



# Induction of Resistance to *Macrosiphum rosae* by Foliar Application of Salicylic Acid and Potassium Sulfate in Rose Plant

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## ABSTRACT

The rose aphid, *Macrosiphum rosae* (L.) (Hemiptera: Aphididae), is one of the most important pests on *Rosa* species, which cause serious damage to plants. Salicylic acid (SA) and Potassium sulfate (PS) have the potential to affect the population of pests on plants. In this study, different concentrations of SA (0.7 and 1.4 mM), PS (11 and 28 mM) and SA (1.4 mM) + PS (28 mM) were used as foliar applications on rose plants to evaluate the life table parameters of *M. rosae*. The chemical constituents of rose leaves were also assessed using Gas Chromatography–Mass Spectrometry (GC/MS). Results showed that the life table parameters of *M. rosae* were significantly affected by various treatments. The longevity of the aphid was longest on control and shortest on SA (1.4 mM). *M. rosae* had the least fecundity on SA (1.4 mM) treatment. The intrinsic rate of natural increase ( $r_m$ ) of *M. rosae* ranged from 0.202 to 0.298 day<sup>-1</sup> on different treatments, which was lowest on SA (1.4 mM) and highest on control. GC/MS analysis showed that Squalene, 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-dimethylpyrazol-1-yl) ethanone, and 9,12,15-octadecatrienoic acid, as secondary metabolite, were the major compounds in SA (1.4 mM) treatment. The results of this study demonstrated that application of SA (1.4 mM) on rose plants has a good potential for reducing the population of *M. rosae* and can be used in integrated pest management programs.

## Introduction

The Rose (*Rosa hybrida* L.) is an important ornamental plant in the world (Blackman and Eastop, 2000). This plant is attacked by several pests causing high annual losses. *Macrosiphum rosae* (L.) (Hemiptera: Aphididae) is one of the pests inhibiting the growth of leaf buds and twigs by sucking plant sap. It also causes weakness of flower buds. Furthermore, the sticky solution “honeydew” excreted by the aphid promotes the growth of sooty-mold on flowers and surface of leaves. In urbanized conditions, this species not only cause measurable economic losses, but also affects urban landscapes by lowering the ornamental

value of shrubs (Jaskiewicz, 2000). The application of synthetic insecticides is practiced to control them throughout the world. However, more attention should be given to other controlling methods with high safety profile.

Plants have an immune system that can be induced by biotic or abiotic inducers (Jones and Dangl, 2006; Shivaji et al., 2010; Li and Zhang 2012; Sattari Nasab et al., 2018; Khoshfarman-Borji et al., 2020). The use of these inducers can limit the pest population and result in the reduced reliance of its management on synthetic insecticides (Nardi et al., 2015). The induction of plant resistance is a method in which, the plant protects itself against pests and pathogens without contamination of the environment. Salicylic acid (SA) is a hormone substance, which plays a vital role in regulating many aspects of

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plant growth and development (Peng et al., 2004; Shivaji et al., 2010). Moreover, its ability to induce systemic resistance in plants is very well-known (Ryals et al., 1996).

Application of fertilizers is a promising way for increasing crop yield. The resistance of plants to pests and diseases is greatly affected by plant nutrition, despite the fact that is also controlled by the genes (Wang et al., 2013; Sunil, 2017). Potassium, especially in the form of PS, ( $K_2SO_4$ ), is one of the essential plant nutrients for the performance of plant primary and secondary metabolites (Marschner, 2012). It has been reported that potassium can decrease insect infestation especially sucking pests mainly through physiological changes in many crops (Wang et al., 2013; Rahman et al., 2014). Crops that are less sensitive may still require PS for optimal growth if the soil accumulates chloride from irrigation water (Schultz et al., 2005).

Secondary metabolites are chemical compounds produced by plants which are mainly used to protect plants against a variety of herbivores (Fürstenberg-Hägg et al., 2013). They also improve plant growth and survival under different environmental stresses (Kliebenstein, 2013). Secondary metabolites can affect the life history traits of the herbivore insects (Price et al., 1980; La Rossa et al., 2013; Pahlavan Yali and Sattari-Nasab 2020). These metabolites may be influenced by application of fertilizers. For example, Mardani Talae et al. (2016) found that adding Zinc sulfate to the growing medium of pepper plant increased the performance of *Myzus persicae* Sulzer. They pointed out that the low amount of secondary metabolites such as flavonoids and phenolic compounds in Zinc sulfate-treated plants compared to control plants with no fertilizer may be responsible for increasing some life table parameters of *M. persicae*.

The role of SA and PS on plant growth and insect's incidence has been well studied (Myers and Gratton, 2006). However, information about their exogenous application on rose plants is rare. Furthermore, most studies about roses have been focused on secondary metabolites from flowers or fruits, while little is known about the kind of secondary metabolites from other plant tissues such as leaves. Therefore, the aim of this study was to evaluate the impact of foliar applications of SA and PS on life table parameters of *M. rosae* and the secondary metabolites of rose plants using GC/Mass method. The findings of this research could provide complementary knowledge on compounds conferring resistance to rose plant against *M. rosae* and could be used for integrated

management of *M. rosae* in urbanized areas and commercial roses.

## Materials and Methods

### *Stock cultures*

All the experiments were conducted in Parks and Green Space Organization of Kerman Municipality (greenhouse collection) at  $23 \pm 2$  °C,  $65 \pm 10\%$  relative humidity, and under natural light. The shrubs of rose plants (cv. Josephine Bruce) used in this study were obtained from Parks and Green Space Organization of Kerman Municipality in Kerman Province, Iran. Each rose treatment was grown in 30-cm diameter pots filled with suitable soil composed of loam soil, peat and sand (2:1:1). To establish aphid colonies, 10 shrubs of rose plant were kept in cages and infested with *M. rosae*. The aphids used here were acquired from the aphid colony reared in the laboratory of the Plant Protection Department of Shahid Bahonar University, Kerman, Iran in October 2017 and transferred onto the potted plants under the above-defined conditions. Aphids were reared for at least three generations on rose plants (cv. Josephine Bruce) before experiments.

### *Inducer treatments*

In this study, six different treatments were used on rose plants comprising aqueous solution of distilled water for control, two concentrations of PS ( $K_2SO_4$ ) (11 and 28 mM) (Merck, Germany), two concentrations of SA (0.7 and 1.4 mM) (84210 Sigma-Aldrich > 99 %), and mixed application of PS and SA (PS 28 mM + SA 1.4 mM). Foliar spraying of treatments was done twice, at 14-day intervals on rose plants. Infestation of the fertilizer-treated plants to *M. rosae* and GC/Mass analysis were performed 48 h after the second spraying.

### *Life table study*

The study was performed based on randomized complete design by placing clip cages (6 cm diameter and 1.5 cm depth) on the leaves of potted rose plants (N=30 potted plants for each treatment) in a growth chamber ( $23 \pm 2$  °C,  $60 \pm 5\%$  RH, and 16/8 light/dark cycles).

Forty-eight hours after the application of the above-mentioned treatments, adult apterous aphids were individually placed on the upper surface of a given leaf inside each clip cage. A total of 30 clip cages were established on a pre-determined leaf of potted rose plants for each treatment. After 24 h, the adult aphids and all nymphs except one were removed from the clip cages. The nymphs were monitored daily until reaching the adulthood to determine nymphal

developmental time and their survivorship on each treatment. After maturity, daily observations for the mortality and fecundity of the aphids were continued and the produced offspring were removed from clip cages until each female aphid died. The obtained data from this experiment was used to assess the population growth parameters.

### Methods of extraction (Maceration)

To aim this, 20 g of rose leaf was dissolved in 200 mL of methanol in an Erlenmayer flask. At first, it became completely homogeneous using a glass agitator and then, the lid was closed. The solution was occasionally stirred with an agitator for 72 h (on a shaker). The methanolic extract was then filtered using a filter paper and the solution sample was used to inject the GC/MS device.

### Gas Chromatography–Mass Spectrometry analysis of extracts

The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (Agilent Technologies 7890 A) in Islamic Azad University of Kerman, Iran. Experimental conditions of GC-MS system were as follows:

HP 5-MS capillary standard non-polar column, dimension: 30m, ID: 250  $\mu$ m, Film thickness: 0.25  $\mu$ m. Flow rate of mobile phase (carrier gas: He) was set at 1.0 mL/min. In the gas chromatography part, temperature program (oven temperature) was 50  $^{\circ}$ C raised to 280  $^{\circ}$ C at 5 $^{\circ}$ C/min and injection volume was 0.1  $\mu$ L. Samples dissolved in Methanol. The results were compared by using WILEY, Demo and NIST Spectral libraries search program.

### Statistical analysis

The raw life history data of all individuals of *M. rosae* were analyzed using the TWOSEX-MSChart program (Chi 2017) based on the age-stage, two-sex life table theory (Chi and Liu 1985). The age-stage specific survival rate ( $s_{xj}$ ) (where  $x$  is the age and  $j$  is the stage), age-stage specific fecundity ( $f_{xj}$ ), age-specific survival rate ( $l_x$ ), age-specific fecundity ( $m_x$ ), and age-specific

maternity ( $l_x m_x$ ) were evaluated from the daily records of the survival and fecundity of all the individuals in the cohort. Furthermore, the fertility life table parameters including intrinsic rate of natural increase ( $r_m$ ), net reproductive rate ( $R_0$ ), mean generation time ( $T$ ), and finite rate of increase ( $\lambda$ ) were calculated. The intrinsic rate of increase was estimated using the iterative bisection method from the Euler-Lotka formula with the age indexed from 0 (Goodman 1982):

$$\sum_{x=0}^{\omega} e^{-r(x+1)} l_x m_x = 1$$

The means and standard errors of life table parameters were determined using the bootstrap technique (Efron and Tibshirani 1993; Huang and Chi, 2012) with 100,000 resampling. The bootstrap method is embedded in the computer program TWOSEX-MSChart. The paired bootstrap test was used to evaluate the differences among treatments.

## Results

### Life table study

There was a significant difference in the nymphal period of *M. rosae* among different treatments (Table 1). The longest (7.04 days) and shortest (5.7 days) nymphal period were obtained in treatments of SA (1.4 mM) and control, respectively. Based on this result, the parameters of adult longevity and total life span were lowest on SA (1.4 mM) treatment and highest on control ( $P < 0.05$ ; Table 1).

Adult pre-reproductive period (APRP) of *M. rosae* was significantly longest on SA (1.4 mM) treatment and shortest on PS (28 mM) and control treatments ( $P < 0.05$ ; Table 2). Moreover, the longest total pre-reproductive period (TPRP) was observed on SA (1.4 mM) treatment ( $P < 0.05$ ; Table 2). The reproductive period and fecundity of *M. rosae* were lowest on SA (1.4 mM) treatment and highest on control ( $P < 0.05$ ; Table 2).

**Table 1.** Mean ( $\pm$ SE) nymph period, adult longevity, and life span of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

Treatments	Nymphal period (d)	Adult longevity (d)	Total life span (d)
Control	5.07 $\pm$ 0.07d	28.21 $\pm$ 1.13a	31.30 $\pm$ 1.72a
PS (11 mM)	5.32 $\pm$ 0.09c	22.86 $\pm$ 0.74bc	26.50 $\pm$ 1.34b
PS (28 mM)	5.21 $\pm$ 0.11cd	23.25 $\pm$ 0.77b	26.80 $\pm$ 1.34b
SA (0.7 mM)	5.96 $\pm$ 0.13b	21.04 $\pm$ 0.85c	25.44 $\pm$ 1.30bc
SA (1.4 mM)	7.04 $\pm$ 0.13a	16.57 $\pm$ 0.62d	22.33 $\pm$ 1.02c
SA (1.4 mM)+ PS (28 mM)	5.29 $\pm$ 0.09cd	22.00 $\pm$ 1.12bc	25.69 $\pm$ 1.47bc

**Notes:** Means followed by different letters within a column are significantly different according to the paired bootstrap test at 5% significance level.

PS= Potassium sulfate, SA= Salicylic acid

**Table 2.** Mean ( $\pm$ SE) reproductive parameters of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

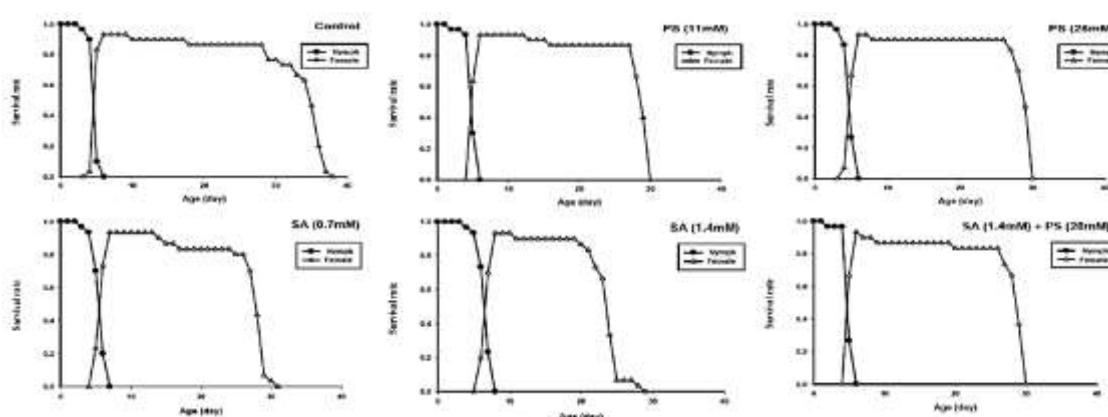
Treatments	Adult pre-reproductive period (days)	Total pre-reproductive period (days)	Reproductive period (days)	Numbers of female progeny
Control	0.96 $\pm$ 0.1b	6.04 $\pm$ 0.12d	25.86 $\pm$ 1.21a	63.00 $\pm$ 3.13a
PS (11mM)	1.18 $\pm$ 0.1ab	6.50 $\pm$ 0.12c	19.75 $\pm$ 0.80b	44.51 $\pm$ 2.17b
PS (28mM)	0.96 $\pm$ 0.1b	6.19 $\pm$ 0.17cd	21.3 $\pm$ 0.21b	43.65 $\pm$ 1.708b
SA (0.7mM)	1.18 $\pm$ 0.12ab	7.14 $\pm$ 0.19b	17.11 $\pm$ 0.93c	30.93 $\pm$ 1.78b
SA (1.4mM)	1.44 $\pm$ 0.16a	8.44 $\pm$ 0.2a	13.26 $\pm$ 0.45d	17.72 $\pm$ 1.02d
SA (1.4mM)+PS (28mM)	1.15 $\pm$ 0.2ab	6.42 $\pm$ 0.2c	20.77 $\pm$ 0.89b	39.56 $\pm$ 2.30b

Notes: Means followed by different letters within a column are significantly different according to the paired bootstrap test at 5% significance level.

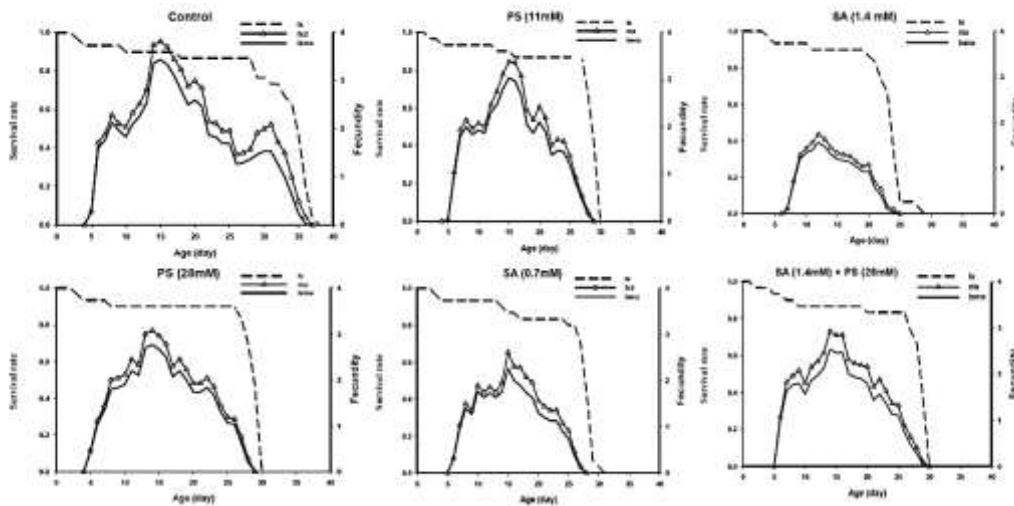
PS= Potassium sulfate, SA= Salicylic acid

The age-stage specific survival rate ( $s_{xj}$ ) represents the probability that a nymph of *M. rosae* will survive to age  $x$  and stage  $j$  (Fig. 1). The variable development rates among individuals in the cohort resulted in an overlapping of the stage specific survivorship curves. The highest survival rate of this pest was observed in the nymphal stage. The age-stage specific survival rate ( $l_x$ ) curve of *M. rosae* showed that the survival rate of this pest declined gradually with increasing the age of the aphid. The death of the last female on control, PS (11 mM), PS (28 mM), SA (0.7 mM), SA (1.4 mM), and SA (1.4 mM) + PS (28 mM) treatments occurred at the days of 38, 30, 30, 31, 29 and 30, respectively (Fig. 2). Based on the age-specific fecundity of population ( $m_x$ ) curves, the aphid started reproduction on day 5 on control, PS (11 mM), and PS (28 mM) treatments, on day 6 on SA (0.7 mM) and SA (1.4 mM) + PS (28 mM)

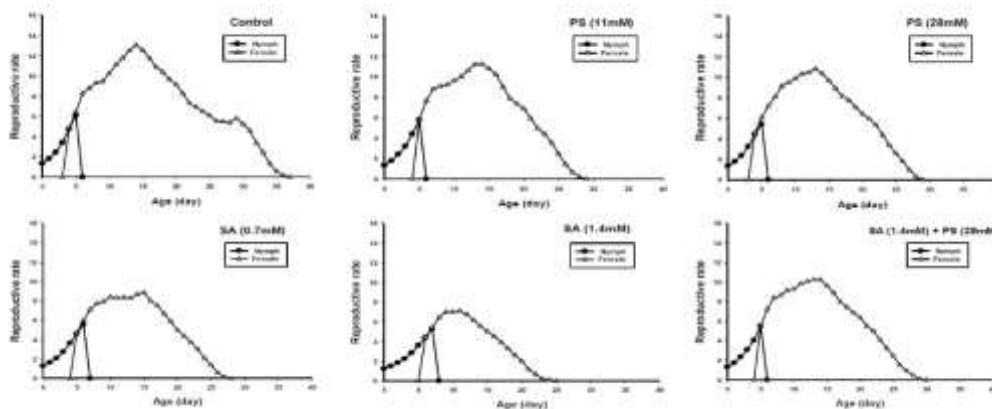
treatments, and on day 7 on SA (1.4 mM) treatment (Fig. 2). The age-specific fecundity ( $m_x$ ) and the age-specific maternity ( $l_x m_x$ ) peaked at the age of 15 d on control, PS (11 mM), and SA (0.7 mM), 14 d on PS (28 mM) and SA (1.4 mM) + PS (28 mM), and 12 d on SA (1.4 mM) treatments (Fig. 2). The contribution of an individual of age  $x$  and stage  $j$  to the future population is shown by the reproductive value ( $v_{xj}$ ) (Fig. 3). The major peaks in reproductive values of females on control, PS (11 mM), PS (28 mM), SA (0.7 mM), SA (1.4 mM), and SA (1.4 mM) + PS (28 mM) treatments were at 13.06 d, 11.25 d, 10.79 d, 8.86 d, 7.09 d, and 10.27 d, respectively (Fig. 3). The age-stage life expectancy ( $e_{xj}$ ) (where  $x$  is the age and  $j$  is the stage) shows the expected lifespan for an individual of age  $x$  and stage  $j$  (Fig. 4). The life expectancy of the aphid on different treatments decreased gradually with aging (Fig. 4).



**Fig. 1.** Age-stage specific survival rate ( $s_{xj}$ ) of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)



**Fig. 2.** Age-specific survival rate ( $l_x$ ), age-specific fecundity ( $m_x$ ), and age-specific maternity ( $l_x m_x$ ) of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)



**Fig. 3.** Age-stage reproductive value ( $V_x$ ) of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

Based on the results, the lowest and highest values of both gross reproductive rate ( $GRR$ ) and net reproductive rate ( $R_0$ ) of *M. rosae* were achieved on SA (1.4 mM) and control treatments, respectively ( $P < 0.05$ ; Table 3). The intrinsic rate of natural increase ( $r_m$ ) of *M. rosae* ranged from  $0.202 \text{ day}^{-1}$  on SA (1.4 mM) treatment to  $0.298 \text{ day}^{-1}$  on control ( $P < 0.05$ ; Table 3). Similar trend was observed for the finite rate of

increase ( $\lambda$ ), being lowest on SA (1.4 mM) treatment and highest on control ( $P < 0.05$ ; Table 3). The mean generation time ( $T$ ) of the aphid was highest on plants treated with SA (0.7 mM) and SA (1.4 mM) and lowest on plants treated with PS (28 mM) ( $P < 0.05$ ; Table 3).

**Table 3.** Mean ( $\pm$ SE) life table parameters of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

Treatments	$GRR$ (offspring)	$R_0$ (offspring)	$r_m$ ( $\text{day}^{-1}$ )	$\lambda$ ( $\text{day}^{-1}$ )	$T$ (days)
Control	$68.28 \pm 1.39a$	$58.81 \pm 4.11a$	$0.298 \pm 0.007a$	$1.347 \pm 0.010a$	$13.67 \pm 0.19ab$
PS (11 mM)	$46.57 \pm 1.21b$	$41.53 \pm 2.87b$	$0.282 \pm 0.006ab$	$1.325 \pm 0.009ab$	$13.21 \pm 0.14bc$

Treatments	<i>GRR</i> (offspring)	<i>R<sub>0</sub></i> (offspring)	<i>r<sub>m</sub></i> (day <sup>-1</sup> )	<i>λ</i> (day <sup>-1</sup> )	<i>T</i> (days)
PS (28 mM)	45.27±0.82bc	40.74±2.58b	0.283±0.008ab	1.327±0.011ab	13.08±0.23c
SA (0.7 mM)	32.99±0.95d	28.87±2.18c	0.243±0.007c	1.275±0.008c	13.82±0.19a
SA (1.4 mM)	18.54±0.78e	16.53±1.25d	0.202±0.006d	1.223±0.008d	13.91±0.15a
SA (1.4mM)+PS (28 mM)	43.05±0.89c	36.92±2.80b	0.273±0.008b	1.314±0.010b	13.22±0.14bc

Notes: Means followed by different letters within a column are significantly different according to the paired bootstrap test at 5% significance level.

PS= Potassium sulfate, SA= Salicylic acid, *GRR*= gross reproductive rate, *R<sub>0</sub>*= net reproductive rate, *r<sub>m</sub>*= intrinsic rate of increase, *λ*= finite rate of increase, and *T*= mean generation time.

#### Chemical analysis of the extract

The GC-MS analysis of the methanolic extract of rose leaves is given in Table 4. Phytol (8.9%) (alcoholic diterpene), Methanamine, N-(diphenylethenylidene) (8.04), and Squalene (7.65) (tertipenoid) were the dominant compounds in leaf extracts of control plants. In leaf extract of PS (11 mM) treatment, main phytochemicals were Squalene (18.12%) and Phytol (11.62). The predominant compounds identified in leaf extract of PS (28 mM) treatment were 2,4-dimethyl-benzoquinoline (19.80%) (sesquiterpene compound), Galactitol (16.31%) (alcoholic sugar), and Squalene (14.44%). The main compounds detected in leaf extract of SA (0.7 mM) treatment were Squalene

(26.90%), 3,3-dimethyl-2-butanamine (21.87%) (an effective-primary metabolite in the synthesis of nitrogen), and B-d-galactopyranoside (10.95%) (flavonoids- derived compound). Squalene (30.89%), 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-dimethylpyrazol-1-yl) ethanone (19.19%) (flavonoids) and 9,12,15-Octadecatrienoic acid (11.07%) (linoleic acid: fatty acid) were detected as major constituents in SA (1.4 mM) treatment. The most important compounds identified in leaf extract of SA 1.4 mM + PS 28 mM treatment were Squalene (41.77%) and 1,2-benzenedicarboxylic acid (10.32) (one of the most important flavonoids).

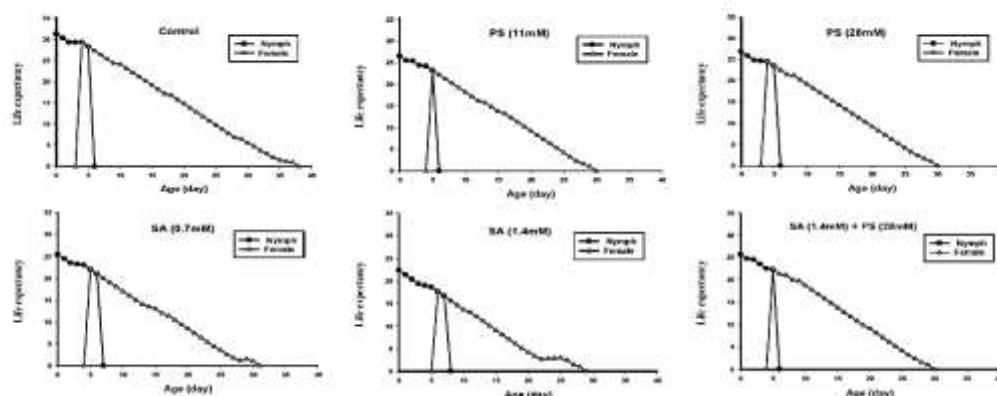


Fig. 4. Age-stage specific life expectancy ( $e_{xj}$ ) of *Macrosiphum rosae* (L.) on rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

Table 4. Components determined in methanolic extract of rose plants treated with Salicylic acid (SA) and Potassium Sulfate (PS)

S.No.	Phytochemical constituents	Retention time (min)	Area (%)
<b>Control</b>			
1	1-Dodecanol	12.073	3.55
2	Butanoic acid	14.233	6.62
3	2-Butanol, 1-(dimethylamino)-	14.787	4.50
4	*Phytol	18.847	8.9
5	Phthalic acid	22.492	4.78
6	*Squalene	24.530	7.65
7	*Methanamine, N-(diphenylethenylidene)-	26.649	8.04
<b>PS (11 mM)</b>			
1	[1,4,7]Trioxonane	22.814	4.42
2	*Phytol	36.734	11.62
3	9,12,15-Octadecatrienoic acid	37.195	5.12

4	1,2-Benzenedicarboxylic	43.902	2.16
5	*Squalene	47.940	18.12
<b>PS (28 mM)</b>			
1	*Galactitol	11.764	16.31
2	B-d-galactopyranoside	13.371	5.84
3	4-methyl decane	13.760	5.46
4	n-hexadecanoic acid	17.384	3.38
5	1,2-benzenedicarboxylic acid	22.501	4.86
6	*Squalene	24.530	14.44
7	Quinoline	26.649	5.51
8	*2,4-dimethyl-benzoquinoline	28.264	19.80
<b>SA (0.7 mM)</b>			
1	*3,3-dimethyl-2-butanamine	11.688	21.87
2	*B-d-galactopyranoside	13.739	10.95
3	3-methyl-3,5-cyanoethyl tetrahydro-4-Thiopyranone	19.079	3.66
4	*Squalene	24.530	26.90
5			
<b>SA (1.4 mM)</b>			
1	.beta.-D-Glucopyranoside	24.159	6.49
2	isoPhytol	31.512	3.21
3	n-Hexadecanoic acid	33.922	3.61
4	Phytol	36.734	3.26
5	*9,12,15-Octadecatrienoic acid	37.204	11.07
6	*Squalene	47.936	30.89
7	4-Amino-3-methoxy-pyrazolo[3,4-d]pyrimidine	51.987	9.03
8	*2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-dimethylpyrazol-1-yl)ethanone	54.896	19.19
<b>SA (1.4 mM)+PS (28 mM)</b>			
1	2-methyl-5-ethyl phenol	18.754	5.75
2	Thymol	19.016	3.52
3	n-hexadecanoic acid	33.922	3.10
4	9,12,15-buthyl-octadecatrienoate	37.203	6.69
5	*1,2-benzenedicarboxylic acid	43.902	10.32
6	*Squalene	47.936	41.77

\* Dominant compounds in leaf extracts

## Discussion

Our results provide strong evidence that SA and PS play a crucial role in influencing the life table parameters of *M. rosae*. Both SA and PS treated rose plants negatively affected the performance of the aphid compared to untreated control plants. Among different treatments, variation in SA concentration tended to have a significant effect on *M. rosae*. Aphids on rose plants sprayed with SA (1.4 mM) had the longest nymphal period and the lowest fecundity; while the shortest developmental time and highest fecundity of the aphid were observed on control plants. The lowest values of net reproductive rate ( $R_0$ ), intrinsic rate of natural increase ( $r_m$ ), finite rate of increase ( $\lambda$ ), and the longest values of mean generation time ( $T$ ) of *M. rosae* were also observed on plants that were treated with SA (1.4 mM). The  $r_m$  can be used as a valuable parameter for evaluating the pest performance on different food-related conditions (Mardani-Talae et al., 2016). It is a reflection of survival and fecundity of the pest, as well as generation time (Southwood and Henderson, 2000). The lower  $r_m$  value of *M. rosae* on SA (1.4 mM) may

be related to poor fecundity and lower survivorship of the aphid on this treatment. SA is a phenolic compound that has a key role in minimizing the infestation of insect pests. Several studies have been documented that SA have decreased the population of many aphid species. For example, Pettersson et al. (1994) reported that cereal crops treated with a slow release formulation of 1% methyl salicylate were avoided by *Rhopalosiphum padi* L. (Hemiptera, Aphididae). Elhamahmy et al. (2016) demonstrated that the foliar application of SA on canola plants was an effective treatment to reduce the *Brevicoryne brassicae* L. (Hemiptera: Aphididae) population. Similarly, Thakur et al. (2016) pointed out that the foliar application of SA was an effective way against mustard aphid, *Lipaphis erysimi* Kalt through positive modulation in activities of defense proteins. Three major groups of secondary metabolites in plants based on their biosynthetic pathway are nitrogen-containing compounds, phenolic compounds, and terpenes (Fang et al., 2011). In this study, GC-MS analysis showed that flavonoids and terpenoids were the common

phytochemicals in the leaf extracts of the tested treatments and among the identified compounds, Squalene and Phytol were detected in all treatments. Phytol, a di-terpene compound, is a hydrophobic tail of chlorophyll which helps them to anchor in thylakoid membrane and play as a photosynthetic pigment (Davies, 1995). In addition, attachment of the phytol tail completes the process of chlorophyll biosynthesis (Ahmed et al., 2017). During the plant growth process, when chlorophyll is decomposed in the plant, Phytol is immediately produced (Davies, 1995). Here, high amount of Phytol in the extract of control plants may be due to the fast degradation of the chlorophyll in rose leaves because of higher population of *M. rosae*. Squalene is a linear triterpene synthesized in plants and animals, which acts as a precursor for the synthesis of secondary metabolites (Ghimire et al., 2016). Furthermore, it is used to form important molecules such as  $\beta$ -sitosterol, campesterol, and stigmaterol, which are precursors of hormones involving in growth and plant adaptation to biotic stress (Nguyen et al., 2013; Azalia Lozano-Grande et al., 2018). According to the results, the highest amount of Squalene was observed in SA (1.4 mM) and SA 1.4 mM + PS 28 mM treatments. Furthermore, 2-(1,3-benzothiazol-2-ylsulfanyl)-1-(3,5-dimethylpyrazol-1-yl) ethanone, as a flavonoid compound, was found in high amount in SA (1.4 mM) treatment. According to Taiz and Zeiger (2006), flavonoid compounds could mediate plant growth and defense response against insects and microbes. Phytochemical studies have shown that the main constituents of *Rosa* species are flavonoids, terpenoids, phenolic acids, and other phenolics (Nowak and Gawlik-Dziki 2007; Bitis et al., 2017). It has also been reported that various extracts and essential oils obtained from different *Rosa* spp. have significant antioxidant and antimicrobial activities (Bitis et al., 2010). Therefore, it seems that decreasing the quality of rose plant, which is caused by increasing the level of secondary metabolites such as flavonoids and terpenoids in SA-treated plants, may be responsible for less performance of *M. rosae* on rose plants.

In the present study, when *M. rosae* reared on plants that were treated with PS, some of its life table parameters (such as  $R_0$ ,  $r_m$  and  $\lambda$ ) decreased compared to control, but increased compared to SA treatments. This trend was also observed when mixed application of SA and PS were used, which implies that SA was more effective in pest reduction than PS. Furthermore, because of less performance of the aphid on SA treatment compared to mixed treatment, its

single application can be recommended on rose plants. According to Sarwar (2012), the reduction of pest damage in plants treated with K may be attributed to the synthesis of defensive compounds against insect pests. These studies are in accordance with the results of the present study, because besides the flavonoids and terpenes in PS-treated plants, some chemicals like 2,4-dimethyl-benzoquinoline, which has a defensive role in plants (Jeon and Lee, 2014) were detected in high amount in PS (28 mM) treatment.

## Conclusions

Based on the obtained results of the present study we showed that the application of SA (1.4 mM) increased the levels of secondary metabolites in rose leaves, and thereby decreased the life table parameters of *M. rosae*. Hence, the findings of the present study can be used as a complementary method to improve the pest management strategies, and to trigger resistance in rose shrubs in urbanized spaces and commercial rose breeding for *M. rosae*.

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## Conflict of interest

No potential conflict of interest was reported by the authors.

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