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Post-harvest Management of *Alternaria* Induced Rot in Tomato Fruits With Essential Oil of *Zanthoxylum armatum* DC

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Alternaria fruit rot is a major disease caused by *Alternaria alternata* (Fr.) Keissl., a prolific fungal pathogen. Among post-harvest diseases of tomato, fruit rot induced by *A. alternata* is the most damaging. Antifungal agents are widely used to control post-harvest management of tomato fruits. However, negative impacts of fungicidal residues in edible fruits and vegetables on human health cannot be over ruled. Eco-friendly ways of controlling Alternaria rot in tomato fruits offer a novel way of tomato rot management. The current study proposes an alternate method in controlling tomato fruit rots through *Zanthoxylum armatum* DC essential oil (EO) application. Gas chromatography-mass spectrometry profiling showed eucalyptol and sabinene as major components of *Z. armatum* EO. Furthermore, EO applied (0.5–4.5 μl/ml) showed significant inhibition of *A. alternata* growth ($p > 0.05$) at 4.5 μl concentration tested. Lipid peroxidation assays revealed significant reduction in membrane damage in tomato fruits treated by EO compared to alone inoculated fruits with *A. alternata.* Elevated activities of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase coupled with enhanced antioxidants such as ascorbic acid, glutathione, proline, and total phenols in EO-treated fruits may be linked with better fruit rot management than control fruits inoculated with *A. alternata*-induced rot alone. Mycelia and spore production was dramatically reduced in EO applied tomato fruits over *A. alternata* alone in tomato fruits ($p > 0.05$). Interestingly, free radical scavenging activities of EO applied tomato fruits showed significant improvement compared to only pathogen-inoculated tomato fruits. Findings propose practical utility of *Z. armatum* EO as a plant-based antifungal for post-harvest management of Alternaria rot in tomato fruits.

Keywords: tomato, fungal pathogens, monetary loss, post-harvest, health issues, fruit rot

INTRODUCTION

Fruits and vegetables play an important role in human nutrition and health, serving as sources of vitamins, fats, minerals, oils, and dietary fibers. The shelf-life of fruits and vegetables is a serious concern of economic importance to horticulturists (Islam et al., 2017; Etana et al., 2019). During storage and transportation, fruits and vegetables act as a suitable substrate for fungal pathogens,

which cause rot, making fruits unfit for marketing use, thereby leading to huge post-harvest monetary losses (de Jesús Salas-Méndez et al., 2019; Ahmad et al., 2020). More than 20–25% of the harvested fruits and vegetables face decay by pathogens during post-harvest handling in the industrialized countries of the world (Kitinoja et al., 2019). This untoward situation is more severe in the developing countries, where post-harvest losses are estimated to be over 35% because of absence of proper storage facilities and transportation (Abano and Sam-Amoah, 2011; Kitinoja et al., 2019).

Attack of fungal pathogens elicits a broad spectrum of innate immunity in plants, which attempt to restrict or prevent pathogen invasion. Upon sensing the invading pathogen, plants mount a set of general defense reactions, such as cell wall reinforcement, accumulation of antimicrobial proteins (pathogenesis-related proteins), and production of phytoalexins (Dangl and Jones, 2001; Ho and White, 2005; de Freitas et al., 2012; Pandey et al., 2016; Jain and Khurana, 2018; Kumar et al., 2018). Plants also produce secondary metabolites including phenolics for curbing the microbial pathogens (Konuk and Ergüden, 2020). Generation of reactive oxygen species (ROS) is an intrinsic trait of any living cell, such that in normal conditions, ROS appear as inevitable by-products, formed as a result of reduction in molecular oxygen in chloroplasts and mitochondria. Moreover, the crucial role of ROS in the plant fungal interactions has been widely demonstrated (Segal and Wilson, 2018; Wang et al., 2019). Improved activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) enzymes upon treatment with *Bacillus subtilis* JK-14 strain proved to play a key role in the control of post-harvest peach diseases caused by *Alternaria tenuis* and *Botrytis cinerea* (Zhang et al., 2019).

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop with high nutritional value worldwide (Ghadage et al., 2019; Salehi et al., 2019; Liang et al., 2021). High moisture content and water-soluble nutrients in tomato fruits make them perishable and susceptible to a number of fungal pathogens causing post-harvest decays (Singh et al., 2017). Rots of tomato fruits are incited by fungal pathogens, such as *Alternaria alternata*, *Phoma* spp., *Didymella lycopersici*, *Geotrichum candidum*, *Botrytis cinerea, Fusarium acuminatum*, and *Rhizopus stolonifer* (Petrasch et al., 2019; Chudinova et al., 2020). Among filamentous fungi implicated in tomato fruit rots, *A. alternata* (Fr.) Keissler is a major storage decay biotic factor (Rizwana et al., 2021; Ventura-Aguilar et al., 2021). Furthermore, *A. alternata* becomes aggressive when the fruit develops injury or becomes debilitated during prolonged storage. Environmental factors have been shown to play crucial role in the development of fungal pathogens in fruits (Davari et al., 2020; Safari et al., 2021). Among these fungal pathogens, tomato fruit rot induced by *Alternaria* spp. appears as dark brown to black, smooth, slightly sunken lesions that are of firm texture and can become several centimeters in diameter (Yang et al., 2020).

Abbreviations: EO, essential oil; ROS, reactive oxygen species; CYA, Czapek yeast extract agar; TCA, trichloroacetic acetic acid; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid); GSH, glutathione; TPC, total phenol content; ASA, ascorbic acid.

To manage and minimize the post-harvest losses caused by various fungal pathogens in tomatoes for long-term storage, application of fungicides is a common preventive strategy. However, fungicidal applications to tomato crop have raised environmental issues and concerns for human safety, development of races resistant to pests, and residual toxicities (Tongnuanchan and Benjakul, 2014; Dagli et al., 2015; Devi et al., 2021). Application of biocontrol agents such as *Trichoderma harzianum* and *B. subtilis* JK-14 strain has been used to control fungal pathogens such as *A. tenuis* and *Alternaria* spp. (Zhang et al., 2019; El-Katatny and Emam, 2021). Leaf and fruit extracts of several medicinal plants have shown promising results in the effective management of tomato fruit rots. For instance, *Annona muricata* fruit extracts could control *Alternaria* spots on tomato fruit during post-harvest management (Rizwana et al., 2021). Plant extracts of *Aloe barbadensis*, *Vitex trifolia*, *Allium sativum*, *Azadirachta indica*, *Acorus calamus*, and *Lantana camara* have been effectively used to control the growth and sporulation of *Alternaria solani* (Devi et al., 2017). Similarly, leaf extracts of *Moringa oleifera* application showed significant reduction in *A. solani*-caused rot in tomato fruits (Mvumi et al., 2018). Plant-derived essential oils (EOs) also offer an effective management control of fungal pathogens in horticultural crops (Brochot et al., 2017; Wan et al., 2019; Kumar et al., 2020; Zhang et al., 2021). Most EOs are conferred with "generally recognized as safe" (GRAS) status by the Food and Drug Administration, USA (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.20; Tongnuanchan and Benjakul, 2014; Dagli et al., 2015; Devi et al., 2021). Antimicrobial activities of *Zanthoxylum armatum* DC (Dhami et al., 2018; Li et al., 2021; Verma et al., 2021) and *Z. alatum* (Guleria et al., 2013) EOs have been widely documented. For instance, antifungal activity of *Z. armatum* EO as an antifungal agent against *Aspergillus flavus* has been demonstrated (Li et al., 2021). However, application of *Z. armatum* EO in the management of *A. alternata*-induced tomato fruit rot is least explored. Green consumerism awareness and desire among common people having fewer synthetic food additives and products and minimum impact on the environment further advocate the use of EOs for the management of fungal pathogens in edible crops (Dangl and Jones, 2001; Anupama et al., 2019; Singh et al., 2021; Tao et al., 2021). Objectives of the present investigation were set forth to evaluate the efficacy of *Z. armatum* DC fruit EO in controlling the *A. alternata*-mediated rot in tomato fruits, *vis-a-vis* its role in modulating antioxidant potential of tomato fruits.

MATERIALS AND METHODS

Survey of Vegetable Markets and Isolation of *A. alternata* From Diseased Tomato Fruits

Various vegetable markets from Jammu city (32.7266° N, 74.8570° E) were surveyed for the collection of visibly cracked and bruised tomato fruits in presterilized polythene bags. *A. alternata* was isolated from visibly infected fruits after incubating them at 28 ± 2°C for 3 days. *A. alternata* was identified

morphologically on the basis of colony characteristics on Czapek yeast extract agar (CYA) medium, conidia in chains, and conidial morphology (shape, size, number of transverse, and longitudinal septa, etc.) as described in Ellis (1971) and Simmons (2007). Isolation and characterization of *A. alternata* were performed as provided in **Supplementary Data 1**. The purified cultures were maintained in duplicates on sterilized potato dextrose agar (PDA) slants. The Koch's postulates were performed for testing the pathogenicity of *A. alternata* (Tomkins and Trout, 1994).

Extraction of EO

The fruits of *Z. armatum* at maturation stage were collected seasonally during from the naturally growing trees at Pancheri region (33.0653° N, 75.1565° E) of Udhampur district of Jammu and Kashmir. Fruits were washed with sterilized distilled water and then dried under aseptic conditions. The dried fruits were then subjected to hydrodistillation in Clevenger's apparatus for EO extraction. The extract was stored in dark clean vial at 4–6°C until further analysis by gas chromatography (GC)-mass spectroscopy (MS).

GC-MS Characterization of *Z. armatum* EO

The EO of *Z. armatum* was analyzed through GC equipped with a flame ionization detector. The GC conditions were as follows: capillary column CP-Sil-8 (30 m × 0.32 mm × 0.25 μm thick film), helium was the carrier gas, injection temperature 280°C, split ratio 1:150, column oven temperature 50°C for 5 min, 250°C, hold for 7 min, total run time 50 min. The identification of various compounds was based on their retention times relative to those of authentic samples in the library and matching spectral peaks available. For the characterization of EO constituents, GC-MS library was used.

In vitro Antifungal Efficacy of EO of *Z. armatum*

The antifungal efficacy of *Z. armatum* EO was performed against *A. alternata* by the poisoned food technique (Perrucci et al., 1994). Requisite amounts of EO were added separately to plates containing 0.5 ml of 5% Tween 20 and 9.5 ml of molten PDA and then mixed to obtain the final concentrations of 0.9, 1.5, 2, 2.5, 3, 3.5, 4, and 4.5 μl/ml. A 5-mm disk from a 7-day-old colony of fungus was separately placed in the center of the plates. Likewise, control sets were prepared using equal amounts of distilled water replacing EO. The prepared plates of both treatment and control sets were incubated at 28 ± 2°C for 7 days. The percentage mycelial inhibition was calculated by using the formula:

$$\text{Percentage mycelial inhibition} = [(dc - dt)/100] \times 100$$

where dc is the mean mycelia growth diameter of colony sets, and dt is the mean mycelia growth diameter of colony of treatment sets.

Calculation of Percent Reduction in the Number of Spores

Reduction in the number of spores by the application of EO was done by counting the number of spores in a hemocytometer.

Preparation of Different Concentrations of EO

Different concentrations of EO were prepared by diluting the stock with 5% Tween 20 (25, 50, 75, and 100%).

Preparation of Spore Suspension of *A. alternata*

Five-day-old cultures of *A. alternata* were flooded with 10 ml of sterilized distilled water. The spores were rubbed from the surface of a Petri dish, and spore density was calculated by using a hemocytometer to obtain a uniform suspension of 1×10^5 spores ml^{-1}.

Treatment

Healthy tomato fruits were weighed and surface sterilized with 1% sodium hypochlorite solution, air dried under aseptic conditions, and rinsed with 70% alcohol. One set of tomato fruits was treated with distilled water only served as control (CN). The second set of tomato fruits was inoculated with tested pathogen without EO (IN). The third set of fruits was dipped in the respective solutions of various concentrations of EO for 30 min. Treated fruits were then incubated at 28 ± 2°C for 12 h and subsequently inoculated with 5 μl of spore suspension of *A. alternata* according to Samyal and Sumbali (2011). The control, inoculated, and treated fruits were again incubated at 28 ± 2°C in sterile polythene bags. After 3 days of incubation, fruits were analyzed for percent rot development, percent rot control, and biochemical analysis.

Calculation of Percent Rot Development and Percent Rot Control

After 3 days of incubation, percentage rot was calculated by using the formula:

$$\text{Percentage rot} = [(W - w)/W] \times 100$$

where W = weight of the fruit before inoculation, and w = weight of the fruit after removal of rotten tissue.

Similarly, percentage rot control was evaluated by using the formula:

$$\text{Percentage control} = \frac{[(\%\text{decay in untreated fruit} - \%\text{ decay in treated fruit}) \times 100}{\%\text{ decay in untreated fruit}]}$$

Biochemical Analyses

Stress Indices

Lipid Peroxidation

The peroxidation of lipids was estimated according to the method of Heath and Packer (1968). Briefly, tomato fruits [0.5 g fresh weight (FW)] were homogenized in 3 ml of 0.1% tricholoroacetic acid (TCA) as previously described in Choudhary et al. (2011).

Proline

Proline content was estimated by following the methods of (Bates et al., 1973) and Choudhary et al. (2011).

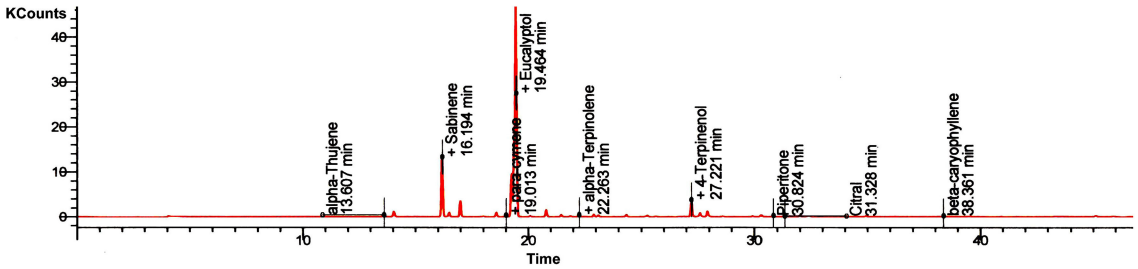

FIGURE 1 | Gas chromatography-Mass Spectrometery chromatogram of the essential oil of *Z. armatum* DC. The y-axis shows peak counts in thousand (K counts), while the x-axis shows retention time in minutes. Standard chromatogram showed presence of important EO constituents' alpha-thujene (13.607 min), sabinene (16.194 min), eucalyptol (19.464 min), alpha-terpinolene (22.263 min), 4-terpinenol (27.221 min), piperitone (30.824 min), citral (31.328 min), and beta-caryophyllene (38.361 min). Besides, these significant peaks, small peaks were also recorded but not shown in the chromatogram.

TABLE 1 | Chemical characterization of *Zanthoxylum armatum* DC essential oil by GC-MS analyses.

S. No	Name of the compound	Area	Retention time	Percentage (%)
1	Eucalyptol	24,045	19.46	39.763
2	Sabinene	14,014	16.19	23.175
3	Beta-pinene	3,407	16.30	5.635
4	4-terpineol	2,426	27.22	4.012
5	Alpha-pinene	1,329	14.01	2.918
6	Piperitone	900	30.82	1.489
7	Alpha-terpenine	844	17.01	1.396
8	Para-cymene	677	19.01	1.119
9	Alpha-thujene	562	13.60	0.929
10	Alpha-terpinolene	399	22.26	0.659
11	Ocimene	204	20.82	0.337
12	Citral	156	31.32	0.258
13	Beta-caryophyllene	113	38.36	0.186

Peak area and retention time of the compound were used to identify constituents of the essential oil using reference library.

TABLE 2 | Efficacy of EO of *Zanthoxylum armatum* DC on %age mycelial inhibition and %age reduction in number of spores of *Alternaria alternata* (Fr.) Keissl.

S. No	Concentration of EO of *Z. armatum* (μl/ml)	%age mycelial inhibition
1	0	0
2	0.5	50.23 ± 1.23
3	0.9	59.15 ± 1.45
4	1.5	52.19 ± 2.10
5	2.0	65.23 ± 1.09
6	2.5	69.43 ± 3.21
7	3.0	76.92 ± 4.21
8	3.5	80.76 ± 9.87
9	4.0	83.45 ± 8.65
10	4.5	100 ± 10.98

Protein Content (PR)

The PR was estimated according to method of Choudhary et al. (2011).

Estimation of Enzymatic Activities

The activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), guaiacol peroxidase (GPOX; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), and glutathione reductase (GR; EC 1.8.1.7) were estimated by following the methods described in Choudhary et al. (2011, 2012).

Non-enzymatic Antioxidants

The estimation of glutathione (GSH), total phenol content (TPC), and ascorbic acid (ASA) contents was done by following the methods as described in Choudhary et al. (2011, 2012).

Free Radical Scavenging Activity of Z. armatum

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Activity. The method of Kwon et al. (2006) was used for calculating DPPH activity. About 300 μl of sample was taken and mixed with DPPH solution (2 ml). The reaction mixture was incubated at 37°C for 30 min. After incubation, the readings were taken spectrophotometrically at 517 nm.

$$\% \text{ inhibition} = \frac{\text{Abscontrol} - \text{Absextract}}{\text{Abscontrol}} \times 100$$

where Abs*control* = absorbance of control, and Abs*extract* = absorbance of extract.

2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-sulphonic acid) (ABTS) Radical Scavenging Assay. ABTS radical scavenging activity was calculated by the method of Wang et al. (2015). About 300 μl of sample was taken and mixed with reagent C [ABTS + potassium persulfate (1:5)] (2 ml). The reaction mixture was incubated at 37°C for 30 min. After incubation, the readings were taken spectrophotometrically at 734 nm.

The percent inhibition was calculated by using the formula:

$$\% \text{ inhibition} = \frac{\text{Abscontrol} - \text{Absextract}}{\text{Abscontrol}} \times 100$$

where Abs*control* = absorbance of control, and Abs*extract* = absorbance of extract.

Statistical Analysis

All the experiments were performed in triplicate. Data shown are the means of three replicates along with standard error ($n = 3$). Student's t-test was carried out, and data were presented at $p \leq 0.05$. All the statistical calculations were performed using IBM SPSS 20.0 software.

RESULTS

Z. armatum EO Characterization

EO of *Z. armatum* was light yellow in color. GC-MS profile showed the presence of 20 different compounds. Retention time and retention percentage of identified compounds are summarized in **Figure 1** and **Table 1**. Eucalyptol (39.763%) and sabinene (23.175%) were the major components of EO, accounting for 62.93% of the total oil composition. Beta-pinene accounted 5.6% of total oil composition. Percentage of each of the remaining constituents of EO was <5%.

Efficacy of EO Against *A. alternata* (*In vitro*)

The EO of *Z. armatum* was tested against *A. alternata* from a concentration of 0.5–4.5 μl ml^{-1}. Mycelial inhibition by 50% was observed at 0.5 μl ml^{-1} (**Table 2**). Other applied concentrations of EO were also effective against *A. alternata*

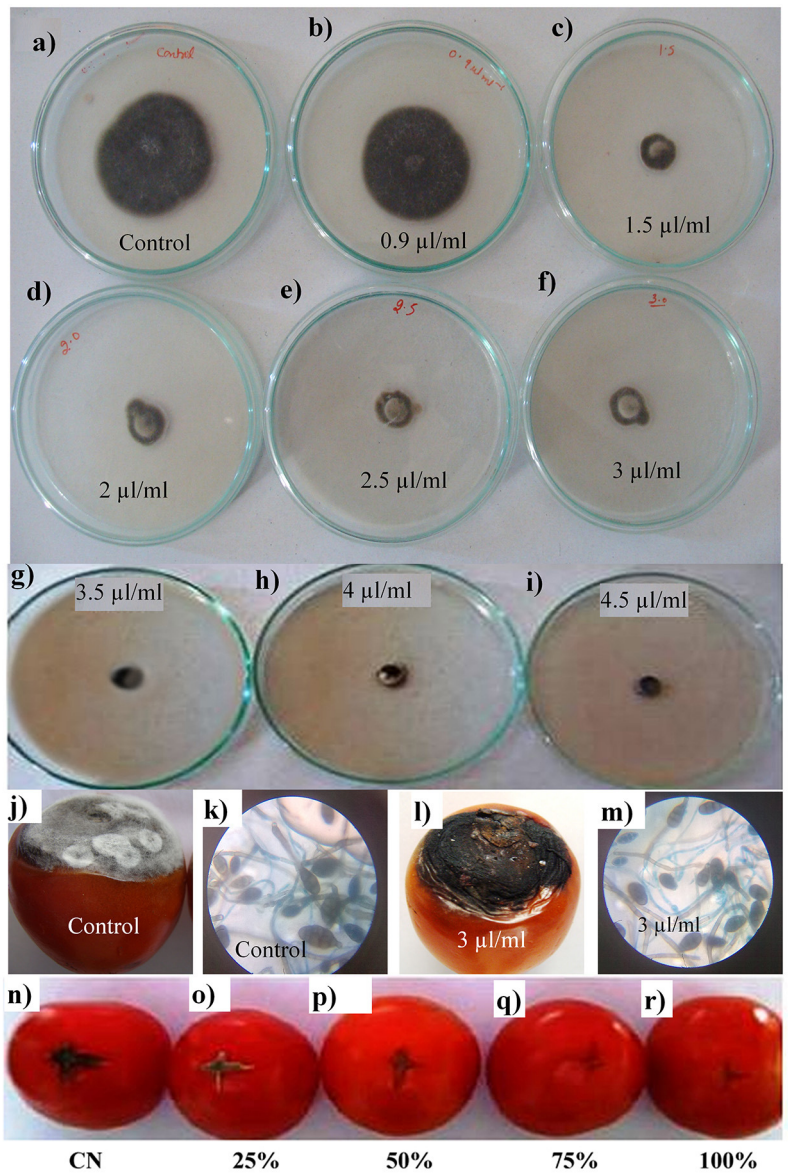

FIGURE 2 | Effects of *Z. armatum* DC. EO applied at 0.9, 1.5, 2, 2.5, 3, 3.5, 4, and 4.5 μl/ml concentrations on percent mycelial inhibition of *A. alternata* isolated from infected tomato fruits **(a–i)**. Effects of *Z. armatum* EO applied at 3 μl/ml concentration show significant inhibition of *A. alternata* growth compared to control fruits (only inoculated with *A. alternata*) **(j,l)** and growth of *A. alternata* fungal mycelia and conidia **(k,m)**. Effects of *Z. armatum* EO applied at 25, 50, 75, and 100% concentration on post-harvest management of *A. alternata*-induced tomato fruit rot growth compared to control fruits **(n–r)**.

TABLE 3 | Effect of EO of *Zanthoxylum armatum* DC applied at different concentrations (25, 50, 75, and 100%) on the percent rot development and percent rot control in tomato fruits inoculated with spores of *Alternaria alternata* (Fr.) Keissl.

S. No	Concentration of EO	Percent rot development	Percent rot control
1	CN	42.31 ± 4.78	
2	25%	15.23 ± 0.98	64.2 ± 2.34
3	50%	14.56 ± 0.67	65.5 ± 1.23
4	75%	6.53 ± 0.23	84.5 ± 454
5	100%	5.23 ± 0.13	87.6 ± 3.23

with maximum percent mycelial inhibition (100%) observed at 4.5 μl ml^{-1} (**Figures 2a–i** and **Table 2**). Moreover, with an increase in the concentration of EO, there was a reduction in the number of spores (**Table 2**). Compared to control, middle range 3.5 μl ml^{-1} showed significant reduction in inhibition of spread of *A. alternata* infection and fungal mycelia and conidia development (**Figures 2j–m**).

Effect of EO on Percent Rot Development and Percent Rot Control

Percent Rot Development

There was about 40% loss in FW of tomato fruit after inoculation with *A. alternata*. EO application significantly reduced rot development (6.4- to 8-fold), with a maximum reduction by 8-fold at 100% EO, followed by 6.47-fold decrease at 75% concentration of EO (**Figures 2n–r** and **Table 3**).

Percent Rot Control

Application of EO significantly reduced rot development, thus increasing rot control such that maximum rot control (87.6%) was recorded at 100% concentration of EO followed by 75% (84.5%) and 50% (65.5%) (**Table 3**).

Effect of EO of *Z. armatum* on PR

A significant decrease in PR was recorded for tomato fruits inoculated with *A. alternata* as compared with control (CN). On the other hand, with the application of EO, a significant increase in PR content was observed at all the applied EO concentrations when compared with tomato fruits inoculated (IN) with *A. alternata* alone (**Figure 3**). Specifically, increase in PR content was recorded at 100% of EO used (2.5-fold), followed by 75% EO (2.7-fold) and 50% EO (2.2-fold) over the IN fruits (**Figure 3**).

Effect of EO of *Z. armatum* on Activity of Antioxidant Enzymes

Superoxide Dismutase (EC 1.15.1.1)

A significant increase in SOD activity was observed in tomato fruits treated with *A. alternata* (IN) in comparison with those (CN) water treated (**Figure 3**). EO supplemented to pathogen-inoculated tomato fruits further increased SOD activity, with maximum increase noted for 100% concentration of EO (1.7-fold), followed by 75% (1.6-fold) and 50% (1.15-fold) EO concentration as compared with pathogen-inoculated (5 μl) tomato fruits without EO application. A small and insignificant change in SOD activity was noticed in tomato fruits treated with both 25% EO and *A. alternata* over IN fruits treated with *A. alternata* alone (**Figure 3**).

Catalase (EC 1.11.1.6)

Specific activity of CAT recorded for tomato fruits treated with *A. alternata* was significantly higher than CN values. EO combined with *A. alternata* was able to strongly evoke CAT activity (from 2.2- to 2.5-fold), with maximum enhancement at 75% concentration of supplemented EO compared with tomato fruits only inoculated with *A. alternata*. No significant change was observed for 25% concentration of EO plus *A. alternata* over IN tomato fruits (**Figure 3**).

Guaiacol Peroxidase (EC 1.11.1.7)

A significant decrease in GPOX (2.3-fold) activity was recorded in tomato fruits inoculated with *A. alternata* (**Figure 3**) as compared with CN tomato fruits. However, a remarkable increase in GPOX activity was recorded in *A. alternata*-inoculated tomato fruits supplemented with EO. Specifically, a maximum increase of 8.29-fold was recorded at 100% EO, followed by 7.37-fold at 75%, 7.25-fold at 50%, and 5.04-fold for 25% EO, when compared with IN tomato fruits (**Figure 3**).

Ascorbate Peroxidase (EC 1.11.1.11)

Enhanced APX activity was noted after inoculation of tomato fruits with *A. alternata* (**Figure 3**). No significant change was noticed in APX activity treated with 25% concentration of EO. On the contrary, a significant increase in APX activity was recorded for tomato fruits supplemented with higher fractions of EO (75% and 100%), with the highest increase in APX activity (2.36-fold) noticed for tomato fruits treated with 100% of EO (**Figure 3**).

Glutathione Reductase (EC 1.8.1.7)

In tomato fruits, inoculation of tomato fruits with *A. alternata* resulted in an increase in GR activity over CN (**Figure 3**). A significant induction in GR activity was noticed after application of EO (75 and 100%) with the maximum induction recorded for 100% EO. No significant changes were observed in tomato fruits treated with EO at 25 and 50% (**Figure 3**).

Effects of EO of *Z. armatum* on Non-enzymatic Antioxidants

A significant increase in ASA was recorded in *A. alternata*-inoculated tomato fruits when compared with CN. An elevation in ASA level was noted after an EO treatment. Specifically, treatment of *A. alternata*-inoculated tomato fruits with 75 and 100% EO increased ASA level by 1.84- and 2.2-fold, respectively. An insignificant increase in ASA content was observed at either 50 or 25% EO over IN tomato fruits (**Figure 4**).

For GSH, a significant decrease in GSH (1.6-fold) content was recorded for tomato fruits infected with the pathogen when compared with CN tomato fruits (**Figure 4**). Application of EO significantly enhanced GSH content in infected tomato fruits at 100, 75, and 50% EO, respectively. A small increase

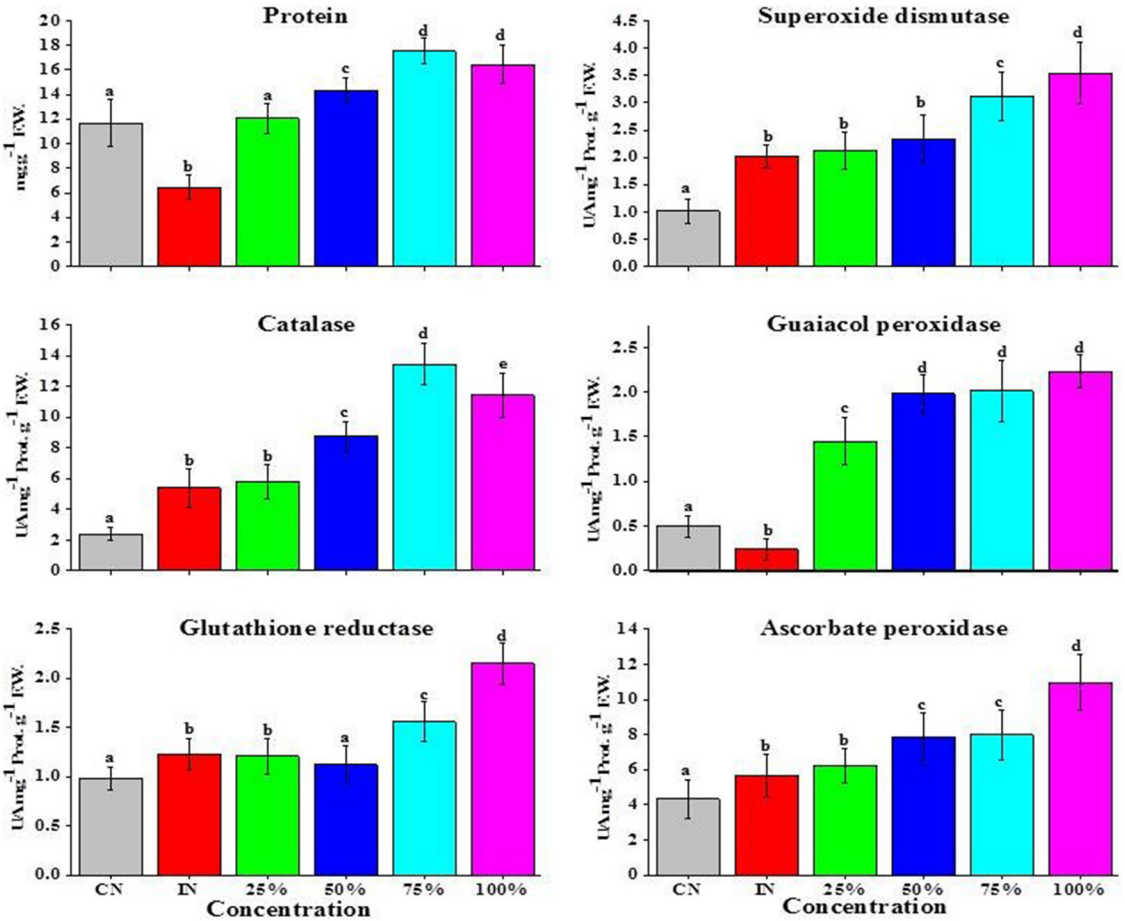

FIGURE 3 | Effects of essential (EO) of *Z. armatum* DC on PR (mg g^{-1} FW) and specific activities (UA mg^{-1} protein g^{-1} FW) of SOD, CAT, GPOX, GR, and APX of tomato fruits inoculated with *A. alternata*. CN, control; IN, inoculated; 25, 50, 75, and 100% indicate concentrations of EO used. Values represent mean ± standard error, where number of biological repeats is five ($n = 5$). Different letters (a–d) indicate significant differences from each other in all combinations at $p < 0.05$.

in GSH content was also observed at 25% (**Figure 4**). Total phenols (TPC) found in *A. alternata*-inoculated tomato fruits were significantly higher than that (1.95-fold) in CN fruits. Tomato fruits treated with both EO and the pathogen showed enhanced TPC content than pathogen-treated tomato fruits (IN) alone (**Figure 4**), with the highest increase observed with 100% EO. A slight increase in TPC content was also observed at either 75 or 50% concentration of EO, while no significant change was noticed in tomato fruits inoculated with *A. alternata* and treated with 25% EO over the control treated with the pathogen alone (IN) (**Figure 4**).

Effect of EO of *Z. armatum* on Stress Indices

Lipid Peroxidation

Membrane damage was calculated by estimating the amount of malondialdehyde (MDA), a product of lipid peroxidation that occurs during stressed conditions. The highest amount of MDA (2.6-fold) was observed in inoculated fruits, while the application of *Z. armatum* EO at any examined concentration could significantly reduce the amount of lipid peroxidation as indicated by the low amount of MDA. Specifically, a gradual reduction in MDA content was observed in *A. alternata*-inoculated tomato fruits treated with EO, with 1.7, 4, 8, and 9.23-fold reduction for 25, 50, 75, and 100% EO, respectively, when compared with IN fruits alone (**Figure 5**).

Proline

A considerable increase in proline (PL) level (1.6-fold) was observed in *Alternaria*-inoculated tomato fruits as compared with CN. The application of EO to *A. alternata*-inoculated tomato fruits further enhanced the PL content such that maximum increase was observed for 100% EO (2.8-fold) when compared with inoculated fruits. Other concentrations of EO also enhance PL content as compared with IN tomato fruits (**Figure 5**).

Effects of EO of *Z. armatum* on Free Radical Scavenging Activity

DPPH

A small reduction in the DPPH activity (0.22-fold) was observed in tomato fruits inoculated with *A. alternata* as compared with

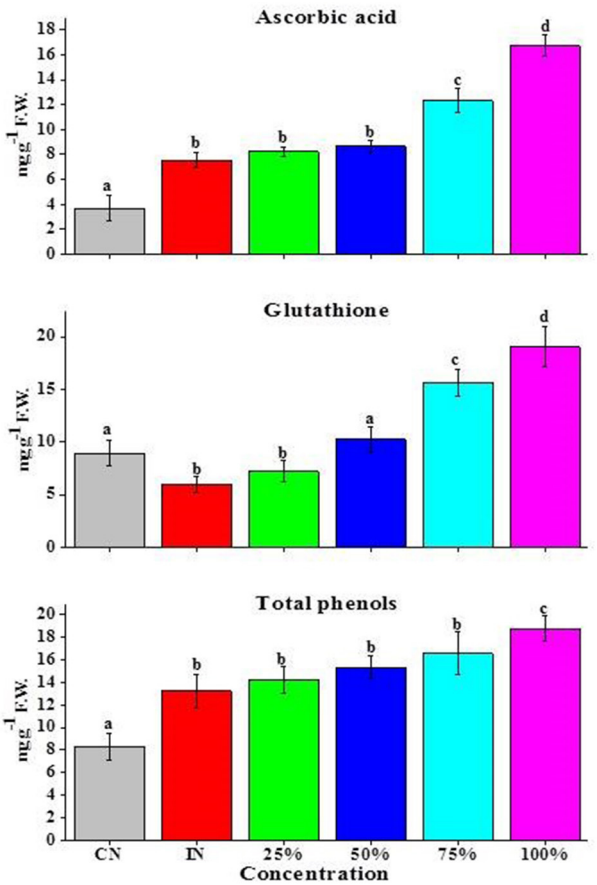

FIGURE 4 | Effects of *Z. armatum* DC EO on endogenous titers (mg g^{-1} FW) of antioxidants (ASA, GSH, and TPC) of tomato fruits inoculated with *A. alternata*. CN, control; IN, inoculated; 25, 50, 75, and 100% indicate concentrations of EO used. Values represent mean ± standard error, where number of biological repeats is five ($n = 5$). Different letters (a–d) indicate significant differences from each other in all combinations at $p < 0.05$.

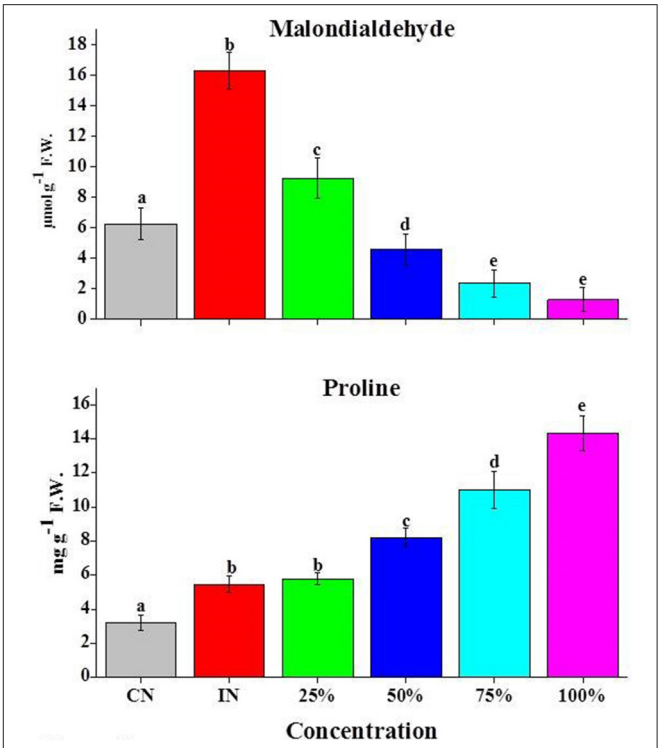

FIGURE 5 | Effects of *Z. armatum* DC EO on PL (mg g^{-1} FW) and MDA (μmol g^{-1} FW) contents of tomato fruits inoculated with *A. alternata*. CN, control; IN, inoculated; 25, 50, 75, and 100% indicate concentrations of EO used. Values represent mean ± standard error, where number of biological repeats is five ($n = 5$). Different letters (a–e) indicate significant differences from each other in all combinations at $p < 0.05$.

CN fruits. On the other hand, after application of EO, there was an increase in DPPH activity with maximum increase observed at 100% followed by 75% when compared with IN tomato fruits (**Figure 6**).

ABTS

There was a reduction in the ABTS activity (1.7-fold) of tomato fruits inoculated with *A. alternata* as compared to CN. Application of EO significantly enhanced the ABTS activity. Maximum enhancement was recorded for 100% EO (2.6-fold) followed by 75% and 50% when compared with IN tomato fruits (**Figure 6**).

DISCUSSION

The present investigation evaluated the efficacy of *Z. armatum* against *A. alternata*, the causal organism of black rot of tomato. Prior to evaluation of the antifungal activity, the EO of *Z. armatum* was characterized by GC-MS, revealing the presence of different compounds, with eucalyptol and sabinene being the major components (**Figure 1** and **Table 1**). Interestingly, the composition of *Zanthoxylum* oil reported in the present investigation varied from an earlier report by Prakash et al. (2012); their report showed 20 compounds with linalool and methyl cinnamate as key constituents of the EO of *Z. armatum* seeds procured from the herbal market of Varanasi, India. Significantly, such chemotypic variations considerably affect biological activity of the oil. While evaluating antimicrobial potential, *Zanthoxylum* EO completely checked the growth of *A. alternata* from a concentration of 0.5–4.5 μl ml^{-1}. Our findings are in concordance with the EO of *Z. armatum* used to inhibit mycelial growth of *Bipolaris sorokiniana* (Manandhar and Tiwari, 2005). From the present study, eucalyptol emerged as a major component of the EO of *Z. armatum*; it may have played role in retarding the growth of the pathogen, *A. alternata*-induced tomato fruit rot. Moreover, besides eucalyptol, sabinenes present in higher concentration in the EO of *Z. armatum* may have also contributed toward the management of *A. alternata*-induced tomato fruit. For instance, the EO of *Foeniculum vulgare* has demonstrated strong inhibition of *A. alternata* (Mahmoudi, 2017). Similarly, the EO of *Ocimum basilicum* showed effective management of *A. alternata*-induced rot in tomato fruits (Perveen et al., 2020). Their findings showed

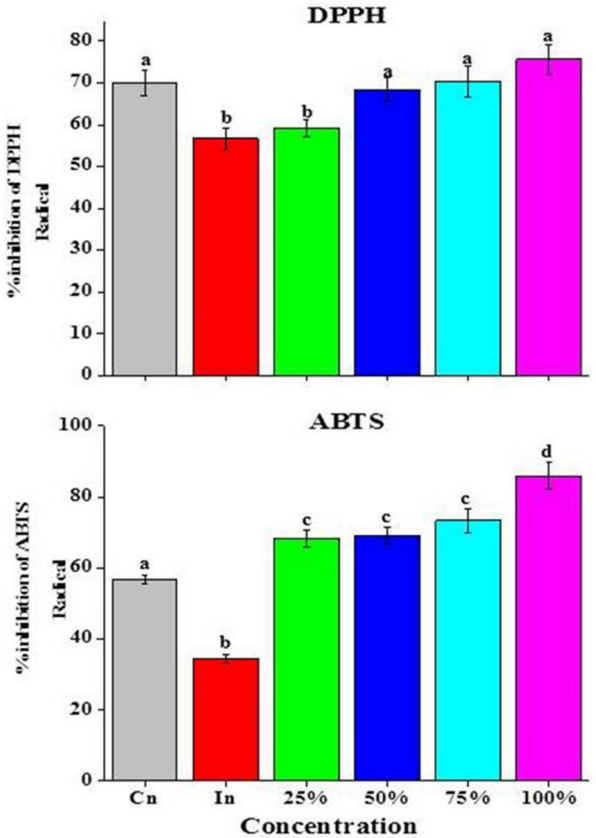

FIGURE 6 | Effects of *Z. armatum* DC EO on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS free radical scavenging activity assays of tomato fruits inoculated with *A. alternata*. CN, control; IN, inoculated; 25, 50, 75, and 100% indicate concentrations of EO used. Values represent mean ± standard error, where number of biological repeats is five ($n = 5$). Different letters (a–d) indicate significant differences from each other in all combinations at $p < 0.05$.

methyl chavicol and linalool as the primary constituents of the EO; moreover, *in vitro* experiments demonstrated 88% reduction in fungus growth at the highest concentration of EO applied (Perveen et al., 2020). These observations are also followed in the present study, where the EO of *Z. armatum* at 75% concentration was able to reduce percent rot development by 84% (**Figure 2**).

Besides EO, the antioxidant system of plant tissues confers resistance to biotic and abiotic stressors (Guleria et al., 2013; Dhami et al., 2018; Zhang et al., 2021). The present study revealed a significant increase in the activity of antioxidant enzymes (SOD, CAT, GPOX, APX, and GR) of tomato fruits inoculated with *A. alternata* and treated with *Z. armatum* EO (**Figure 3**). These antioxidant enzymes may have worked synergistically to promote scavenging of ROS produced during abiotic and/or biotic stress (Staerck et al., 2017; Zhang and Feng, 2018; Dvorak et al., 2020). Although a plethora of information is available on the use of plant extracts for fungal stress amelioration (Prakash et al., 2012; Zhang and Feng, 2018), there is a paucity of information about the effects of plant extracts on mitigation of fungal stress in plants via modulating an antioxidant (enzymatic) system. Likewise, various non-enzymatic antioxidants (ASA, GSH, and TPC) evaluated in tomato fruits inoculated with *A. alternata* and treatment with *Z. armatum* fruit extract also showed a rising trend (**Figure 4**). Presumably, their accumulation helped in decimating the negative effects of oxidative stress. Among non-enzymatic antioxidants, rapid production of phenols at the infection site depicts initial activation of the defense mechanism in plants, thereby restricting or slowing down pathogen growth (Taheri and Kakooee, 2017). In addition, phenols also restrict the growth of invading pathogen by binding with hydrolytic enzymes released by fungal pathogens during cell division (Taheri and Kakooee, 2017; Zhang and Feng, 2018). Similarly, GSH is a rapidly accumulated antioxidant after fungal attack that may act as a systemic signal, carrying information concerning the attack to non-infected tissues (Staerck et al., 2017; Taheri and Kakooee, 2017; Zhang and Feng, 2018; Dvorak et al., 2020). Furthermore, GSH also functions as a reducing agent for other antioxidants such as ASA (Taheri and Kakooee, 2017). While a correlation between antioxidant capacities and abiotic stress has been demonstrated in several plant systems (Staerck et al., 2017; Taheri and Kakooee, 2017; Zhang and Feng, 2018; Dvorak et al., 2020), similar investigations on the correlation between antioxidant potential and biotic stress have been lacking. A high level of MDA was observed in tomato fruits inoculated with *A. alternata*. Further, MDA content was decreased by application of *Z. armatum* EO, suggesting its protective role on membrane damage by fungal infection (**Figure 5**). Similarly, an increase in PR was observed in EO-treated tomato fruits compared to control. This decrease in MDA upon EO application may be due to the free radical scavenging activity of *Z. armatum* that scavenge the ROS held responsible for lipid peroxidation (Guleria et al., 2013). An increase in PL content was observed during the present investigation (**Figure 5**). It might be due to its increased synthesis and reduced degradation of PL during stress. Moreover, it also acts as a molecular chaperon stabilizing the structure of proteins (Verbruggen and Hermans, 2008). Furthermore, there was a reduction in the free radical scavenging activity in the *A. alternata*-inoculated tomato fruits (**Figure 6**), which improved in EO-treated tomato fruits due to the improved antioxidant profile.

CONCLUSIONS

Application of *Z. armatum* fruit EO in the management of *A. alternata* rot at the physiological level showed that EO could decrease incidence of *Alternaria* rot of tomato considerably. The approach of using botanicals to manage various post-harvest losses without any adverse effects on the consumer's health can be extended to other post-harvest rots of fruits and vegetables.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YPS and SP conceived and designed the experiments. SS, HRH, and MU performed experimentation. YPS, SP, and NSY analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.679830/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.