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Full Length Article

Management of Tomato Leaf Spot Caused by *Alternaria tenuissima* Wiltshire using Salicylic Acid and Agrileen

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Abstract

Alternaria tenuissima was isolated from tomato showing symptoms of leaf spot disease cultivated under greenhouse conditions from Asir region, Saudi Arabia. The ability of salicylic acid (SA) and the newly prepared bioactive matter "Agrileen" (AG) to suppress the leaf spot disease of tomato was evaluated. Exogenous application of SA (0.5 and 1.0 mM) or AG (2.5 and 5%) enhanced the growth and plant yield of tomato in addition to the reduction of infection with *A. tenuissima*. Photosynthetic pigments, total proteins, free proline and the relative water content of leaves were also enhanced when either SA or AG was applied. The protein pattern showed an increase in band intensity in plants treated with SA that express the increase in gene expression involved in resistance against stress conditions. The results confirmed that the application of SA or AG could protect tomato plants against *A. tenuissima* infection either through the direct strength of the defense system and a reduction of the severity of the pathogen. © 2013 Friends Science Publishers

Keywords: Agrileen; *Alternaria tenuissima*; Leaf spot; Salicylic acid; Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide (Abd-El Kareem *et al*., 2006). It is cultivated essentially in all countries either in fields or in protected cultures. Leaf spot of tomato caused by *Alternaria* spp. is one of the common factors that reduce tomato production (Faheed *et al.,* 2005). This pathogen infects the plant leaves at all stages of plant growth, and causes destructive necrotic symptoms, that lead to yield losses. *Alternaria tenuissima* is an opportunistic pathogen and causes diseases of several plant species (Reddy *et al.,* 2002). During the last few years early blight has been occurring almost every year, essentially due to the soil-borne survival of the fungus, local overwintering/ oversummering of inoculum, cultivation of susceptible varieties and favorable environmental conditions (Khan *et al.,* 2003).

Application of chemical pesticides is still the common method of management of many crop diseases. Moreover, the application of these pesticides to reduce the food shortage is constrained, because of their possible carcinogenicity and toxicity. In addition, they have long degradation periods lead to environmental pollution (Lingk, 1991). The growing public admiration and the challenges encountered in the treatment of fungal infections have stimulated the researchers to find alternative synthetic fungicides.

Biological control and resistance-induction inducers are promising approaches to control plant diseases (Yu and Zheng, 2006). The use of the chemical inducers; jasmonic acid (JA) and salicylic acid (SA) represent interesting and new sources to control fungal and bacterial pathogens within the environmental friendly integrated crop protection systems (Ellis *et al.,* 2002). When exogenously applied, such molecules have been shown to move systemically through plants. Many factors (biotic and abiotic) could affect host physiology and metabolism, leading to the defense-gene activation in plants, which are expressing susceptibility to the pathogen (Ali *et al.,* 2007). Induced resistance (IR) is known to activate the cellular defense responses against pathogenic attack. The classic form of IR is systemic acquired resistance (SAR) controlled by signaling mechanisms that depend on the internal accumulation of SA (Sticher *et al.,* 1997; Durrant and Dong, 2004), and could be associated with the formation of defense compounds, such as PR proteins. The appearance of pathogenesis related proteins (PR) (Pieterse *et al.,* 1998) was suggested to be controlled by action of SA. In addition, free proline accumulation was greatly stimulated by the addition of lower concentrations of salicylic acid in plants (Fabro *et al.,* 2004). Recent studies have shown that some natural compounds (growth regulators and systemic chemicals) could act as defensive barriers against several

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pathogens (Schkalikov and Schekhovtcova, 1994; Hashem and Hamada, 2002).

In this study, the hormonal inducer SA and newly biologically active matter "Agrileen" were used to manage leaf spot disease of tomato caused by *A. tenuissima.* The study also investigated the possible mode of action of such compounds through changes in growth, yield and some metabolic activities related to the induced resistance in the infected tomato plants.

Materials and Methods

Plant Growth Conditions

Seeds of tomato (*Lycopersicon esculentum* Mill. cultivar Supemarmind), were surface sterilized by immersing in 0.5% sodium hypochlorite for 5 min and washing in sterilized water. After drying, the seeds were sown in 25 cm diameter plastic pots filled with a sandy clay soil. Each pot was sown with 10 seeds. After germination, they were thinned to two plants. When the plants were 45 days old, the pots were divided into six groups (12 pots/group) and were treated as follows:(1) plants without any treatments were referred as healthy control, (2) plants infected with *A. tenuissima* (20 mL/plant containing 10^6 cfu/mL) as infected control, (3) whereas the third and fourth groups, plants were infected with *A. tenuissima* and sprayed 3 times (15 day intervals) with 20 mL of 0.5 and 1 mM SA and 4) in the fifth and sixth groups, plants were infected with *A. tenuissima* and sprayed 3 times (15 day intervals) with 20 mL of 2.5 and 5% of Agrileen. Tween-20 was added (0.1%) as a surfactant solution. All pots were arranged in a complete randomized design in a greenhouse and watered regulatory to near field capacity with tap water.

Pathogen Isolation and Identification

A. tenuissima HQ905482 was isolated from leaves of tomato plant showing leaf spot symptoms on potato dextrose agar (PDA) at 25°C. Identification of the pathogen was carried out based on the macro and micromorphological characteristics (Ellis, 1976). Identification was confirmed by amplification of ITS gene. The fungal DNA was extracted using the Qiagene DNA extraction kit (Qiagene, Germany). The DNA sequences were analyzed using the DNA Blast and the obtained nucleotide sequences were deposited in the GenBank under accession number "HQ905482".

Bioactive Matter "Agrileen"

The bioactive matter Agrileen is a derivative of animal blood protein of fibrinogen, albumin, globulin, Arabic gum, calcium chloride and some trace elements. This preparation was prepared in our laboratory for the first time and its LD_{50} is 2.5%.

Effect of Agrileen on the Growth of *A. tenuissima in vitro*

Agrileen was amended with sterilized but cooled PDA to obtain final concentrations 0, 1, 2, 3, 4 and 5%. Petri plates containing a desired concentration of Agrileen was inoculated with 0.5 cm plugs from 4-days-old culture of *A. tenuissima*. Each treatment was carried out in three replicates and repeated twice. After incubation for 7 days at 25°C, the fungal growth was measured.

Pathological Measurement

Ninety days after sowing, 10 plants from each treatment were examined for the number of infected and uninfected leaves. The incidence of disease (ID%) was calculated according to the following equation:

ID% = (NO. of infected leaves/No. of both infected and healthy leaves) x 100.

The disease index (DI) of leaf spot on tomato plants was detected as the percentage of the infected area of leaves, using the rating 0-4 scale (0 = no symptoms, 1 = 0-10%, 2=10-25%, 3 = 25-50%, and 4 = 50-100%).

Morphological Measurements

The growth parameters including plant height, leaf number, fresh and dry weight and fruit yield were assessed as indicators of plant health, after 90 days from cultivation.

Physiological Analysis

After 90 days, the following physiological analyses were examined:

Photosynthetic pigments: Chlorophyll a, b and carotenoids were estimated in fresh leaf samples. One-half g of fresh leaves were ground in acetone (90% v/v), filtered and made up to a final volume of 50 mL. Pigment concentrations were calculated (absorbance of extract at 663, 648 and 470 nm) using the formula of Lichtenthaler (1987).

$$\text{Chlorophyll a (mg/g FW)} = [(11.75 \times A663 - 2.35 \times A645) \times 50]/500.$$

$$\text{Chlorophyll b (mg/g FW)} = [(18.61 \times A645 - 3.96 \times A663) \times 50]/500.$$

$$\text{Carotenoids (mg/g FW)} = [(1000 \times A470) - (2.27 \times \text{Chl a}) - (8.14 \times \text{Chl b})/227 \times 50]/500.$$

Total free proline content: Free proline was estimated as described by Bates *et al.* (1973). Briefly, samples of 0.5 g of fresh leaves were ground in 10 mL of 3% aqueous sulfosalicyclic acid. The solution was then filtered through Whatman's No. 2 filter paper. In a test tube, two mL of filtrate was added to two mL acid-ninhydrin and two mL of glacial acetic acid. The tubes containing the mix were placed in a water bath for 1 h at 100°C. The mixture was extracted with four mL of toluene and the chromophore containing toluene was aspirated, cooled to room temperature. The absorbance was measured at 520 nm with a Shimadzu UV 1601 Spectrometer. Free proline was calculated on fresh weight basis.

Total soluble phenol content: The soluble content of

phenol in tomato leaves was extracted as described by Hsu *et al.* (2003). Five grams of fresh leaves were homogenized in 80 mL methanol and kept overnight. The filtrates were diluted to 100 mL, and served as a stock solution. According to Slinkard and Singleton (1997), 200 µL of the stock solution was added to 1.4 mL distilled water, and 0.1 mL of 50% (1N) Folin- Ciocalteu phenol reagent. After three min., 0.3 mL of 20% (w/v) sodium carbonate was added. The mixture was allowed to stand for two h. After gentle vortex, the absorbance was determined at 765 nm. Total soluble phenol content was standardized against tannic acid.

Total soluble protein content: Bradford's method (Bradford, 1976) was used to determine the protein amount in the leaf crude extracts. Bradford's reagent was prepared as follows: 500 mg Coomasie Brillant Blue G-250 in 250 mL ethanol (95%) and 500 mL phosphoric acid (85%). The solution was finally diluted to one L and filtered. The prepared reagent was 5 x. It was diluted to 1x before using. From each sample, 20 mL was transferred to a test tube and completed to 500 mL with distilled water. Five mL of 1x Bradford reagent was added to the diluted sample. After 10 minutes, the absorbance was measured at 595 nm using Schimadzu UV-1201 spectrophotometer. The standard curve was constructed using bovine serum albumin (BSA).

Electrolyte leakage percent: To determine electrolyte leakage, 100 mg fresh leaf were cut into discs (5 mm in diameter) and placed in test tubes containing 10 mL deionized water. The closed tubes were placed in a water bath at 32°C. After two h, the initial electrical conductivity of the medium (EC1) was measured using an EC meter. The samples were autoclaved at 121°C for 20 min and cooled to 25°C. The final EC2 was measured. The electrolyte leakage (EL) was expressed following the formula:

$$L = EC1/EC2 \times 100$$ (Dionisio-Sese and Tobita, 1998).

Relative water content of the leaf (RWCL): RWCL was estimated using as described by Yamasaki and Dillenburg (1999). The leaf samples were prepared from the mid height of each plant to minimize age effect on the variability of results. Individual leaves were removed and weighed for fresh mass (FM). To determine the turgid mass (TM), whole leaves were floated in distilled water in closed petri plates. During the imbibition period, leaf samples were weighed periodically, after gently wiping water from the leaf surface with tissue paper. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80°C for 48 h, to obtain dry mass (DM). Values of FM, TM and DM were used to calculate LRWC. The equation below was applied:

$$LRWC\ (\%) = [(FM - DM)/(TM - DM)] \times 100$$

Sodium Dodicyl Sulphate-Polyacrylamide-gel Electrophorasis (SDS-PAGE)

One gram of healthy, infected and treated tomato leaves was dissected, collected on ice, and ground in two volumes of protein extraction buffer consisting of 2 M KPO_4 (pH 7.8), 0.5 M EDTA, 1% Triton X-100 and 12.5% of 80% glycerol. The mixture was passed through double layer of Miracloth and the filtrate was used. Seven µL of each sample was analyzed by SDS-PAGE (Abe and Davies, 1991; 1995). Samples were diluted with 2x sample loading buffer to yield final concentrations of 2% Sodium dodicyl sulphate (SDS), 0.01 M Tris-HCl (pH 6.8), 20% glycerol, 1% β-mercaptoethanol, and 0.01% BPB, heated at 95°C for 5 min, and separated by SDS-PAGE in 10% acrylamide gel.

Plant Harvest

Plants with different treatments were harvested after 120 days and their productivity was estimated.

Statistical Analysis

Treatments were arranged in a completely randomized design with 6 treatments. Experiments were carried out twice. Analysis of variance (ANOVA) was performed using the SPSS software package to determine the least significant difference (LSD) among treatment at $P < 0.05$.

Results and Discussion

Effect of Agrileen on the Pathogen Growth

Fig. 1 shows the inhibitory effect of Agrileen on the radial growth of *A. tenuissima*. A clear correlation between the concentration of Agrileen and the reduction of the fungal growth was obtained. The concentration of 5% Agrileen had a lethal effect on the growth of the tested fungus, where it was completely inhibited. This proves the fungicidal effect of Agrileen, which could be used as a safe alternative compound to control leaf spot disease.

Effect of Salicylic Acid (SA) and Agrileen (AG) on Leaf Spot Disease Progress

The results indicated that application of SA and AG had significantly reduced the progress of leaf spot disease in plants infected with *A. tenuissima*. The number of infected leaves was reduced to 93% of the infected plants when AG was applied at a level of 5%, however SA, when applied at one mM, reduced the number of infected leaves to 64% compared to the infected control (Fig. 2). Accordingly, the disease incidence percent was minimized to 4.85% and 19.58% after application of AG at 1 mM and SA at 5%, respectively (Fig. 3). Thus, the disease index achieved a minimum value (0.8) as a result of 5% AG compared to control infected plants only (3.6) (Fig. 4). *A. tenuissima* is considered as a secondary invader rather than a primary plant pathogen. However, Feng *et al.* (2007) mentioned that *A. tenuissima* is a main leaf spot causal pathogen. On eggplant, leaf spot of *A. tenuissima* was recorded as small, circular, brown necrotic spots all over the shoot (Raja *et al.*, 2005). These symptoms are similar to the present study. *A. tenuissima* was also found to be the causal pathogen of the

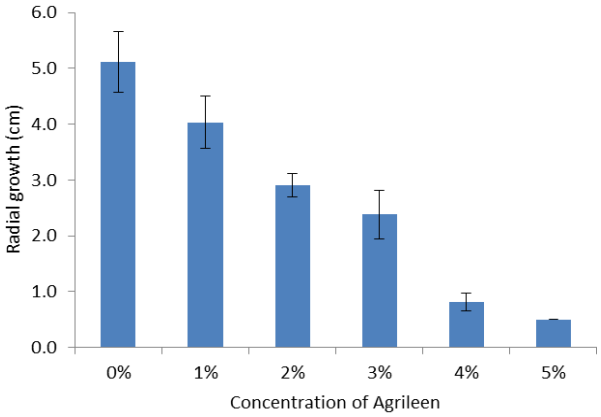

Fig. 1: Effect of Agrileen on the radial growth of *A. tenuissima* after 7 days incubation at 25°C

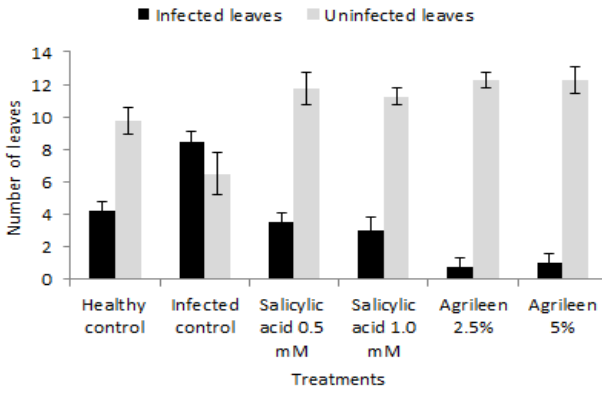

Fig. 2: Effect of application of salicylic acid and Agrileen on number of infected and healthy leaves of tomato

leaf spot of *Amaranthus hybridus* (Blodgett *et al.*, 1999). *A. tenuissima* can survive under various environmental conditions and must have developed sophisticated mechanism to adapt itself to different environmental niches (Wan *et al.*, 2008). Therefore it has a wide range of hosts (Feng *et al.*, 2007). The humid climate of some areas in Saudi Arabia is seemed to be more favorable for infection by *A. tenuissima* of several crop plants.

Growth Parameters and Yield

All growth parameters and yield were significantly reduced in infected tomato plants compared to uninfected controls (Table 1). Plant height, number of leaves/plant, fresh and dry weight of shoots and fruits yield were significantly reduced by 15.9, 21.82, 54.83, 66.35 and 18.02%, respectively compared to control. The noticeable decrease in growth and yield of the infected plants is in agreement with results of Faheed *et al.* (2005). Aducci *et al.* (1997) explained that the decrease in fresh weight of infected tomato shoots may be due to the toxins produced by the pathogen, which affected K uptake and stomata functions leading to uncontrolled transpiration and excessive loss of

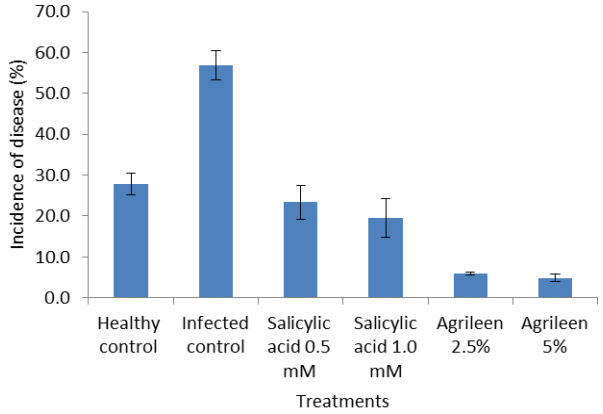

Fig. 3: Effect of application of salicylic acid and Agrileen on percentage of incidence of the leaf spot disease on tomato

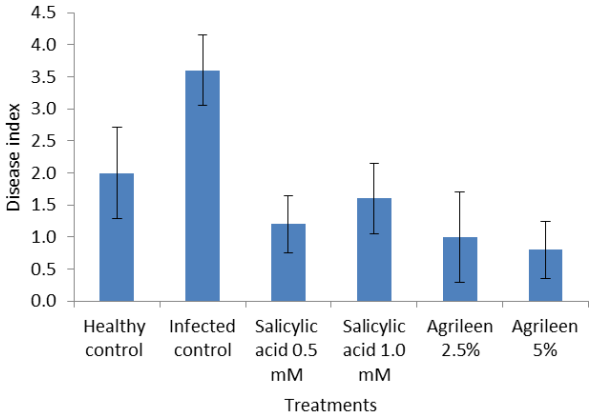

Fig. 4: Effect of application of salicylic acid and Agrileen on disease index of leaf spot of tomato

water leading to plants wilting. However, reduction in shoot dry weight might be related to an increased rate of respiration and decompartementalization due to membrane degradation (Orcutt and Nilsen, 2000). This finding greatly supports our results, where the electrolyte leakage percent was 13.14% in the infected plants (Table 1).

Application SA or AG significantly alleviated the hazardous effect of the pathogen and enhanced the all growth parameters of infected tomato plants as compared to infected and uninfected controls. Also, infected plants treated with SA or AG significantly increased fruits yield/plant over healthy control (Table 1). Results of the present study indicated that both SA and AG reduced the negative impact of *A. tenuissima* on growth and yield. Our investigation also could confirm at least a double action of salicylic acid, by enhancing plant resistance and as a foliar fertilizer. The results coincide with those of Hossain *et al.* (2002) who reported that salicylic acid promotes cell elongation and increases cell wall extensibility. Moreover, the results proved the considerable efficacy of salicylic acid against *Alternaria* leaf spots (Colson-Hanks and Deverall,

2000). It was reported that salicylic acid is involved in regulation of resistance against *A. solani* (Faheed *et al.*, 2005). Since the new bioactive matter AG was applied for the first time in this study, its exact mode of action in protection the plants against the fungal pathogens is still not understood. We recommend further studies to explore the mode of action of this material.

Relative Water Content of Leaf (LRWC)

Leaf water content expresses the relative amount of water present in the plant tissues, so it is a good indicator of water balance (Yamasaki and Dillenburg, 1999). Infection of tomato plants with the pathogen led to a significant reduction in LRWC by 10.51% as compared to healthy control (Table 1). However, significant increase in LRWC was achieved when either SA or AG was used; however, the highest increase was achieved when AG was applied at the rate of 2.5%. The reduction in LRWC is suggested to be related to an increased rate of respiration and membrane injury (Orcutt and Nilsen, 2000). In agreement with our results, Maiceeva (1999) has proved the ability of both Strom (organic compound) and F-760 (growth regulator) to increase the water maintenance capacity for 4 h after uprooting of wheat plants. She pointed out that the increase of water maintenance capacity of plants results in induction against dryness and improves the physiological processes. This leads in general to the improvement of plant performance to enhance their resistance against facultative parasites attacking the weak plant organs.

Electrolyte Leakage Percent (ELP)

Electrolyte leakage percent (EL%) represents the degree of cell membrane injury. Table 1 shows that ELP of the infected plants increased significantly up to two folds that of healthy plants. This means that membrane stability of infected plants was severely deteriorated by fungal infection, which is based on a relationship between cellular constituents and the fraction that leaked out (Hamada and Hashem, 2003). It is assumed that toxins excreted by the fungal pathogen play an important role in cell membrane injury (Achor *et al.*, 1993). Our results revealed that the application of both SA and AG significantly reduced ELP to almost the values of healthy plants. The highest reduction was achieved with high dose of AG (7.5%) and low dose of SA (7.68%). These results prove the efficiency of both SA and AG in alleviating the cell membrane injury through reduction of fungal infection and/or inducing the resistance of tomato plant against the infection.

Photosynthetic Pigments

The rate of photosynthesis is one of the most important signs on physiological status in plants that is related to the chlorophyll content. Data in Table 2 show that, in infected plants with *A. tenuissima*, chlorophyll a, b and carotenoids were significantly reduced by 80.28, 100 and 97.30% for chlorophyll a, b and carotenoids, respectively compared to control plants. Reduction in chlorophyll and carotenoids in tomato leaves because of *Alternaria* infection may be a consequence of the fungal effect on the release of transported toxins leading to the liberation of reactive oxygen species causing programmed cell death (Howlett, 2006).

On the other hand, applications of SA or AG

Table 1: Effect of Salicylic acid and Agrileen on growth parameters, fruit yield, leave relative water content percent (LRWC %) and electrolyte leakage percent (EL%) of tomato plants infected with *Alternaria tenuissima*

Treatments	Plant height (cm)	No. of leaves /plant	FW of shoots/plant(g)	DW of shoots/plant (g)	Yield of fruits plant(g)	LRWC%	EL%
Healthy control	55.67b	22.00a	58.73a	8.65a	159.90d	79.51a	6.71e
A. tenuissima	48.33d	18.00c	37.93c	5.20d	135.40e	71.95d	13.14c
A. tenuissima +0.5 mM SA	58.33a	22.00a	58.40a	8.52a	174.00c	76.32bc	7.68d
A. tenuissima +1 mM SA	52.67c	20.00b	51,93b	7.34b	163.90d	75.38bc	8.14b
A. tenuissima +2.5% AG	53.67bc	21.00b	51.67b	7.32b	203.00b	78.04ab	9.07a
A. tenuissima +5% AG	50.00d	18.67c	43.23c	5.94c	213.80a	74.95c	7.50c
LSD ($P < 0.05$)	2.25	1.18	3.09	0.57	9.29	2.74	0.37

SA = salicylic acid and AG = Agrileen. Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P < 0.05$)

Table 2: Effect of salicylic acid and Agrileen on photosynthetic pigment content, total soluble phenols content, total soluble proline content and total soluble protein content of tomato plants infected with *Alternaria tenuissima*

Treatments	Photosynthetic pigments (mg/g fresh leaves)			Total soluble metabolites (mg/g fresh leaves)		
	Chl. a	Chl. b	Carotenoids	Phenols	Proline	Proteins
Healthy control	1.280a	0.320a	0.730b	0.980b	0.290e	7.19d
A. tenuissima	0.710e	0.160e	0.370f	1.18a	0.147f	5.42e
A. tenuissima +0.5 mM SA	0.960b	0.230b	0.540d	0.830d	0.393d	7.70bc
A. tenuissima+1 mM SA	0.950b	0.227bc	0.560c	0.783e	0.447c	7.88b
A. tenuissima +2.5% AG	0.750d	0.180d	0.440e	0.890c	0.713b	8.72a
A. tenuissima +5% AG	0.880c	0.210c	0.847a	0.870cd	0.777a	7.62c
LSD (P <0.05)	0.02	0.01	0.01	0.20	0.04	0.01

SA = salicylic acid and AG = Agrileen. Different letters indicate significant difference among treatments within the same column according to least significant difference test ($P < 0.05$)

significantly increased chlorophyll a, b and carotenoids contents compared to the infected control. This finding is supported by the results obtained by El- Khallal (2007), Hamada and Hashem (2003) and Hibar *et al.* (2007). The high chlorophyll content in SA-treated plants could be attributed to its stimulatory effect rubisco (ribulose 1,5-bisphosphate carboxylase) activity (Khodary, 2004). It is worthy to mention that enhancement of photosynthetic pigments in the plant treated with AG induced the absence of toxic effect due to application of such bioactive material. This encourages us to use and suggest it as a safe bioagent to control the target disease.

Total Soluble Phenols

Results presented in Table 2 indicate that the formation of soluble phenols was significantly increased in the infected plants by 20.41% of the healthy controls. On the other hand, there was a significant reduction in the phenolic content in infected plants which followed by these treated with SA or AG compared to either infected or healthy controls. The role of phenolic compounds in defense mechanism against stress by plant pathogens is well established (Khatun *et al.*, 2009). However, low level of phenols in treated plants with SA or AG indicates the role of them in preventing stress due to the fungal pathogens.

Total Free Proline and Total Soluble Protein Content

Results in Table 2 indicate that total free proline and total soluble protein levels were significantly decreased by 97.28 and 32.66%, respectively in infected leaves of tomato plants as compared to uninfected controls. The significant decrease in the protein content in leaves as a result of pathogen infection might be attributed to some activities related to a hypersensitive response (Chandra and Bhatt, 1998). Application of SA and AG significantly increased total free proline and soluble protein contents not only to achieve but also to exceed their content in healthy controls. This finding is supported by the results of (El- Khallal, 2007; Abo-Elyousr *et al.*, 2009). El-Bahay and Moursy (2003) reported that close correlation between the levels of total soluble proteins in response to SA could be attributed to its action on DNA-RNA synthesizing protein machinery at transcriptional and/or translocational levels.

The SDS-PAGE could confirm the above mentioned results since the number of protein bands, which appeared in the infected plants were lower than in healthy and treated ones. The most clear bands in the examined samples were; 69, 50, 43, 29, 26, 24 and 20 kDa (Fig. 5). Protein bands of 69 and 50 kDa were clearly expressed in the healthy samples and those treated with SA and AG than infected samples. In tomato, several apoplastic proteases have a relation to defense response to biotic and abiotic stresses. Serine proteases of the P69 subtilase family have been linked to pathogen defense, and two isoforms, P69B and

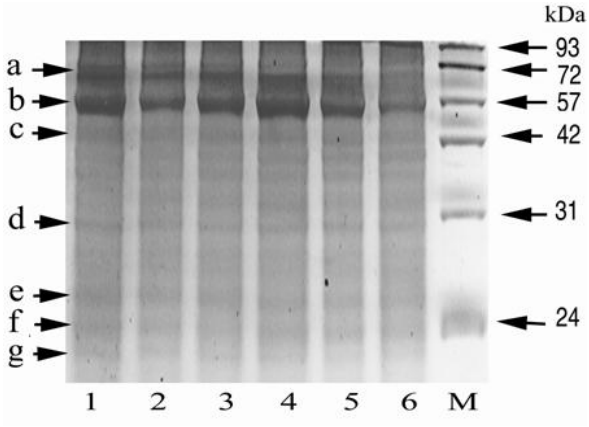

Fig. 5: SDS-PAGE showing the effect of application of salicylic acid and Agrileen on protein profile of tomato. Samples correspond to: lane 1, healthy control (uninfected plants); lane 2, infected control (infected with *A. tenuissima*); lane 3, *A. tenuissima* +0.5 mM SA; lane 4, *A. tenussima*+1 mM SA; lane 5, *A. tenuissima* +2.5% AG; lane 6, *A. tenussima*+5% AG; M, molecular weight marker. Notations with arrows are: a, 69 kDa; b, 50 kDa; c, 43 kDa; d, 29 kDa; e, 26 kDa; f, 24 kDa and g, 20 kDa

P69C, specified as pathogenesis-related proteins (Jordá *et al.*, 1999; Van Loon and Van Strien, 1999). The protein of 50 kDa is a systemin-binding protein (SBP), identified in the cell membrane of tomato leaves. It plays an important role in expression of furin-like protease in the mechanism of defense gene in a membrane bound (Schaller and Ryan, 1994). Twenty-nine kDa in tomato plants enhanced phosphorylation process in response to the polyuronide. Edward *et al.* (1989) suggested that this protein could be related to the mechanism of signal transduction leading to defense gene expression in the plant infected with a pathogen.

Conclusion

Our results confirmed that *A. tenuissima* is a causal pathogen of tomato leaf spot in Saudi Arabia. Salicylic acid and the new bioactive material "Agrileen" showed promising effect as safe biological alternatives in the control of *Alternaria* leaf spot. Application of SA or AG can protect tomato plants against *A. tenuissima* infection either through the direct strength of the defense system or reduction of severity of the pathogen. We strongly recommend using of these compounds in management of other diseases on other crops to approve their efficiency as alternatives to the chemical pesiticides in controlling the plant diseases.

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