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ORIGINAL ARTICLE

Defense priming of tomato plants by *Streptomyces* metabolites to combat *Corynespora cassiicola* and *Pseudomonas syringae* infestations

Shalini Devi Suvala[1] · **Venkata Bhaskara Rao Kokati**[2]

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Abstract
Induction of disease resistance in plants can be a better option in the management of alarming microbial plant diseases. *Streptomyces albolongus* isolate which proved to produce antifungal compounds exhibited antagonism against notorious plant pathogens in our past research. The present work focuses on the utility of *S. albolongus* metabolites in activating tomato plant defense system challenged by *Pseudomonas syringae* and *Corynespora cassiicola*. Metabolites extracted by ethyl acetate were found to contain a high amount of linolenic acid, azelaic acid, hexadecanoic acid and propyl ester of octadec-9-enoic acid as analyzed by Fourier-transform infrared spectroscopy and Gas chromatography–mass spectrometry techniques. Disease severity was significantly low in tomato plants that received metabolite application. Raised peroxidase and polyphenoloxidase enzyme activities were recorded in plants that received metabolite application compared to control plants. Gene expression studies indicated that parallel activation of pathogenesis related and Proteinase inhibitor (PinII) genes contributed for resistance against *P. syringae*. We report the possible role of C 9 and C 18 fatty acids in triggering salicylic acid and jasmonic acid pathways of tomato plants in response to *P. syringae* and *C. cassiicola* inoculation.

Keywords *Streptomyces* metabolites · Plant defense · Linolenic acid · Azelaic acid · *Corynespora cassiicola* · *Pseudomonas syringae*

Introduction

Tomato is one of the most popular vegetables worldwide, however, its cultivation has been limited by pathogens. *Pseudomonas syringae* is an easily cultured Gram-negative bacterial pathogen. It is a biotrophic plant pathogen that derives energy from live plants. It causes bacterial speck disease of tomato. Disease symptoms include small necrotic lesions surrounded by chlorotic halos on foliage. This disease sign decreases fruit marketability and thereby causes economic losses (Preston 2000). *Corynespora cassiicola* is a globally distributed necrotrophic plant pathogen (Lopez et al. 2018; Leilani et al. 2018). It can affect various parts of the plant. This pathogen has a wide host range including more than 530 plant species from 380 genera. It causes target spot disease of tomato. Due to resistance developed by this pathogen, most of the registered fungicides failed in controlling target spot by *C. cassiicola* (Farr and Rossman 2013; Aveline et al. 2014). Uncontrolled use of agrochemicals has become a major cause of food poisoning by pesticides and evolution of microbicide resistant plant pathogens (Sheila et al. 2013; Aveline et al. 2014). Hence there is an urgent need to investigate eco-friendly measures to control these plant pathogens.

The control of plant pathogens by biocontrol microorganism may involve direct growth inhibition of pathogens and induction of defense mechanisms in the plant. In this scenario, beneficial microbes and their metabolites are gaining importance for their preventive and curative effects in the management of many plant diseases (Shalini Devi et al. 2015). Capabilities of fungal and bacterial

✉ Shalini Devi Suvala
suvvalashalinidevi@gmail.com

Venkata Bhaskara Rao Kokati
kokatibhaskar@yahoo.co.in

[1] Department of Microbiology, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad 500 094, India

[2] Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore 632 014, India

compounds in triggering the plant immunity has been explored by many researchers. Picard et al. (2000) proved the tomato plant resistance inducing potential of oligandrin, a protein of microbial origin using pot culture experiment. Ryu et al. (2003) reported in vitro plant defense activation capabilities of 2,3-butanediol extracted from bacteria. Volatile compounds like *m*-cresol and methyl benzoate (Naznin et al. 2014) were identified as plant resistance elicitors of fungal origin by experimenting on Arabidopsis plants grown in the hydroponic culture system. Even though *Streptomyces* spp. are well known as biocontrol agents (Gopalakrishnan et al. 2015; Kurth et al. 2014), very few research publications described the involvement of *Streptomyces* metabolites in the induction of plant resistance. The induced defense system is one of the methods that allow plants to protect themselves from plant pathogens. Finding new systemic resistance inducers is of great interest in agriculture. Hence, in the present research study, we have focused on the application of metabolites from *Streptomyces albolongus* isolate for induction of systemic resistance against plant diseases caused by *P. syringae* and *C. cassiicola*. To the extent of our knowledge, this is the first report exploiting fatty acids from *Streptomyces* for inducing plant defense.

Materials and methods

Collection and maintenance of pathogens

Plant pathogenic fungi, *C. cassiicola* (MTCC 2123) and bacteria, *P. syringae* (MTCC 2730) were collected from Regional Agricultural Research Station, Tirupati, India and maintained on potato dextrose agar and nutrient agar respectively.

Extraction and analysis of metabolites

Metabolites were extracted from *Streptomyces* isolate S9 that was previously isolated in our laboratory from rhizospheric soils (*S. albolongus* KX247006). *S. albolongus* S9 was inoculated in Glucose peptone broth (pH 7) and incubated at 28 °C for 120 h in an incubator. The fermented broth was centrifuged at 8400*g* for 10 min. Further, the supernatant was added to an equal volume (1:1) of ethyl acetate and shook vigorously for 2 h. Ethyl acetate layer containing secondary metabolites was separated. Ethyl acetate was evaporated using rotary evaporator, the residue obtained was dissolved in water (1 g/ml). This is referred to as ethyl acetate extract. Extracted metabolites were subjected to Fourier-transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GCMS) analysis (GCMS-QP 2010 PLUS Quadrupole mass spectrometer) (detailed protocol given below).

Metabolite application and challenge inoculation

Seeds of tomato (*Solanum lycopersicum* L.) cv. PKM-1, a susceptible tomato variety, were sterilized, sowed and maintained according to Martinez et al. (2015). Tomato seedlings were transplanted to pots containing peat moss and red soil (1:1 ratio) and maintained up to 40 days until the complete opening of 6 leaves. Four experimental treatments (T1–T4) were maintained in triplicates. Treatments T1 and T2 were designed to evaluate the efficacy of extracted metabolites in triggering resistance against *P. syringae*. Tomato plants were decapitated above the sixth fully expanded leaf and 30 µl of metabolites was applied on the fresh wound for T1 plants and T2 plants were treated similarly with water. After 7 days, 100 µl of pathogenic bacterial suspension (10^8 CFU/ml) (Picard et al. 2000) was sprayed on the first two leaves present at the base (T1 and T2). Seven days later, disease severity was evaluated by using the below mentioned scoring scale.

1. 10 lesions per leaf, mild chlorosis
2. 10–50 lesions per leaf, mild chlorosis
3. 50–100 lesions per leaf, mild chlorosis
4. More than 100 lesions per leaf, severe chlorosis, flower abortion
5. More than 100 lesions per leaf, lesions on stems, curling of leaves, severe chlorosis, defoliation, flower abortion.

A similar experiment was done with *C. cassiicola* as a challenge organism using treatments T3 and T4. T3 plants were treated with metabolites and T4 with water. Fungal spore suspension (3×10^4 ml^{-1}) (Romeiro et al. 2010) was sprayed on the first two leaves present at the base (T3 and T4) after 5 days of metabolites/water infiltration. Disease severity was evaluated 7 days later by using the below mentioned scoring scale.

1. 1–5 small lesions per leaf
2. 5–25 lesions per leaf
3. More than 25 lesions per leaf, lesions coalesce, leaf curling begins
4. 25–50 lesions per leaf accompanied by leaf blight, reduction of leaf area
5. Grade 4 symptoms + stunted plant growth + large lesions on stem.

Plant disease index, intensity and percent efficacy were calculated by using the following formula.

$$\text{Percentage of disease index (PDI\%)} = \text{Sum of ratings} \times 100/\text{Total no. of leaves}$$

$$\text{(PE\%)} = \text{PDI\% in control} - \text{PDI\% in treatment} \times 100/\text{PDI\% in control.}$$

Determination of enzyme activities

Leaf samples from tomato plants (T1, T2, T3 and T4) were collected after 7 days of pathogen inoculation for determination of enzyme activities. Fresh leaf material (2.0 g) was homogenized by adding 3 ml of ice-cold 50 mM sodium acetate buffer, pH 5.2. Homogenate was filtered through cheesecloth and centrifuged at 10,000 rpm for 15 min at 4 °C. Supernatant (crude extract) was used as the source of enzymes (Roberto et al. 2013). Peroxidase (POX) and polyphenol oxidase (PPO) enzyme activities of collected leaf samples were measured using spectrophotometric estimation procedure.

Spectrophotometric estimation of peroxidase was done by adding 25 µl of the crude extract to 2 ml of a solution containing 50 mM sodium acetate buffer, pH 5.2, 20 mM guaiacol, and 20 mM hydrogen peroxide (H_2O_2). After incubation at 30 °C for 10 min, the absorbance was read at 480 nm. One POX unit of activity (UA) was expressed as the variation of 1U of absorbance at 480 nm mg^{-1} of soluble protein per minute (UA mg P^{-1} min^{-1}).

PPO activity of tomato leaf samples was determined by adding 50 µl of the crude extract to 3 ml of a solution containing 100 mM potassium phosphate buffer, pH 6.5, and 25 mM pyrocatechol. The increase of absorbance at 410 nm, for 10 min at 30 °C, was measured. One PPO unit was expressed as the variation of absorbance at 410 nm mg^{-1} of soluble protein per min and expressed as UA per milligram protein per min (UA mg P^{-1} min^{-1}) (Roberto et al. 2013).

Analysis of gene expression

Expression of defense related genes were studied by amplification using quantitative polymerase chain reaction (qPCR). Tomato plant leaves were collected from four different treatments (T1, T2, T3 and T4) after 7 days of pathogen inoculation. Total RNA was extracted from these samples using Tri-Reagent (Sigma) and cDNA was synthesized with 3 µg of purified total RNA using cDNA synthesis kit (Thermo Scientifics) according to the manufacturer's instructions. Real time quantitative PCR (RT-qPCR) was performed according to the method of Martinez et al. (2015) to study the expression levels of two defense related genes viz., Pathogenesis related gene acidic PR-1 (Block et al. 2005) and proteinase inhibitor gene PinII. Expression values were normalized using actin, a housekeeping gene (Herman et al. 2008).

Fourier-transform-infra red spectrum (FTIR)

FT-infra red spectrum of ethyl acetate extract was analyzed by using Shimadzu FTIR 8400s. The sample was mixed with KBr (potassium bromide) and a disc was prepared for analysis, this disc was applied on FTIR instrument for analysis and the peaks obtained were observed and interpreted (Mohan 2005).

Gas chromatography mass spectroscopy analysis (GCMS)

The active fractions obtained in column chromatography were pooled and subjected to GCMS analysis by following a modified method of Strom et al. (2002). 100 µl of the sample was dried in vacuum, derivatized using BSTFA-TMCS for 30 min at 60 °C and injected onto the GCMS (GCMS-QP 2010 PLUS quadrupole mass spectrometer). GC was performed on a DB-5 column with 0.25 mm i.d. and 0.25 m film thickness (Supelco). Injection temperature was 230 °C, the interface set to 250 °C, and the ion source adjusted to 200 °C. The carrier gas used was helium set at a constant flow rate of 1 ml min^{-1}. The oven temperature was set to 100 °C and held for 4 min. The temperature was increased at a rate of 5 °C, till it reached 280 °C and held for 12 min.

Results and discussion

Disease severity

The role of *S. albolongus* metabolites in preventing the plant diseases caused by *P. syringae* and *C. cassiicola* was evaluated by calculating percent disease index and percent efficacy. Control tomato plants (T2) that received water application and inoculated with *P. syringae* exhibited severe speck symptoms like lesions on leaves and stems, curling of leaves, severe chlorosis, defoliation, flower abortion with 96.6% PDI (Fig. 1a). In tomato plants (T1) treated by metabolites prior to *P. syringae* inoculation, PDI and PE were calculated as 13.3% and 86.63%, respectively.

T4 plants that received water application and then infected by *C. cassiicola*, expressed severe disease symptoms including large lesions on leaves and stem accompanied by leaf blight and reduction of leaf area (Fig. 1b). Higher levels of target spot symptoms were observed in T4 plants is also evident from calculated PDI (98.3%). However

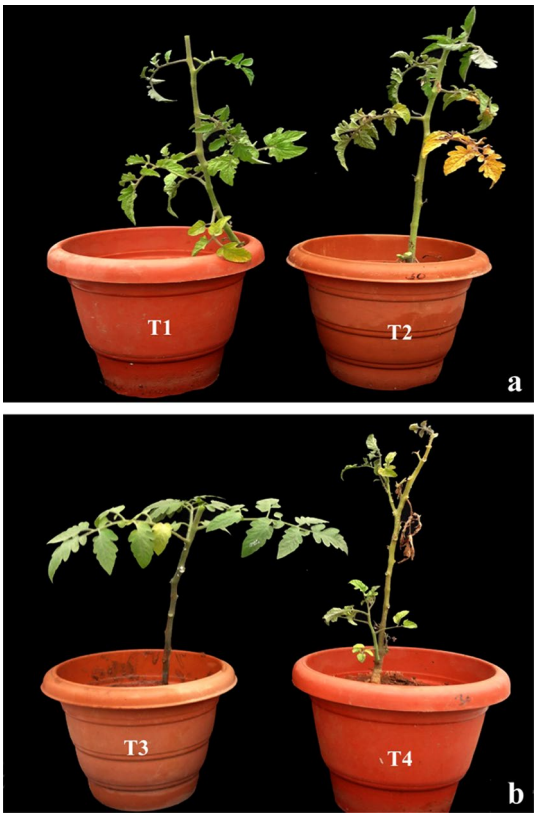

Fig. 1 Pot culture experiments for evaluation of **a** Bacterial speck disease prevention by *S. albolongus* S9 metabolites, T1-metabolites treated and T2-control plant. **b** Target sport disease prevention by *S. albolongus* S9 metabolites, T3-metabolites treated and T4-control plant

only 10% PDI was recorded in T3 plants. Efficacy of target spot prevention was noted as 89.83%.

These results indicate that *S. albolongus* metabolites exhibited induced systemic resistance to leaf infection by *P. syringae* and *C. cassiicola*. Many research and review reports in the last decade support the present findings (Oliveira et al. 2016; Romeiro et al. 2010).

Rudrappa et al. (2010) reported the role of Acetoin, a metabolite secreted by *Bacillus subtilis* strain FB17 in inducing strong resistance against *P. syringae* pv. tomato in *Arabidopsis thaliana*. This study also demonstrated the inability of acetoin biosynthetic mutants of *B. subtilis* strain FB17 in conferring plant resistance against challenge pathogen. Fatima and Anjum (2017) explored induced systemic resistance (ISR) active metabolites like 3-hydroxy-5-methoxy benzene methanol (HMB), eugenol and tyrosine secreted by *Pseudomonas aeruginosa* PM12. This study proved the ability of these bacterial metabolites in combating Fusarium wilt disease of tomato.

Determination of enzyme activity

An increase in activity of the enzymes was observed in plants treated with *S. albolongus* metabolites and then challenged with pathogen when compared to water treated control plants.

Enzyme activities in plants challenged by *P. syringae*

High level of PPO was observed in T1 [8.9 UA (mg P^{-1} min)$^{-1}$] compared to control (T2) [2.66 UA (mg P^{-1} min)$^{-1}$] (Fig. 2a). Application of *S. albolongus* metabolites (T1) raised PPO synthesis by 234% than control (T2). T1 plants exhibited 2.65 UA (mg P^{-1} min)$^{-1}$ of POX levels while T2 plants showed 0.38 UA (mg P^{-1} min)$^{-1}$(Fig. 2b). POX levels were more in T1 plants than T2 plants by 597%.

Enzyme activities in plants challenged by *C. cassiicola*

An increase in activity of enzymes, PPO and POX was observed in T3 plants that received metabolites when compared to water treated plants (T4). T3 plants were found to exhibit 8.05 UA (mg P^{-1} min)$^{-1}$ and 3.28 UA (mg P^{-1} min)$^{-1}$of PPO and POX activates, respectively. However, control plants (T4) showed 2.46 UA (mg P^{-1} min)$^{-1}$ and 0.33 UA (mg P^{-1} min)$^{-1}$ of PPO and POX activates, respectively (Fig. 2c, d). PPO levels were higher in T3 plants by 233% than T4 water treated plants. Activities of POX enzyme was found to be raised by 893% in T3 compared to T4 samples.

The increase of these enzymes is associated with cell wall reinforcement and systemic resistances (Anterola and Lewis 2002). Earlier publications also suggest the higher expression of various defense enzymes in plants upon exogenous application of salicylic acid. The efficacy of salicylic acid (SA) as a therapeutic agent against *Xanthomonas axonopodis* pv. phaseoli was investigated by Ali et al. (2016) using common bean as a model plant. High expression of superoxide dismutase (SOD), malate dehydrogenase (MDH) and phenylalanine ammonia-lyase (PAL) enzymes in SA pre treated plants was reported in this study.

Gene expression studies

Plants exhibit defense responses through various mechanisms/pathways. SA, jasmonic acid (JA), ethylene (ET), abicisic acid (ABA) are known to play prominent roles in these pathways. Hence compounds that trigger the production of these hormones can act as promising agents in inducing resistance to plants. PRI gene is a marker gene that indicates the activation of SA mediated responses. PinII gene is

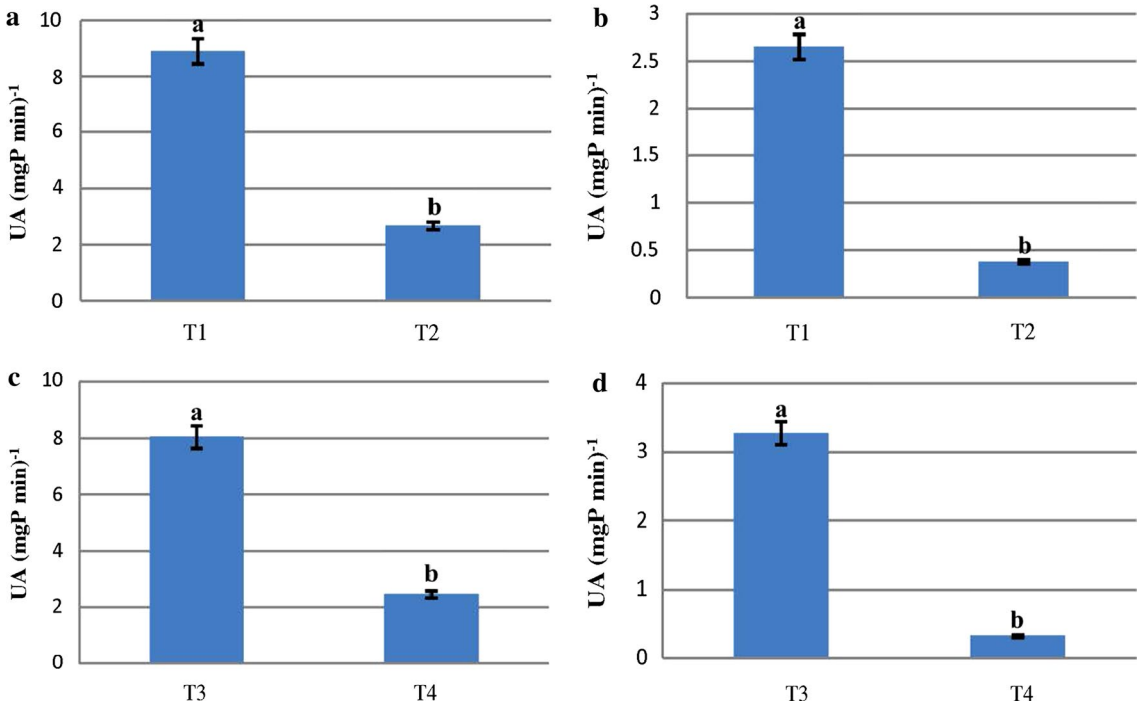

Fig. 2 Graphical representation of enzyme activities in tomato plants **a** PPO levels and **b** POX in tomato plants inoculated with *P. syringae*. **c** PPO levels and **d** POX in tomato plants inoculated with *C. cassiicola.* The data are the average of six replication ± SD. Data in the same column followed by same letter are not significantly different ($P < 0.05$) according to analysis of variance

utilized to measure the induction of JA regulated pathway (Herman et al. 2008).

Challenge inoculation by *P. syringae* was found to induce PRI and PinII genes. Expression of these genes was more pronounced in plants prior treated with metabolites than control plants (Fig. 3a, b). PRI gene expression was elevated in the case of T1 plants by 60.5% than control treatment (T2). In T1 plants, extract infiltration was found to induce PinII gene expression by 62% relative to it's corresponding (T2) control plants.

Metabolite application was found to trigger expression levels of PRI and PinII genes, indicating that both SA and JA pathways have operated in the defense against *P. syringae*. This suggests the cooperative effect of these two signals. This is in agreement with studies of Clarke et al. (2000) who reported the positive combinational effect of SA, JA and ET mediated responses in conferring resistance against *P. syringe* and *P. parasitica* in *Arabidopsis*.

T3 plants exhibited elevated levels of PinII gene expression compared to control plants (T4) that received water application (Fig. 3c, d). Levels of PRI gene transcript was noticed to be higher in plants treated with metabolites and challenge inoculated with *C. cassiicola.* Infiltration of metabolites (T3 plants) led to a 28% higher expression of PRI than control plants (T4). Metabolites induced (T3) plants raised expression of PinII transcripts by 36.7% when compared with control plants (T4).

PinII gene was found to be highly activated in *C. cassiicola* inoculated plants suggesting that JA mediated defense responses could have played a major role in resistance against this necrotrophic pathogen. In a study with tomato-*Botrytis cinerea* plant pathosystem, authors have demonstrated the activation of PinII and LoX a genes in plants induced by *Micromonospora* spp. Since *B. cinerea* is a necrotrophic pathogen JA is expected to play a key role in the defense mechanism (Martinez et al. 2015).

The above results were in agreement with the statement that plants develop defense systems to detect the effectors and set appropriate responses, termed as effector triggered immunity (Chisholm et al. 2006). Hence we assume that even though metabolite application efficiently reduced the severity of both infections, expression levels of evaluated defense genes were different. This means that although the plants are primed by the same elicitor they respond differently to different pathogens. Depletion of SA levels in transgenic plants resulted in increased susceptibility to biotroph, *P. syringe,* but not necrotrophs like *B. cineria* (Dempsey et al. 1999; Thomma et al. 1999). This explains the importance of SA in controlling biotrophic plant pathogens. Experiments with coi1 mutants explained the significance of JA-dependent defense against necrotrophs. However, the

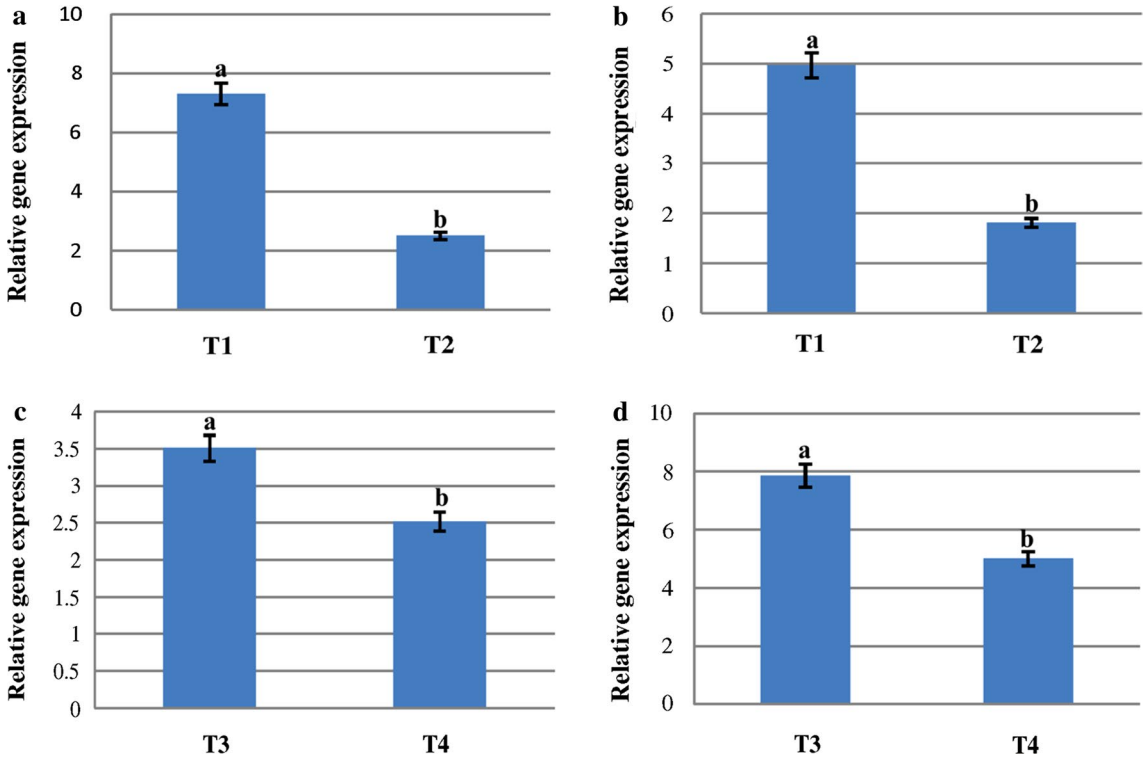

Fig. 3 Gene expression levels in experimental tomato plants. Graphs indicate expression of **a** PRI and **b** PinII in experimental tomato plants inoculated with *P. syringae* **c** PRI and **d** PinII in experimental tomato plants inoculated with *C. cassiicola*. The data are the average of six replication ± SD. Data in the same column followed by same letter are not significantly different ($P < 0.05$) according to analysis of variance

role of JA was found to be dispensable for resistance against biotrophic fungus, *Phytophthora parasitica* (Lawton et al. 1994).

Analysis of metabolites from *S. albolongus*

In the present study culture filtrate of *S. albolongus* was extracted by ethyl acetate as this organic solvent has medium polarity and minimum toxicity. In a study done by Fatima and Anjum (2017), different solvent extracts of *P. aeruginosa* were used for inducing tomato plant resistance. Among all the extracts, metabolites extracted using ethyl acetate were found to exhibit resistance inducing capabilities against fusarium wilt disease.

Metabolites obtained from *S. albolongus* culture filtrate were analyzed by FTIR. The infra red (IR) spectrum of the ethyl acetate extract showed adsorption bands at 1242.2, 1759.14 and 2995.5 which revealed the presence of functional groups esters (C–O), carboxylic acid (C=O) and alkane (C–H) respectively. GCMS chromatogram shown in Fig. 4, revealed the presence of four major peaks (high abundance). The *m/z* ratio and available library data suggested

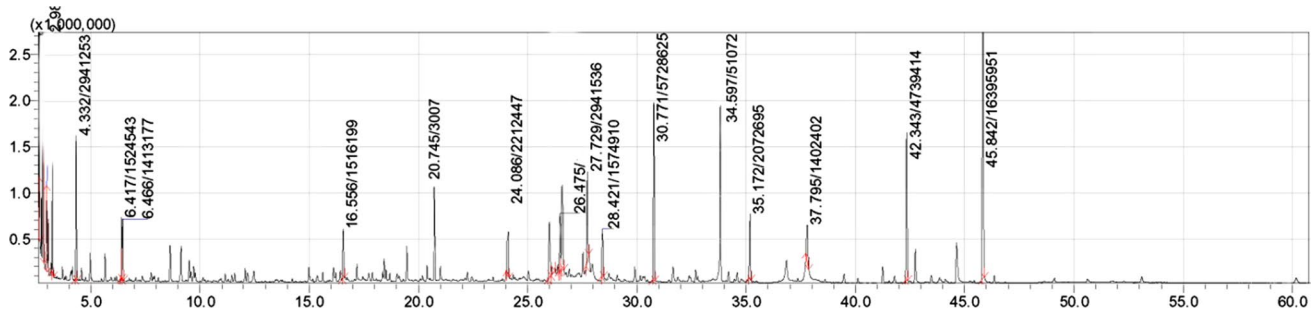

Fig. 4 Gas chromatography mass spectroscopical analysis of *Streptomyces* S9 isolate metabolites extracted by ethylacetate

Table 1 List of compounds present in ethylacetate extract detected by GC–MS analysis

S. no.	Name of compound	*m/z*	RT
1	Cyclohexanecarboxylic acid	73	6.418
2	2-Ethyl-1-imethyl(isopropyl)silyloxyhexane	75	6.467
3	Uracil	73	17.184
4	Azelaic acid	73	20.735
5	1-Dodecanamine, *N*-methyl-*N*-nitroso	73	24.092
6	Hexadecanoic acid	256	26.189
7	Hydrocinnamic acid	192	28.421
8	3,3′-Thiobispropanoic acid	117	30.772
9	Linolenic acid	73	34.591
10	Octadecane-12-on-1-ol	117	35.173
11	Phenethylamine, 3-benzyloxy-4-fluoro-*N*-formyl-beta-hydroxy-	125	37.792
12	Octadecanoic acid ethyl ester	371	42.342
13	Octadec-9 enoic acid, propyl ester	399	45.84

that a major portion of applied metabolites composed of azelaic acid, hexadecanoic acid, linolenic acid and propyl ester of Octadec-9-enoic acid (Table 1).

Literature review evidences the role of these compounds in various defense mechanisms in plants (De Bigault et al. 2016). Azelaic acid is a C9 dicarboxylic acid. It is a mobile signal. In plants, free radicals are known to oxidize free fatty acids (C 18) that contained a double bond at C 9 position to yield 9-0x0 nonanoic acid, which is converted to a dicarboxylic acid, azelaic acid upon addition of carboxylic group. Azelaic acid is known to act as a long-distance resistance-priming signal and plays a role in triggering the accumulation of SA, a well known defense signal (Gao et al. 2014). It also induces AZI1 gene, whose product AZI1 protein is responsible for conferring disease resistance by generating vascular sap. Jung et al. (2009) have reported specific loss of immunity in *Arabidopsis* plants in which AZI1 gene was mutated. They have also suggested that azelaic exhibits poor antibacterial activity and no antifungal action in this case. Yu et al. (2013) confirmed that C 18 fatty acids (FAs), such as oleic acid (18:1), its desaturated derivatives, linolenic acid (18:2) and linolenic acid (18:3) serve as precursors of azelaic acid. Scalschi et al. (2014) employed hexanoic acid to induce resistance in tomato plants against *P. syringae*.

Jasmonates are identified as a class of defense signaling hormones which includes JA. Biosynthesis of JA occurs either by octadecanoid pathway or hexadecanoid pathway. These pathways are initiated by lipoxygenases which catalyze oxygenation of unsaturated C 18 or C 16 FAs within the plastids of plant cells. Subsequent steps are catalyzed by allene oxide synthase and allene oxide cyclase leading to production 12-oxophytodienoic acid, which moves into peroxisomes. Further biochemical reactions (reduction and β-oxidation) which occur in peroxisomes results in the synthesis of JA (Schaller and Stintzi 2008). We assume that desaturases of plant cell could have involved in the introduction of double and triple bonds in the structure of C 18 or C16 saturated FAs present in applied metabolites. It is hypothesized that although enzymes of the octadecanoid pathway are abundant in an unstressed tomato plant, production of jasmonates was limited/regulated by substrate availability (Stenzel et al. 2003). Application of substrates (C 18 or C16 FAs) might have geared the jasmonate pathway in the present experiment that conferred resistance capacity to metabolite treated plants. Cohen et al. (1991) observed the role of C18 FAs viz, linoleic acid and oleic acid in inducing systemic resistance to potato plants against *Phytophthora infestans*. Oxygenated C18 FAs were known to protect rice plants from rice blast disease by resistance induction and inhibition of blast fungus spore germination (Namai et al. 1993).

The results of the present study suggest the possible role of azelaic acid and FAs in triggering SA and JA synthesis thereby disease resistance (Fig. 5). Simultaneous induction of these defense chemicals might be the possible reason for systemic resistance in tomato plants challenged by *P. syringae* and *C. cassiicola*.

A recent study conducted by Fatima and Anjum (2017) reported the disease resistance inducing ability of 3-hydroxy-5-methoxy benzene extracted from *P. aeruginosa* against Fusarium wilt in tomato. Balmer et al. (2018) highlighted the role of tricarboxylic acids in defense priming against *P. syringae* in *Arabidopsis*.

Conclusion

Most methods of plant disease management include curative measures. Prevention is better than cure, hence prevention of plant diseases could reduce the crop losses. Emerging research on plant resistance induction paves path for sustainable and environment friendly agricultural practices.

The present study presents an eco-friendly method of plant protection. Utilization of microbial metabolites for prevention of plant diseases demonstrated in this study minimizes the use of hazardous agrochemicals thereby reducing their ill effects on the environment and thus confers environmental sustainability. Since the metabolites reported in this

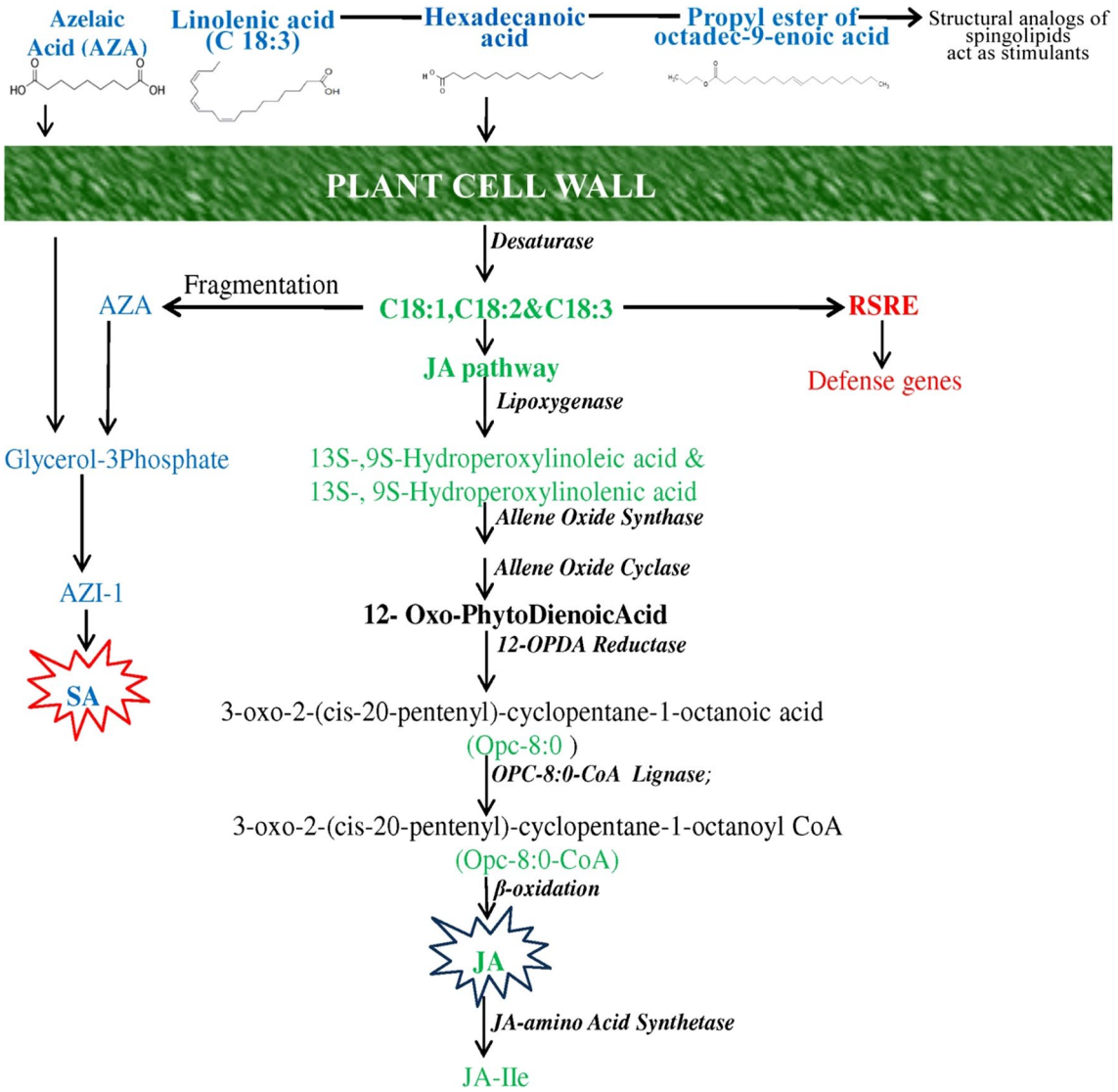

Fig. 5 Upon external application of *Streptomyces* S9 isolate metabolites, azelaic acid, octadecanoic acid, linolenic acid are expected to enter the plant cell. Aforementioned reports evidences the infiltration of fatty acids into plant cell as identified by deuterium labeling (Jung et al. 2009).These metabolites can participate in following metabolic pathways conferring fitness benefit to plant. (1) Azelaic acid is a component of applied metabolites and also produced by fragmentation of other fatty acids. This compound is known to signal Salicylic acid mediated responses (Walley et al. 2013). (2) Octadecanoic acid, linolenic acid are acted upon by denaturizes (present in plant cell) that introduces double and triple bonds. 18:1, 18:2, 18:3 can be utilized as precursors of jasmonate synthesis (Upchurch 2004) or (3) can activate rapid stress responsive element (RSRE) which regulates defense gene expressions (Walley et al. 2013). (4) Fatty acids can mimic the spingolipids and stimulate lipase (octadecanoic acid and Linolenic acid are the structural analogs of spingolipids). Spingolipids are naturally produced by fungal plant pathogens which are known to elicit defense responses (Umemura et al. 2004)

study showed prevention of pathogen infection and pathogen spread, agricultural losses can also be minimized which can lead to agricultural sustainability.

The present research work demonstrates the capability of *S. albolongus* metabolites in activating tomato plant defense system. However, biosafety of these metabolites, least effective dose, effect of individual compounds present in the culture filtrate, the effect of metabolites application on yield of the plant, responses of induced plants to other abiotic stress factors and studies on transgenerational resistance must be carried out to extend the scope of these metabolites for commercial usage.

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