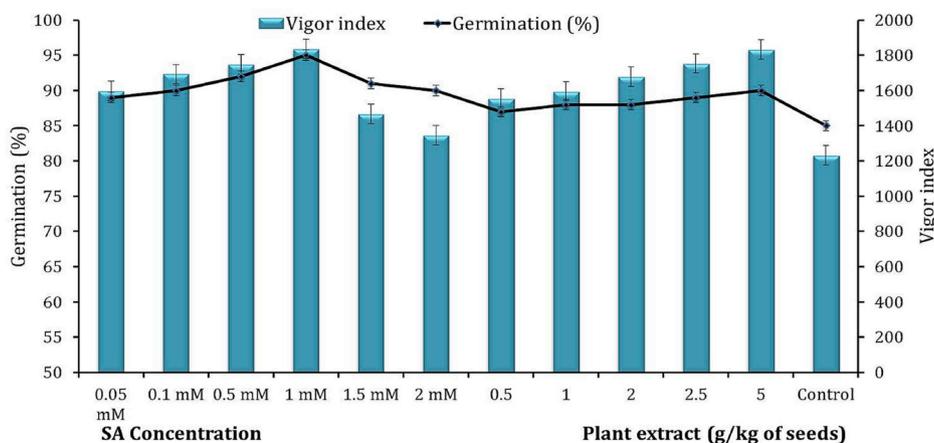


Fig. 1. Effect of seed treatment with salicylic acid and *A. nilgiricum* leaf powder on seed germination and seedling vigour of tomato under laboratory conditions.

Table 1

Effect of salicylic acid and *A. nilgiricum* leaf powder for their potential to protect against *R. solanacearum* under greenhouse conditions.

Treatments	Disease incidence (%)
0.05 mM	63.4 ± 2.54 ^l
0.1 mM	57.3 ± 1.79 ^f
0.5 mM	54.8 ± 2.13 ^e
1.0 mM	46.6 ± 1.57 ^a
1.5 mM	58.5 ± 1.66 ^g
2.0 mM	64.1 ± 2.54 ^l
0.5 g kg ⁻¹ seeds	61.6 ± 3.12 ^b
1.0 g kg ⁻¹ seeds	54.3 ± 1.89 ^e
2.0 g kg ⁻¹ seeds	52.7 ± 1.71 ^d
2.5 g kg ⁻¹ seeds	49.6 ± 1.13 ^c
5.0 g kg ⁻¹ seeds	48.5 ± 1.66 ^b
Control	96.5 ± 1.39 ^k

Means of three replications, followed by the letters according to Duncan's multiple range tests (DMRT). Means sharing different alphabetical superscripts (a-k) in a column are significantly different (P < 0.05).

3.5. Effect of combination seed treatment of salicylic acid and *A. nilgiricum* on *R. solanacearum* incidence in tomato under field conditions

Under field conditions, combination seed treatment with SA and *A. nilgiricum* leaf powder resulted in significant decrease in bacterial wilt incidence. Among seed treatments, highest protection against bacterial wilt was observed in combined treatment with SA and *A. nilgiricum* leaf powder at 1.0 mM + 5 g kg⁻¹ seeds followed by 1.0 mM + 0.5 g kg⁻¹ seeds treated plot. The combined seed treatments (SA + *A. nilgiricum* leaf

powder) 1.0 mM + 0.5 g kg⁻¹, 1.0 mM + 1.0 g kg⁻¹, 1.0 mM + 2 g kg⁻¹, 1.0 mM + 2.5 g kg⁻¹ and 1.0 mM + 5 g kg⁻¹ seeds significantly reduced wilt incidence by 41%, 44%, 48%, 61% and 57% respectively in comparison to control (95%) (Table 3).

3.6. Estimation of peroxidase and polyphenol oxidase activity

The combined seed treatment with salicylic acid and *A. nilgiricum* leaf powder influenced changes in defense-related POX and PPO enzymes and the maximum activity of these enzymes was recorded at different time point post-challenge inoculation. POX activity was extended to the maximum level in all the treatments at 36 h post pathogen inoculation and then gradually reduced (Fig. 4). The POX activity was higher in combination seed treatment with 1.0 mM + 5 g kg⁻¹ seeds challenged with *R. solanacearum* as compared to other treatments,

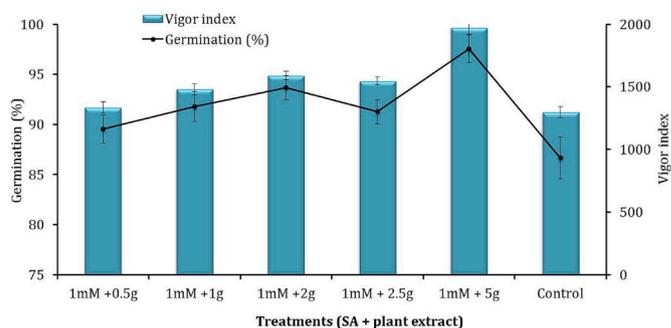


Fig. 3. Effect of combination seed treatment of salicylic acid and *A. nilgiricum* on seed germination and seedling vigour of tomato.



Fig. 2. Screening of salicylic acid for their potential to protect tomato against bacterial wilt under greenhouse conditions a). Untreated control, b). *R. solanacearum* treated control and c). Seed treatment with salicylic acid and challenge inoculated with *R. solanacearum*.

Table 2

Effect of combination seed treatment of salicylic acid and *A. nilgircicum* on *R. solanacearum* in tomato under greenhouse conditions.

Treatments (SA + <i>A. nilgircicum</i> leaf extract)	Disease incidence (%)
1.0 mM 0.5 g kg ⁻¹ seeds	51.7 ± 1.78 ^c
1.0 mM + 1.0 g kg ⁻¹ seeds	48.9 ± 2.12 ^d
1.0 mM + 2.0 g kg ⁻¹ seeds	46.3 ± 1.66 ^c
1.0 mM + 2.5 g kg ⁻¹ seeds	32.7 ± 0.89 ^a
1.0 mM + 5.0 g kg ⁻¹ seeds	37.6 ± 1.79 ^b
Control	95.2 ± 1.39 ^f

Means of three replications, followed by the letters according to Duncan's multiple range tests (DMRT). Means sharing different alphabetical superscripts (a–f) in a column are significantly different ($P < 0.05$).

Table 3

Effect of combination seed treatment of salicylic acid and *A. nilgircicum* on *R. solanacearum* incidence in tomato under field conditions.

Treatments (SA + plant extract)	Disease incidence (%)
1 mM + 0.5 g kg ⁻¹ seeds	54.7 ± 1.78 ^c
1 mM + 1.0 g kg ⁻¹ seeds	50.9 ± 2.12 ^d
1 mM + 2.0 g kg ⁻¹ seeds	47.3 ± 1.66 ^c
1 mM + 2.5 g kg ⁻¹ seeds	38.6 ± 1.74 ^b
1 mM + 5 g kg ⁻¹ seeds	34.7 ± 0.83 ^a
Control	95.2 ± 1.38 ^f

Means of three replications, followed by the letters significantly according to Duncan's multiple range tests (DMRT). Means sharing different alphabetical superscripts (a–f) in a column are significantly different ($P < 0.05$).

whereas no change was observed in control. The activity of PPO increased significantly in combination seed treatment with 1.0 mM + 5 g kg⁻¹ seeds up to 48 h post-challenge inoculation (Fig. 5). The activity of PPO enzyme from the combination seed treatment with SA and *A. nilgircicum* leaf powder treated tomato seedlings challenge inoculated with *R. solanacearum* revealed higher activity in comparison to individual seed treatment or control.

4. Discussion

Present-day agriculture worldwide is dependent on resistance breeding and synthetic chemicals to fight against crop pathogens. Alternatively, inducers of plants own immunity through abiotic and biotic sources are environmentally safe and are reliable. One of the main constraints of tomato cultivation today is losses caused by bacterial wilt. The extensive genetic diversity of strains responsible for the various bacterial wilt diseases of plants has led to the concept of a *Ralstonia solanacearum* species complex (Genin and Denny, 2012).

Pre-treatment with several biotic and abiotic inducers induce plants

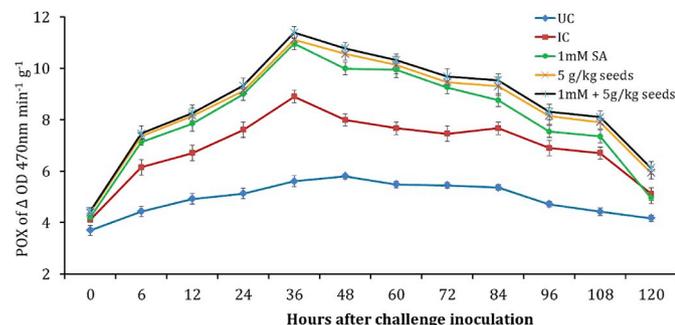


Fig. 4. The combined effect of SA and *A. nilgircicum* leaf powder on activity of peroxidase in tomato plants. Values are the mean of three replications and bars represent standard errors. UC: Uninoculated control; IC: Inoculated control; 1 mM SA and 5 g kg⁻¹: Seedlings treated with SA and leaf powder alone and 1 mM SA and 5 g kg⁻¹: Combined treatment with SA and leaf powder.

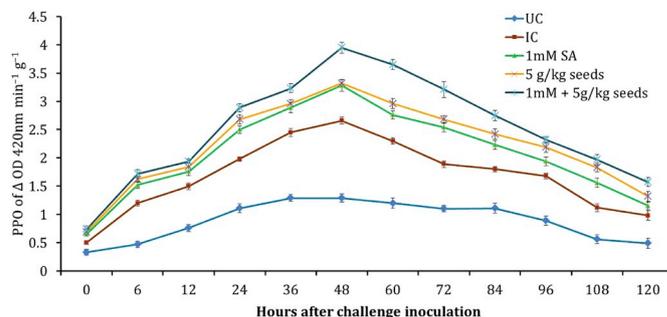


Fig. 5. The combined effect of SA and leaf powder on activity of polyphenol oxidase in tomato plants. Values are the mean of three replications and bars represent standard errors. UC: Uninoculated control; IC: Inoculated control; 1 mM SA and 5 g kg⁻¹: seedlings treated with SA and leaf powder alone and 1 mM SA and 5 g kg⁻¹: Combined treatment with SA and leaf powder.

defense response against pathogen attack in plants against viruses (Udayashankar et al., 2009, 2012), fungi (Jogiaha et al., 2018) and bacteria (Narasimhamurthy et al., 2018). This response is mediated through many bio-chemicals, physiological and molecular mechanisms (Idrees et al., 2011). Plants are the major pools of antimicrobial compounds that can be used as an alternative source for the development of natural pesticides to act against different phytopathogens (Jeyaseelan et al., 2010). The treatment of eco-friendly and bioactive compounds against plant pathogens, in the form of dried leaf powders/green manures of many plants, could be added to soil as organic amendments or the soil could be drenched with plant extracts (Gasić and Tanović, 2013).

In the present study, seed treatment with salicylic acid and *A. nilgircicum* leaf powder improved tomato seed germination and vigour index of germinated seedlings in comparison to control. The exogenous application of SA has been reported as a germination stimulator (Sakhabutdinova et al., 2003). In the present study, seed treatment with SA and *A. nilgircicum* leaf powder reduced bacterial wilt incidence in tomato under greenhouse conditions. Earlier reports indicate that exogenous application of SA increases membrane permeability which would enable absorption and use of nutrients, stimulates root formation and passage of assimilates (Aftab et al., 2010). The aqueous extract of *Psidium guajava*, *Aloe vera*, and *Allium sativum* treatments are reported to protect tomato seedlings against bacterial wilt (Bora and Semual, 1998). Vu et al. (2013) reported *Sedum takesimensis* powder formulation successfully protected tomato seedlings against bacterial wilt under greenhouse conditions.

The elicitors are ecologically harmless chemicals that can induce defense responses in plants. In the present study, we report induction of resistance against *R. solanacearum* upon pre-treatment with salicylic acid. Plant materials have been deliberated as one of the foremost varieties of compounds that induce resistance in host plants. The combination seed treatment of salicylic acid and *A. nilgircicum* leaf powder elevated the activity of defense-related enzymes. The SA stimulates defense genes intricate in cell wall modification, production of secondary metabolites in plants and PR proteins (Mishra et al., 2012). In tomato plants, bacterial wilt has been reduced by many defense mechanisms, which include ET and SA related defense signaling pathways (Milling et al., 2011; Chen et al., 2009).

In the present study, tomato seed treatment with SA and *A. nilgircicum* leaf powder significantly increased POX and PPO activities post-challenge inoculation with *R. solanacearum*. Peroxidases are vital enzymes that play a key role in regulating processes like elongation of plant cell, oxidation of phenolic compounds, cross-linking of extension monomers, polysaccharide cross-linking, oxidation of hydroxyl cinnamyl alcohols into free radical intermediates, wound healing and oxidation of IAA (Haluskova et al., 2009; Jadesha et al., 2012; Thakker et al., 2013). POX enzyme is also involved in the biosynthesis of lignin

which offers a physical barrier and/or limits the degree of pathogen invasion in plants (Thilagavathi et al., 2007).

In the present study, PPO activity increased upon seed treatment with SA and *A. nilgircum* leaf powder as compared to control. The increase in PPO activity might have occurred for blocking the spread of the pathogen in plants by catalyzing phenolics oxidation which is long known to have antimicrobial properties and for inhibiting the dispersal of pathogens (Ngadze et al., 2012). SA is well known to be intricate in signal transduction and shows a significant role in plant defense reactions of local and acquired resistance. Besides, to reduce the disease incidence, SA also affected the accumulation of phenolic compounds that are associated with plant resistance (El-Hadrami et al., 1997).

Exogenous foliar application of salicylic acid, H₂O₂, and chitosan elicitors in *C. annuum* significantly increased endogenous H₂O₂ as well as gene expression and enzymatic activities related to plant defense as phenylalanine ammonia-lyase and catalase 1 (Mejía-Teniente et al., 2013). Bacterial wilt incidence was reduced by 40% and 57% by silicon and chitosan treatments respectively in tomato (King Kong 2). Combined application of silicon and chitosan treatments reduced the wilt incidence by 75% in King Kong 2 followed by 47% in L390 respectively (Kiririka et al., 2013). Evidence of their synergistic effect was analysed by gene expression analysis conducted at 72 h post-inoculation via TOM2 microarray revealed regulation of 204 and 126 genes. The defense genes chitinases and peroxidases were highly up-regulated in combined silicon and chitosan treatments. Under greenhouse conditions, tomato plants pre-treated by soil drenching with DL-3-aminobutyric acid (BABA) reduced disease severity of bacterial wilt compared to control plants receiving water. BABA treatment significantly reduced the population of *R. solanacearum* in stems of tomato plants (Hassan and Abo-Elyousr, 2013).

The elicitors, chitosan, salicylic acid and jasmonic acid-induced resistance in hydroponic tomato against wilt pathogen, *R. solanacearum*. The induction by elicitors was supported by defense responses in tomato plants against *R. solanacearum*, evident from reduced vascular browning and wilting symptoms of tomato plants treated with SA, chitosan and challenged subsequently with *R. solanacearum* (Mandal et al., 2013). The reduced disease incidence in tomato by SA and chitosan may be a result of cell wall strengthening through the deposition of lignin and coincident induction of defense enzymes. The combination of soil fumigant (thymol), a monoterpene phenol compound originating from thyme, and acibenzolar-S-methyl (foliar spray) significantly reduced disease and increased tomato yield compared to control, whereas acibenzolar-S-methyl or thymol alone did not significantly reduce disease or increase yield compared to the control (Hong et al., 2011).

The *Allium fistulosum* extract (100 and 50%) suppressed the growth of *R. solanacearum* under *in vitro* inhibition assay. Under *in vivo* experiments in a growth chamber, pre-plant treatment of soil with *A. fistulosum* extract significantly reduced *R. solanacearum* populations. The wilt pathogen was not detected in the soil after treatment with 100% concentrated extract from the third day after application until the end of the experiment (Deberdt et al., 2012). The methanol and chloroform extracts of *Chromolaena odorata* inhibited the growth of *R. solanacearum* under *in vitro* conditions using agar disc diffusion method (Sukanya et al., 2009). Soil drenching treatments of aqueous plant extracts of *Hibiscus sabdariffa*, *Punica granatum* and *Eucalyptus globulus* significantly reduced bacterial wilt incidence in potato under greenhouse and field conditions (Hassan et al., 2009).

The *in vitro* antibacterial activity by disc diffusion sensitivity test of aqueous, acetone and methanol extracts of *Eichhorina crassipes*, *Mimosa diplotricha*, *Lantana camara* and *Prosopis juliflora* was studied against *R. solanacearum*. Aqueous extract of *E. crassipes* provided the highest inhibition zone, followed by *M. diplotricha*. Tomato plants treated with leaf extract of *E. crassipes* reduced percent disease severity index under field conditions (Alemu et al., 2013). The plant extracts were effective in inhibiting the growth of the bacterial pathogen, not only *in vitro* but also

in the stem of potato plants as compared with the inoculated control. The activity of defense-related enzymes, including peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase were significantly increased in plants treated with plant extracts compared to control. In greenhouse experiments, chemical elicitor acibenzolar-S-methyl (ASM) applied as foliar spray and/or soil drench (3 µg/mL) before and as foliar spray (30 µg/ml) after transplanting was effective in reducing bacterial wilt incidence on moderately resistant cultivars inoculated with *R. solanacearum* (Pradhanang et al., 2005).

5. Conclusions

As one of the approaches for bacterial wilt management caused by *R. solanacearum*, botanicals can be a promising and eco-friendly choice. Chemical based companies may also use the findings as a preliminary study for the preparation and development of plant-based green technology for the management of bacterial wilt of tomato. The results presented here show that tomato seed treatment with SA, *A. nilgircum* leaf powder individually and in combination protect tomato plants against bacterial wilt under greenhouse and field conditions. Thus, seed treatment with SA could be a valuable alternative to bacterial wilt disease control. Also, it is necessary to examine the toxicity of the *A. nilgircum* leaf powder for endorsing its use on a commercial scale.

Acknowledgments

The authors are gratefully acknowledging the financial assistance granted by Post-Doctoral fellowship (No.F./PDFSS-2014-15-7487), University Grant Commission (UGC), New Delhi for carrying out this research. The authors also like to Department of Forests and Wildlife, Govt. of Kerala for providing necessary for sample collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101414>.

References

- Aftab, T., Khan, M.M.A., Idrees, M., Naeem, M., Moinuddin, M., 2010. Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisin in production in *Artemisia annua* L. *J. Crop Sci. Biotech.* 13, 183–188.
- Alemu, D., Lemessa, F., Wakjira, M., Berecha, G., 2013. Antibacterial activity of some invasive alien species extracts against tomato (*Lycopersicon esculentum* Mill) bacterial wilt caused by *Ralstonia solanacearum* (Smith). *Plant Pathol.* 12, 61–70.
- Angulo, C., Leyva, M.O., Finiti, I., López-Cruz, J., Fernández-Crespo, E., García-Agustín, P., González-Bosch, C., 2015. Role of dioxygenase α-DOX2 and SA in basal response and in hexanoic acid-induced resistance of tomato (*Solanum lycopersicum*) plants against *Botrytis cinerea*. *J. Plant Physiol.* 175, 163–173.
- Anitha, C., Soumaya, J.D., Ajitha, R.K., Remaya, M.P., Sreja, J.R., Suvachan, A., 2016. Augmentative biopotential efficiency of leaf extracts on the tropical medicinal plant *Cardiospermum helicacabum*. *World J. Parasitol. Pharm. Sci.* 5, 1557–1568.
- Atkinson, N.J., Urwin, P.E., 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot.* 63, 3523–3543.
- Baki, A.A., Anderson, J.D., 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci.* 13, 630–633.
- Bhuvaneshwari, V., Paul, P.K., 2012. Transcriptional and translational regulation of defense enzymes induced by neem fruit extract in tomato. *Arch. Phytopathol. Plant Prot.* 45, 1374–1385.
- Bora, L.C., Semual, J., 1998. Use of medicinal plants for management of bacterial blight in rice and bacterial wilt of tomato in Assam. In: Golden Jubilee Symposium on Spice, Medicinal & Aromatic Plants Biodiversity, Conservation & Utilisation, Calicut, India, 36, pp. 10–12.
- Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A., 2014. Biostimulants and crop responses: a review. *J. Biol. Agri. Horti.* 31, 1–17.
- Chen, Y.Y., Lin, Y.M., Chao, T.C., Wang, J.F., Liu, A.C., Ho, F.L., Cheng, C.P., 2009. Virus induced gene silencing reveals the involvement of ethylene, salicylic acid and mitogenactivated protein kinase-related defense pathways in the resistance of tomato to bacterial wilt. *Physiol. Plant.* 136, 24–335.
- Chen, D., Liu, B., Zhu, Y., Zhang, H., Chen, Z., Zheng, X., Xiao, R., Chen, Y., 2017. Complete genome sequence of *Ralstonia solanacearum* FJAT-91, a high-virulence pathogen of tomato wilt. *Genome Announc.* 5, e00900–e00917.

- Deberdt, P., Perrin, B., Coranson-Beaudu, R., Duyck, P.F., Wicker, E., 2012. Effect of *Allium fistulosum* extract on *Ralstonia solanacearum* populations and tomato bacterial wilt. *Plant Dis.* 96, 687–692.
- Dempsey, D.A., Klessig, F., 2017. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans? *BMC Biol.* 15, 1–11.
- El-Hadrami, I., Ramos, T., El Bellaj, M., El Drissi-Tourane, A., Macheix, J.J., 1997. A sinapic derivative as induced defense compound of date palm against *Fusarium oxysporum* f. sp. *albedinis*, the agent causing bayoud disease. *J. Phytopathol.* 145, 329–333.
- FAO, 2013. FAOSTAT. Food and Agriculture Organization of the United Nations. Rome.. 2013.
- Gašić, S., Tanović, B., 2013. Biopesticide formulations, possibility of application and future trends. *Pestic. Phytomed.* (Belgrade) 28, 97–102.
- Genin, S., Denny, T.P., 2012. Pathogenomics of the *Ralstonia solanacearum* species complex. *Annu. Rev. Phytopathol.* 50, 67–89.
- Haluszkova, I., Valentovicova, K., Huttova, J., Mistrik, J., Tamas, L., 2009. Effect of abiotic stresses on glutathione peroxidase and glutathione S-transferase activity in barley root tips. *Plant Physiol. Biochem.* 47, 1069–1074.
- Hammerschmidt, R., Nuckles, E.M., Kuc, J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.* 20, 73–82.
- Hashempour, A., Ghasemzhad, M., Fotouhi, G., Sohani, M.M., 2014. The physiological and biochemical response to freezing stress olive plants treated with salicylic acid. *Russ. J. Plant Physiol.* 61, 443–450.
- Hassan, M.A.E., Bereika, M.F.F., Abo-Elnaga, H.I.G., Sallam, M.A., 2009. Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. *Plant Pathol. J.* 25, 352–360.
- Hassan, M.A.E., Abo-Elyousr, K.A.M., 2013. Activation of tomato plant defense responses against bacterial wilt caused by *Ralstonia solanacearum* using DL-3-aminobutyric acid (BABA). *Eur. J. Plant Pathol.* 136, 145–157.
- Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. *Environ. Exp. Bot.* 68, 14–25.
- Hindi, N.K.K., 2013. *In vitro* antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar garlic extract combination. *Am. J. Phytomed. Clin. Ther.* 1, 42–51.
- Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., Matsui, H., 2001. A large family of class III plant peroxidases. *Plant Cell Physiol.* 42, 462–468.
- Hong, J.C., Momol, M.T., Ji, P., Olson, S.M., Colee, J., Jones, J.B., 2011. Management of bacterial wilt in tomatoes with thymol and acibenzolar-S-methyl. *Crop Protect.* 30, 1340–1345.
- Hu, G., Fan, J., Xian, Z., Huang, W., Lin, D., Li, Z., 2014. Over expression of SIREV alters the development of the flower pedicel abscission zone and fruit formation in tomato. *Plant Sci.* 229, 86–95.
- International Seed Testing Association (ISTA), 2005. Proceedings of the international seed testing association. International rules of seed testing. *Seed Sci. Technol.* 15 A, 1–9.
- Idrees, M., Naeem, N., Aftab, T., Khan, M.M.A., 2011. Moinuddin. Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. *Acta Physiol. Plant.* 33, 987–999.
- Jadesha, G., Hemachandra, H., Manish, K.M., Manjunath, H.K., Prabakar, V.P., 2012. Role of plant extracts in inducing the systemic acquired resistance in harvested banana against anthracnose disease. *Ann. Biol. Res.* 3, 5413–5419.
- James, D., Mathew, S.K., 2017. Compatibility studies on different endophytic microbes of tomato antagonistic to bacterial wilt pathogen. *Int. J. Adv. Biotechnol. Res.* 7, 190–194.
- Jeyaseelan, E.C., Pathmanathan, M.K., Jeyadevan, J.P., 2010. Inhibitory effect of different solvent extracts of *Vitex negundo* L. and *Allium sativum* L. on phytopathogenic bacteria. *Arch. Appl. Sci. Res.* 2, 325–331.
- Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., Macho, A.P., Ding, W., Liao, B., 2017. Bacterial wilt in China: history, current status and future perspectives. *Front. Plant Sci.* 8, 1549.
- Jogaiah, S., Abdelrahman, M., Tran, L.S.P., Ito, S.I., 2018. Different mechanisms of *Trichoderma virens*-mediated resistance in tomato against *Fusarium* wilt involve the jasmonic and salicylic acid pathways. *Mol. Plant Pathol.* 19, 870–882.
- Kiirika, L.M., Stahl, F., Wydra, K., 2013. Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against *Ralstonia solanacearum*. *Physiol. Mol. Plant Pathol.* 81, 1–12.
- Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance & underlying mechanisms in plants. *Front. Plant Sci.* 6, 1–17.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265.
- Mandal, S., Kar, I., Mukherjee, A.K., Acharya, P., 2013. Elicitor-induced defense responses in *Solanum lycopersicum* against *Ralstonia solanacearum*. *Sci. World J.* 561056.
- Mayer, A.M., Harel, E., Shaul, R.B., 1965. Assay of catechol oxidase a critical comparison of methods. *Phytochemistry* (Oxf.) 5, 783–789.
- Milling, A., Babujee, L., Allen, C., 2011. *R. solanacearum* extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. *PLoS One* 6, 1.
- Mishra, A.K., Sharma, K., Misra, R.S., 2012. Elicitor recognition, signal transduction and induced resistance in plants. *J. Plant Interact.* 7, 95–120.
- Mejía-Teniente, L., Durán-Flores, F.D.D., Chapa-Oliver, A.M., Torres-Pacheco, I., Cruz-Hernández, A., González-Chavira, M.M., Ocampo-Velázquez, R.V., Guevara-González, R.G., 2013. Oxidative and molecular responses in *Capsicum annum* L. after hydrogen peroxide, salicylic acid and chitosan foliar applications. *Int. J. Mol. Sci.* 14, 10178–10196.
- Miura, K., Tada, Y., 2014. Regulation of water, salinity, and cold stress responses by salicylic acid. *Plant Sci. J.* 5, 410.
- Narasimhamurthy, K., Chandra Nayaka, S., Soumya, K., Brijesh, S., Niranjana, S.R., 2017. Phytochemical screening and antimicrobial activity of leaf extracts of *Amomum nilgiriicum* (Thomas) (*Zingiberaceae*) from Western Ghats, India. *J. Biol. Prod. Nat.* 7, 311–330.
- Narasimhamurthy, K., Soumya, K., Chandra Nayaka, S., Niranjana, S.R., Srinivas, C., 2018. Evaluation of biological efficacy of *Trichoderma asperellum* against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Egyptian J. Biol. Pest Control.* 28, 63.
- Narasimhamurthy, K., Srinivas, C., 2012. *In vitro* screening of bioantagonists agents and plant extracts to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*). *J. Agri. Technol.* 8, 999–1015.
- Narasimhamurthy, K., Malini, M., Fazilath, U., Soumya, K., Chandra, N.S., Niranjana, S. R., Srinivas, C., 2016. Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against *Ralstonia solanacearum* causing bacterial wilt. *Scient Horti* 207, 183–192.
- Newman, S.M., Tantasawat, P., Steffens, J.C., 2011. Tomato polyphenol oxidase B is spatially and temporally regulated during development and in response to ethylene. *Molecules* 16, 493–517.
- Ngadze, E., Icishahayo, D., Coutinho, T.A., Waals, J.E., 2012. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. *Plant Dis.* 96, 186–192.
- 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 Dis.* 89, 989–993.
- Percival, G.C., 2001. Induction of systemic acquired disease resistance in plants: potential implications for disease management in urban forestry. *J. Arboric.* 27, 181–192.
- Sakhabutdinova, A.R., Fatkhutdinova, D.R., Bezrukova, M.V., Shakirova, F.M., 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.* 314–319.
- Sales, M.D.C., Costa, H.B., Fernandes, P.M.B., Ventura, J.A., Meira, D.D., 2016. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. *Asian Pac. J. Trop. Biomed.* 6, 26–31.
- Singh, R., Jagtap, G.P., 2017. Effect of Selected Plant Extracts on *in vitro* growth of *Ralstonia solanacearum* (Smith) the causal agent of bacterial wilt of ginger. *Technofame A J. Multidisc. Adv. Res.* 6, 32–39.
- Singh, N., Kumar, R., Ray, S.K., 2018. An Innovative Approach to Study *Ralstonia solanacearum* pathogenicity in 6 to 7 days old tomato seedlings by root dip inoculation. *Bio Protoc.* 8, e3065.
- Srijita, D., 2015. Biopesticides: an eco-friendly approach for pest control. *World J. Pharm. Pharm. Sci.* 4, 250–265.
- Sudisha, J., Mostafa, A., Lam Son, P.T., Ito, S., 2013. Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. *J. Exp. Bot.* 64, 3829–3842.
- Sukanya, S.L., Sudisha, J., Hariprasada, P., Niranjana, S.R., Prakash, H.S., Fathima, S.K., 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *Afr. J. Biotechnol.* 8, 6677–6682.
- Suffredini, J.B., Sader, H.S., Goncalves, A.G., Rais, A.O., Gales, A.C., Varella, A.D., Younes, R.N., 2004. Screening of antimicrobial extracts from plants native to the Brazilian Amazon rainforest and Atlantic forest. *Braz. J. Med. Biol. Res.* 37, 379–384.
- Tarchevsky, I.A., Yakovleva, V.G., Egorova, A.M., 2010. Proteomic analysis of salicylate induced proteins of pea (*Pisum sativum* L.) leaves. *Biochemistry* 75, 590–597.
- Thakker, J.N., Patel, S., Samiksha, D., Pinakin, C., 2013. Induction of defense-related enzymes in banana plants: effect of live and dead pathogenic strain of *Fusarium oxysporum* f. sp. *cubense*. *SRN Biotechnol.* 1–6.
- Thilagavathi, R., Saravanakumar, D., Ragupathi, N., Samiyappan, R., 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. *Phytopathol. Mediterr.* 46, 157–167.
- Thomas, V.P., Sabu, M., Prabhu Kumar, K.M., 2012. *Amomum nilgiriicum* (*Zingiberaceae*), a new species from western Ghats, India. *PhytoKeys* 8, 99–104.
- Udayashankar, A.C., Nayaka, C.S., Niranjan-Raj, S., Kumar, B.H., Reddy, M.S., Niranjana, S.R., Prakash, H.S., 2009. Rhizobacteria mediated resistance against *Bean common mosaic virus* strain blackeye cowpea mosaic in cowpea (*Vigna unguiculata*). *Pest Manag. Sci.* 65, 1059–1064.
- Udayashankar, A.C., Nayaka, C.S., Archana, B., Nayak, U., Niranjana, S.R., Prakash, H.S., 2012. Seed treatment with strobilurins enhances resistance of common bean against *Bean common mosaic virus*. *J. Phytopathol.* 160, 710–716.
- Vallad, G.E., Goodman, R.M., 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci.* 44, 1920–1934.
- Vu, T.T., Kim, J.C., Choi, Y.H., Choi, G.J., Jang, K.S., Choi, T.H., Yoon, T.M., Lee, S.W., 2013. Effect of gallotannins derived from *Sedum takesimensis* on tomato bacterial wilt. *Plant Dis.* 97, 1593–1598.
- Yuliar, N.Y.A., Toyota, K., 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microb. Environ.* 30, 1–11.
- Zhang, Y., Zhao, L., Zhao, J., Li, Y., Wang, J., Guo, R., Gan, S., Liu, C.J., Zhang, K., 2017. S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine tunes salicylic acid homeostasis. *Plant Physiol.* 175, 1082–1093.