Journal of
Current Opinion in Crop Science

Journal homepage: www.jcocs.com; ISSN(O):2583-0392

RESEARCH ARTICLE

Role of salicylic acid in disease resistance against *Alternaria* spp in tomatoes (*Solanum esculentum* L.)

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Article history:

Received: September 09, 2021
Accepted: December 12, 2021
Published: December 31, 2021

Citation:

Matengu, T., Makaza, W., Ngadze, E., Rugare, J. T., Upenyu, M., & Gasura, E. (2021). Role of salicylic acid in disease resistance against *Alternaria* spp in tomatoes (*Solanum esculentum* L.). *Journal of Current Opinion in Crop Science, 2*(4), 391-401.

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ABSTRACT

Early blight and Alternaria leaf spot are among the major diseases limiting productivity of tomatoes. Evolvement of pathogen into resistance form and detrimental effect of fungicides have spawned efforts to develop management strategies that are economic and environmentally friendly. Salicylic acid is a natural plant hormone known to induce plant defense against various biotic and abiotic stresses. Therefore, the main focus of this study was to evaluate the role of salicylic acid on tomato crop against *Alternaria* spp infection. A range of salicylic acid concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mM) and distilled water (control) were laid in a randomized complete block design. Peroxidase (POD), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) enzyme activities were recorded from one to four weeks after inoculation with virulent *Alternaria* spp spores. The results showed that plants treated with 2.5 mM responded rapidly and significantly showed higher induction of POD, PAL and PPO enzyme activities. Plants sprayed with salicylic acid at 3 mM showed phytotoxic symptoms and reduced enzyme activity was recorded. In addition, foliar application of salicylic acid at 2.5 mM significantly ($p<0.001$) reduced disease severity (25.5 %) compared to plants treated with distilled water (85.46 % disease severity). These results showed that 2.5 mM of salicylic acid is safe and can be used in inducing tomato plant disease resistance.

Keywords: *Alternaria* spp, plant defence mechanisms, salicylic acid, tomato

INTRODUCTION

Global tomato (*Solanum lycopersicum* L.) production is severely limited by abiotic and biotic stresses where significant reduction in economic yield can be attributed to microbial infections (Meitei et al., 2014). Early blight disease of tomatoes is one of the major challenges that affect tomato production. Early blight has been reported in many countries such as Australia, Brazil, Israel, India and United States of America where it significantly reduces yield by up to 78 % (Chourasiya et al., 2013; Adhikari et al., 2017; Kokaeva et al., 2018). Yield losses due to Alternaria early blight disease infection of up to 79% annually have been reported in tomato (Molina et al., 2014). In cases of severe infection where the disease is left uncontrolled, yield losses can be as high as 100 % (Stevenson, 1994; Fontem, 2003; Wharton and Kirk, 2007). Alternaria leaf spot is also of significant importance as about 20 % of yield reduction is accounted for by the disease but up to 80 % is responsible for economic fruit yield loss depending on prevailing environmental conditions (Kirk et al., 2013).

Early blight of tomato is a devastating foliar disease caused by *Alternaria solani*. It is prominent mostly under conditions of high relative humidity and in high rainfall areas, though it can occur in a wide range of climatic conditions (Wharton and Kirk, 2007; Kumar et al., 2017). Symptoms are generally expressed on older leaves as they start as small lesions (1 - 2 mm) which are brown to black in colour (Adhikari et al., 2017). The spots develop characteristic concentric rings which are surrounded by yellow halo as the disease progresses, whilst the entire leaves may become chlorotic and coalesce as the lesions increase (Meitei et al., 2014; Ramanujam et al., 2015). Later, a significant defoliation can be recorded which can have impact on high respiration rates of the plant which expose fruits to direct sun resulting in physiological disorders such as sunscald in fruits (Bandici, 2008; Bhonwang and Stout, 2009; Soleimani and Kirk, 2011; Bessadat et al., 2014; Adhikari et al., 2017). The pathogen invades the plant via the fruit's point of attachment with the stem and via cracks caused by either growth processes or pest attack (Misheck et al., 2007; Ramanujam *et al*., 2015). Its progression may affect susceptible tomato varieties such that it can decrease the distribution and partitioning of the photo-assimilates, concomitantly resulting in low yields (Misheck et al., 2007; Gondal et al., 2012; Bessadat et al., 2014; Chandiposha et al., 2014; Rodrigues, 2014).

Cultural practices such as crop rotation, destruction of infected plant residues and removing weeds which act as alternate hosts for the pathogen help to decrease the inoculum levels of the pathogen in subsequent plantings especially of tomato varieties which are more susceptible to the disease (Agrios, 2005; Chaerani and Voorrips, 2006; Constabel and Barbehenn, 2008). Others practice drip irrigation and avoid overhead irrigation in cool and cloudy periods to reduce the spread of the disease (Chandiposha et al., 2014; Adhikari et al., 2017). Furthermore, during harvesting, bruising of the crop and other mechanical damages should be minimized as this facilitates entry of the pathogens in the fruit or tuber (Bandici, 2008; Fernando et al., 2011). In addition, since the disease is prevalent when the crop is suffering from inadequate nitrogen, adequate soil fertility should be maintained to reduce its incidence *(*Vicente and Plasencia, 2011; Gondal et al., 2012; Gosh, 2015).

The use of synthetic chemicals in the management of *Alternaria solani* has been widely documented (Falcioni et al., 2014; Desta, 2015; Delaney et al., 2017). However, chemical control is considered as the last resort in integrated management of early blight (Chaerani and Voorrips, 2006; Bessadat et al., 2014). The efficacy of commonly used fungicides has been reduced due to injudicious use of chemicals to manage early blight *(*Ganie et al., 2013; Mamgain et al., 2013; Czajkowski et al., 2015). However, the development of chemical resistance by *Alternaria* spp due to continuous use of fungicides became the major central problem (Fontem, 2003; Espinardo, 2004; Kumar et al., 2017). Nonetheless, Hayat et al. (2008) and Javaheri *et al.* (2012), reported the impact of these chemicals as they contribute to climate change, whilst some have low degradability and are persistent in soils, causing decrease in the populations of beneficial microbes (Josh et al., 2010; Ngadze et al., 2012; Nayem et al., 2017).

Plant phyto-hormones such as salicylic acid, ethylene, jasmonic and abscisic acids play an important role in signaling various pathways (Wang et al., 2007; Nikoo et al., 2014). Various roles included are regulation of processes of germination, photosynthesis, respiration, flower formation and also in post-harvest processes such as ethylene biosynthesis, fruit ripening and firmness (Thakur and Sohal, 2013). Among them, localized and systemic response against infection by virulent pathogens is a major role of salicylic acid in the plant

(Javaheri et al., 2012). Salicylic acid is produced by the plant in response to stress and exhibits a great capacity in controlling diseases (Delaney et al., 2017; Awan et al., 2019).

Activation of transduction pathway after elicitation by exogenous salicylic acid application, activate inducible plant defences like reactive oxygen species (ROS), resulting in hypersensitive response (HR) during pathogen attack (Hayat et al., 2008; Thakur and Sahal, 2013). Oxidative stress causes rapid rise in the plant defence mechanism as the cell wall of the pathogen is altered hence the pathogen will not secrete its pathogenicity factors such as enzymes and toxins (Bhonwong and Stout, 2009; Ngadze et al., 2012). In addition, free radicals generated by salicylic acid applied exogenously causes oxidative damage to proteins of the pathogen thus destroying the invading pathogen directly (Czajkowski et al., 2015; Nayem et al., 2017). Moreover, oxidative burst following salicylic acid application results in hydrogen peroxide being released which causes cross-linkage between cell wall components of the plant and hydroxyproline- rich glycoproteins thus making the plant cell wall tougher for digestion by pathogens (Zhang et al., 2011; Nikoo et al., 2014). Hydrogen peroxide can also cause biological damage of membranes by reacting with polyunsaturated lipids present in the cell membrane resulting in lipid peroxides being formed (Mandal et al., 2009; Zhang et al., 2011; Awan et al., 2019). Other remaining ROS such as hydroxyls (OH), produced after application of salicylic acid react with biological molecules thus creating an environment unfavourable for pathogen development (Agnos, 2005; Na et al., 2006; Fernando et al., 2011). Plant tissue cells are protected from ROS as they contain detoxifying enzymes such as peroxidase, catalase and superoxide dismutase (Ngadze et al., 2012; Nikoo et al., 2014).

Salicylic acid application induces systematic acquired resistance (SAR) which is a defence response against biotic and abiotic stresses (Soleimani and Kirk, 2011; Ngadze et al., 2012; Kumar, 2014). SAR provides the plant with sufficient protection against microbes ranging from several days to few weeks as it causes various cellular defence response to be released such as phytoalexins, modification of the cell wall and rapid accumulation of pathogenesis related proteins (PRP) (González et al., 2009; Falcioni et al., 2014; Ramanujam et al., 2015). These proteins accumulate in the intercellular and extracellular of the leaf and whilst others are enzymes which have antimicrobial activities as well as other non-enzymatic based proteins involved in defence (Falcioni et al., 2014; Nikoo et al., 2014; Awan et al., 2019). These phytoalexins can accumulate in both resistant and susceptible plants but resistance occurs when they reach a concentration that is sufficient to suppress pathogen development (Joshi et al., 2010; Gondal et al., 2012; Gosh, 2015). Exogenous application of salicylic increased protein synthesis which was attributed to synthesis of defence enzymes and other protein based compounds (Hayat et al., 2017; Awan et al., 2019). The aim of this study was to evaluate the effect of salicylic acid in protecting tomato against *Alternaria* spp infection.

MATERIALS AND METHODS

Study site, plant materials and pathogen

The laboratory and greenhouse experiments were conducted at the University of Zimbabwe, Department of Plant Production Sciences and Technologies during the period of 2018-2019. *Alternaria* spp were isolated from infected leaves of tomato plants showing characteristic symptoms of spots with concentric rings inside the lesion. The leaves were dissected into small pieces (3-5 mm), surface sterilized using 0.1 % sodium hypochlorite (NaOCl) solution for four minutes, rinsed thrice using sterilised distilled water to remove traces of NaOCl and dried using sterile blotter papers. The plant tissues were then transferred aseptically into Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25 ± 2 °C (Ngadze et al., 2012; Kumar et al., 2017). Certified tomato seeds (variety Floridade) were used in the study.

Experimental design

Six treatments were arranged in a randomized complete block design (RCBD) and each treatment was replicated three times in four blocks. Treatments were assigned randomly in each block and were blocked according to distance from the door to reduce variation between the treatments.

Soil preparation and planting

Planting media was prepared by mixing red clay soil collected from the Department of Plant Production Sciences and Technologies experimental fields with pine bark at a ratio 2:1, respectively. The planting media was then sterilized by incubating at 80 ºC in an oven for 48 hours. Greenhouse pots measuring 23 cm in diameter and 24 cm in height were disinfected using 0.1 % sodium hypochlorite and filled with sterile planting media. Five tomato seeds were sown in each pot and thinning was done at three weeks after emergence to remain with one healthy plant per pot.

Agronomic practices

Basal fertilizer compound S (7 % N: 21 % P: 8 % K) was applied at the rate of 8 g per pot at planting. 10 g of ammonium nitrate (34 %) was split applied equally at three and six weeks after seedling emergence as topdressing per pot. The pots were supplied with 200 mL of autoclaved water after every two days. Weeding was achieved mechanically through hand pulling. Tomato plants were stacked with 1.4 m bamboo poles which were disinfected using 0.1 % sodium hypochlorite. Tying the plants to the pole was achieved using strings to aid in support. Suckers were removed when necessary and performed at all growth stages of the tomato plants.

Exogenous application of salicylic acid

Plants were grouped into six treatments and each replicated thrice. Tomato plants were sprayed with 1, 1.5, 2, 2.5, and 3-mM salicylic acid per respective treatments and water was used as the control. Salicylic acid was sprayed using a 500 ml hand held sprayer until the leaf surface area was completely wet.

Artificial inoculation of plants

Spore suspension of Alternaria strains was prepared and adjusted to a concentration of 1.0 x 10^6 $CFUmL^{-1}$ with sterile water using 14-days-old culture. Spore suspension was applied per pot at the rate of 10 mL per pot and was applied at four leaf stage over the leaf surface till runoff. This was done eight hours after application of salicylic acid. In the control pots, water was used instead of spore suspension. Plants were then covered with a transparent polythene bag for 48 hours to maintain high humid conditions required for accelerating the infection processes.

Enzyme assays

To determine peroxidase activity, the method described by Ngadze et al. (2012) was used. Peroxidase enzyme activity was presented as $U\mu l^{-1}$ absorption per minute. Polyphenol oxidase activity was determined by Kuvalekar et al. (2011) and activity of enzyme was presented as $U\mu g^{-1}\ g^{-1}$. Phenylalanine ammonia lyase (PAL) activity was determined according to the method described by Ngadze et al. (2012) and the enzyme activity is represented as µg PAL g^{-1} leaf tissue.

Disease incidence

Disease scoring technique was used to collect data in the laboratory experiment. Disease incidence was based on Alternarosis symptoms on the plants. Disease incidence was calculated using the following formula: Disease incidence = $\frac{n}{N}$ x 100 %; Where n is number of plants infected by diseases and N is number of the assessed plant.

Disease severity

Disease severity was scored using the scale described by Falcioni et al. (2014).

Vegetative growth and yield parameters

Plant height was measured using a ruler and expressed in centimetre (cm). Number of leaves was obtained by counting individual plants at weekly interval. Tomato fruit yield was obtained by weighing using an analytical balance (Model Analytical Balance Sartorius Research R200D).

Statistical analysis

Data exploration for normality was done using Shapiro-Wilk test at 5 % probability level and residuals were plotted using Minitab version 16 to check assumptions for Analysis of Variance (ANOVA). Genstat 14th edition was used to generate ANOVAs and for statistically significant different treatments ($p<0.05$), Fisher's least significant difference (LSD) at 5 % significance level was used to separate the means.

RESULTS

Peroxidase enzyme activity (POD)

Peroxidase activity significantly increased ($p < 0.001$) in plants sprayed with 2.5 mM of salicylic acid from one to four weeks as compared to plants treated with 1 mM, 1.5mM, 3mM and distilled water (Table 1). No significant differences in enzyme activity were recorded in plants sprayed with 1 mM, 1.5 mM and water from four weeks after inoculation ($p > 0.05$). The highest POD activity was recorded in plants sprayed with 2.5 mM while significantly lowest was observed in water treated control plants at 3 WAI (Table 1).

Table 1. Activity of POD in leaves of tomato variety Floridade at 1 to 4 WAI of *Alternaria* spp spores and exogenous application of salicylic acid

Treatment	POD ($U\mu l^{-1}\ min^{-1}$) at 1 WAI	POD ($U\mu l^{-1}\ min^{-1}$) at 2 WAI	POD ($U\mu l^{-1}\ min^{-1}$) at 3 WAI	POD ($U\mu l^{-1}\ min^{-1}$) at 4 WAI
1 mM	0.184^{bc}	0.211^{a}	0.261^{a}	0.238^{a}

1.5 mM	0.178^{b}	0.223^{a}	0.300^{ab}	0.308^{bc}
2 mM	0.229^{d}	0.273^{b}	0.358^{b}	0.328^{c}
2.5 mM	0.272^{e}	0.328^{c}	0.451^{c}	0.335^{c}
3 mM	0.200^{c}	0.271^{b}	0.293^{ab}	0.293b^{c}
Control	0.143^{a}	0.222^{a}	0.234^{a}	0.268^{ab}
P-value	< 0.001	< 0.001	< 0.001	0.001
LSD	0.021	0.044	0.074	0.064
CV %	13.0	21.3	28.7	20.7

*Means followed by different letters within a column are significantly different (p < 0.05)

Phenylalanine ammonia lyase (PAL) activity

Significant differences (p<0.05) in enzyme activity of PAL were observed across all treatments (Table 2). PAL activity decreased significantly with time (p<0.05), No significant (p>0.05) differences in enzyme activity were recorded in plants treated with 1 mM, 3 mM salicylic acid and in the control.

Table 2. Phenylalanine ammonia lyase activity in response to the effect of different concentration of salicylic acid (mM) from 1 to 4 WAI with *Alternaria* spp spores

Treatment	PAL (μgg^{-1}) at 1 WAI	PAL (μgg^{-1}) at 2 WAI	PAL (μgg^{-1}) at 3 WAI	PAL (μgg^{-1}) at 4 WAI
1 mM	0.222^{b}	0.237^{a}	0.253^{ab}	0.058^{a}
1.5 mM	0.235^{b}	0.246^{ab}	0.260^{bc}	0.068^{a}
2 mM	0.238^{b}	0.260^{bc}	0.268^{bc}	0.068^{a}
2.5 mM	0.311^{c}	0.274^{c}	0.2550^{c}	0.093^{b}
3 mM	0.213^{a}	0.248^{ab}	0.255^{ab}	0.063^{a}
Control	0.160^{a}	0.233^{a}	0.239^{a}	0.063^{a}
P- value	< 0.001	< 0.001	0.004	0.001
LSD	0.046	0.016	0.014	0.016
CV %	24.8	8.0	6.5	29.3

Data presented are means of three replications. Means bearing different letters within a column are significantly different at (p<0.05).

Polyphenol oxidase (PPO) activity

Polyphenol activity was significantly (p<0.05) affected by application of salicylic acid (Table 3). PPO activity was significantly higher in plants treated with 2.5 mM at 3 WAI than in the control.

Table 3. Effect of different salicylic acid concentration PPO activity from 1 to 4 WAI of *Alternaria* spp spores in tomato Floridade variety

Treatment	PPO ($U\mu l^{-1}\,m^{-1}$) at 1 WAI	PPO ($U\mu l^{-1}\,m^{-1}$) at 2 WAI	PPO ($U\mu l^{-1}\,m^{-1}$) at 3 WAI	PPO ($U\mu l^{-1}\,m^{-1}$) at 4 WAI
1 mM	0.046^{b}	0.142^{ab}	0.208^{b}	0.172^{abc}
1.5 mM	0.053^{b}	0.143^{ab}	0.223^{b}	0.172^{abc}
2 mM	0.069^{c}	0.175^{c}	0.221^{b}	0.177^{abc}
2.5 mM	0.078^{d}	0.190^{c}	0.248^{c}	0.183^{c}
3 mM	0.048^{b}	0.168^{bc}	0.207^{b}	0.168^{ab}
control	0.037^{a}	0.138^{a}	0.175^{a}	0.162^{a}
P-value	< 0.001	< 0.001	< 0.001	0.031
LSD	0.009	0.028	0.023	0.013
CV %	19.3	20.9	13.1	9.2

Data represents mean value of three replicates. Means bearing different letters are significantly different at 5 % probability level.

Disease severity

There was a significant ($p<0.05$) time x salicylic acid concentration interaction on disease severity (Figure 1). Disease severity was significantly higher in the control than in plants treated with salicylic acid. Significantly lower disease severity was observed in plants treated with 2.5 mM salicylic acid compared to the other treatments at week 5.

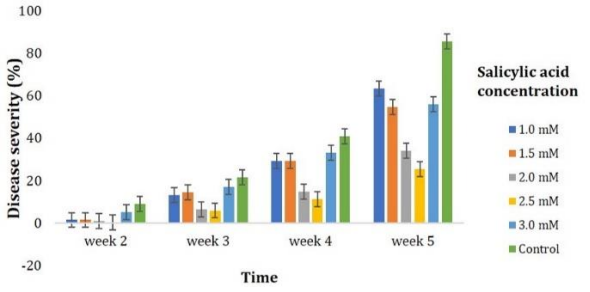

Figure 1. Effect of different salicylic acid concentrations on disease severity (%) of Floridade tomato variety from 2 to 5 WAI with *Alternaria* spp.

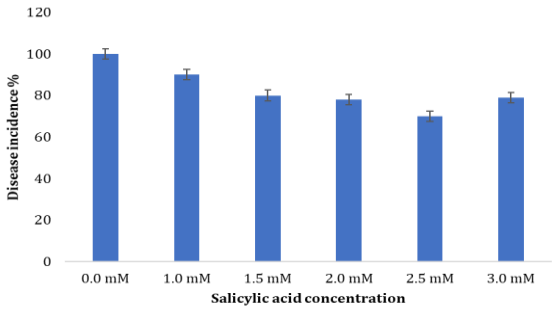

Figure 2. Disease incidence (%) of tomato at 2 WAI after inoculation with different salicylic acid concentrations. Error bars represent LSD at $p<0.05$.

Effect of salicylic acid on height and number of leaves of tomato plants inoculated with *Alternaria* spp

There was a significant ($p<0.05$) time x salicylic acid concentration interaction on plant height of tomato. Significant differences in plant height were observed from 3 to 5 WAI (Figure 3). During this period, salicylic acid treated plants at 1.5 mM, 2 mM, and 2.5 mM and 3 mM significantly increased plant height compared to the control plants. However, no significant differences in plant heights were observed in plants sprayed with 1 mM of salicylic acid and the control (Figure 3).

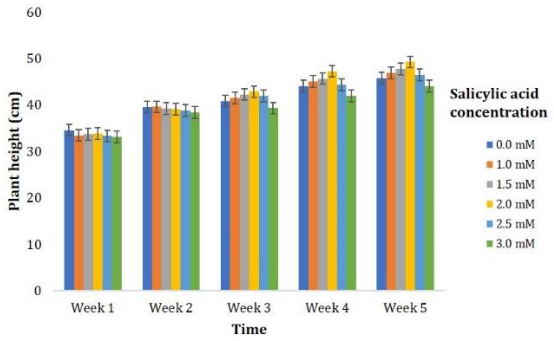

Figure 3. Plant height (cm) as influenced by different concentrations of salicylic acid after 1 to 4 WAI with *Alternaria* spp spores

There was a significant ($p<0.05$) time x salicylic acid concentration interaction on number of leaves. Plants treated with 3 mM of salicylic acid produced significantly fewer leaves compared to plants treated with 2 mM and 2.5 mM of salicylic acid from 3 to 4 WAI. However, no significant difference was observed in plants treated with 1 mM, 1.5 mM and 3 mM of salicylic acid (Figure 4).

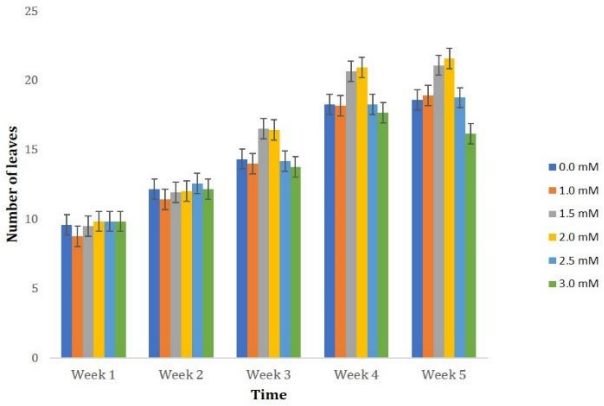

Figure 4. Effect of different concentrations of salicylic acid on number of leaves of tomato Floridade variety from 1 to 5 WAI. Error bars represents LSD value at 5 % significance level.

Effect on total fruit yield in response to different salicylic acid concentrations

It is apparent from Figure 5 that tomato plants treated with 2.5 mM had highest yield of 2.411 kg. Plants inoculated with 3 mM, however, did not corresponded to increased yield. The lowest yield of 1.367 kg was recorded in control plants treated with water. However, there was no significant differences

observed in plants treated with 1 mM, 3mM and control plants (Figure 5).

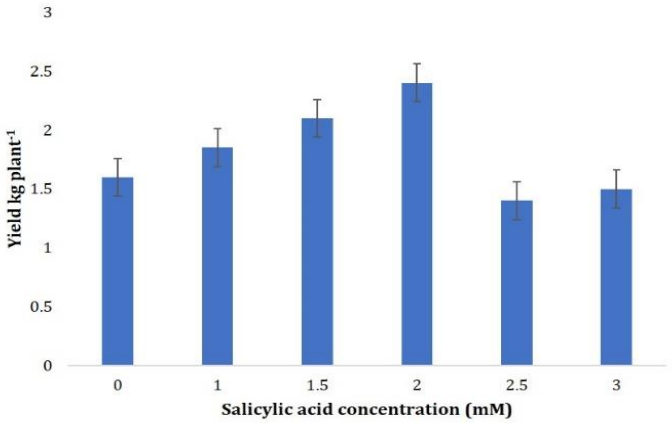

Figure 5. Tomato fruit yield as influenced by different concentration of salicylic acid. Error bars represent LSD at 5 % significance level

DISCUSSION

In the present study, POD activity exhibited marked increase in plants sprayed with different concentrations of salicylic acid over their control. POD enzyme activity increased with increase in salicylic acid concentration up to 2.5 mM while a reduction in enzyme activity was shown from 3 mM but this decrease was not below that of control treated plants. These results concur with the findings of Maksimov et al. (2010) and Awan et al. (2019) who reported an increase in peroxidase enzyme activity levels in potato tubers inoculated with late blight disease. The increase in POD levels has been linked to plant defense mechanism where POD oxidatively polymerize hydroxycinnamyl alcohols yielding lignin and isodityrosine cross linking bridges in the host cell walls (Wang et al., 2007; Ngadze et al., 2012; Nikoo et al., 2014). These structures form barriers to pathogens invading the host cells thus effectively contributing resistance of plants against diseases (Wai et al., 2014; Kumar et al., 2017). Peroxidase is also involved in the production of free radicals including hydrogen peroxide which create an environment that does not favor growth of pathogens thus constituting part of defence mechanisms (Rai et al., 2011; Kumar, 2014).

The present results also illustrated that POD activity was highest in plants sprayed with 2.5 mM. Contradictory results were reported by War et al. (2011) in their study on comparison of different salicylic acid concentrations in inducing chickpea plant defense. In their study, 1.5 mM of salicylic acid was found to induce higher peroxidase activity. These differences could be attributed to differences in factors such as plant age when salicylic acid was sprayed which affects enzyme activity. In the current study plants were sprayed at the four leaf stage whereas twenty-day old plants were sprayed in their study.

In this study, the activity of PAL was found to significantly differ in salicylic acid treated plants and untreated control plants. First, activity of PAL was notably high during the first week after inoculation in all treatments. The results from the current study support the findings reported by Ghosh (2015) who recorded high activity of PAL during the initial 28 days of infection by *Pythium* spp. Phenylalanine ammonia lyase catalyzes the formation of cinammic acid, a precursor that is required in the biosynthesis of phenols and lignin which bind to the fungal tip of the hyphae stopping its further movement and growth contributing to plant resistance mechanism (Mohammad et al., 2013; Yusuf et al., 2013).

However, for PAL to be effective it should be produced in high amounts during the initial stages of inoculation to effectively suppress the invading pathogen (Ngadze et al., 2012). Slow activity of PAL during initial infection encourages the pathogen to grow and express its pathogenicity factors leading to development of severe symptoms on the plant (Falcioni et al., 2014). Secondly, PAL enzyme activity was notably high in plants treated with 2.5 mM and the results from this study support the findings of Nayem et al. (2017) who reported high enzyme activity at this concentration. It was interesting to

note that enzyme activity at 3 mM was lower than that of plants treated with 2.5 mM of salicylic acid. Reduced PAL activity could be due to phyto-toxicity that was observed in the plants treated with 3 mM salicylic acid.

This study clearly illustrates that PPO activity was induced in all salicylic acid treated plants and reached peak activity three weeks after inoculation with spores of *Alternaria* spp. The results from the current study concur with the findings of Kamal (2009) who studied the influence of applying salicylic acid at different doses in onion inoculated with *Stemphylium* spp. In their study, they revealed that treatment with salicylic acid induced high PPO activity. During infection by the pathogen, damaged membranes stimulate the release of phenols in host cells and these phenols are oxidized in a reaction catalyzed by PPO into free radicals that react with biological molecules as a mechanism of defence. In some plant and pathogen interactions, high levels of induced PPO activity were linked closely to enhanced lignification as a mechanism of resistance (Bhonwong and Stout, 2009). Another mechanism of resistance to *Alternaria* spp was postulated to the involvement of PPO in catalyzing oxidation of polyphenol to produce quinones required in lignification of plant cells (Meena et al., 2011; Adhikari et al., 2017). Therefore, it is reasonable enough to suggest that salicylic acid concentrations that resulted in reduced PPO activity were insufficient to confer resistance in tomato plants inoculated with a virulent strain of *Alternaria* spp.

The present results indicate that foliar spraying of salicylic acid at 1 mM and 1.5 mM was insufficient for induction of disease resistance as disease severity was high in plants treated with these dosages compared to plants treated with 2 mM and 2.5 mM of the same hormone. It was also interesting to note that salicylic acid concentrations that stimulated high POD, PPO and PAL activity also lessened *Alternaria* disease development as shown by reduced disease incidence and severity. These concentrations exhibited high disease resistance as compared to plants treated with water. Lower enzyme concentration can result in inadequate oxidation of phenols to toxic substances and structures that confer plant resistance to disease (Delaney et al., 2017). This depicts that high enzyme activity plays a role in suppression of *Alternaria* spp. The result from the current study corresponds with the findings of Thanh et al. (2017) who studied the effect of salicylic acid in inducing resistance against *Xanthomonas oryzae* in rice. In their study, salicylic acid reduced disease severity by more than 38 %. Enhanced cell wall lignification and increased free radicals in host cells as a result of increased enzyme activity reduce disease symptom expression on tomato plants that were inoculated with early blight (Awadalla, 2008).

Foliar applied salicylic acid was found to positively influence growth and this was ascribed to enhanced cell division and cell enlargement (Bandici, 2008; Kumar et al., 2017). The same author also suggested that salicylic acid can alter auxin, cytokinin and abscisic acid hormonal balances that will consequently improve plant growth and yield. In this study, fruit yield was notably high with increase in salicylic acid concentration up to 2.5 mM. From the current study, plants with reduced disease severity had high yield than those with high disease incidence and severity. Increased yield could be due to increased photosynthetic capacity and better water use efficiency in leaves that were least affected by the pathogen (Javaheri et al., 2012; Awan et al., 2019).

CONCLUSION

The study showed that salicylic acid at 2.5 mM was the best elicitor concentration to induce enzyme activity (PAL, POD and PPO) against *Alternaria* spp. These results clearly suggest that defence enzymes including PPO, POD and PAL possess different activity at different times after inoculation with *Alternaria* spp in tomato plants. Salicylic acid concentrations at 2 mM and 2.5 mM performed the best in the suppression of disease severity and incidence in tomatoes under greenhouse conditions.

ACKNOWLEDGEMENTS

Funding for this research was obtained from the University of Zimbabwe's Department of Plant Production Sciences and Technologies.

AUTHORS CONTRIBUTION

MT: methodology, data collection and writing; WM and JTR: Data analysis and interpretation, drafting manuscript. NE, MU and EG: Project leaders, conceptualization, and student supervision, writing and correction, final approval for submission. All authors wrote and revised the manuscript.

DECLARATION STATEMENT

The authors declare that they do not have any conflict of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing', and was approved by the University of Zimbabwe.

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