

## Determination of Salicylic and Jasmonic Acids's Median Lethal Concentration and its affecting in *Tetranychus urticae* Life cycle on Eggplant Leaves

Yusra Jamal Talib 

Marshes research Centre- University of Thi-Qar, Iraq.

Email: [yosra.iraq83@gmail.com](mailto:yosra.iraq83@gmail.com)

### Abstract

The current study was conducted to determine the efficiency of salicylic acid concentrations 0, 0.5, 0.75 mM and jasmonic acid concentrations 0, 5, 50 and 100 $\mu$ M in the adult *T. urticae* life stages. In addition to determining the median lethal concentration for both acids. This study was applied using the eggplant leaves under laboratory conditions. The results showed that the salicylic acid concentrations significantly influenced the adult longevity and life cycle of the mite *T. urticae*. The control treatment recorded the highest adult longevity and life cycle periods of 10.33 and 18.67 days, respectively. In contrast, the adult longevity decreased to 7 days when treated with a 0.75 mM concentration. While exposed to 0.5 mM of acid, the average life cycle period scored 12.67 days, significantly different from other salicylic acid concentrations. The lower acid concentrations were more effective in shortening the adult longevity and life cycle. The statistical study revealed the substantial impact of different concentrations of jasmonic acid on the adult longevity, egg stage period, and life cycle of the mite *T. urticae*. However, the control group registered the longest average adult longevity and the shortest egg stage period, with values of 10.33 days and 1 day, respectively. Moreover, jasmonic acid is most effective at decreasing the adult longevity, with the individual rearing on leaves that treated with 100 $\mu$ M of jasmonic acid reaching a minimum longevity of 6.33 days. It was also noted that the highest efficacy in prolonging the egg stage period resulted from 50 and 100 $\mu$ M jasmonic acid concentration, reaching a maximum of 2.67 days. Jasmonic acid. The lethal concentration of salicylic acid was found to be 1.53 mM, while the lethal value of jasmonic acid was reported to be 43.75  $\mu$ M.

As a result of the efficiency of these compounds in influencing pests, this study aimed to determine the several concentrations toxicity of salicylic acid and jasmonic acid in the two spotted spider mite *T. urticae*'s life stages. In addition to calculate the lethal concentrations of salicylic and jasmonic acids on adult *Tetranychus urticae*, two-spotted spider mites.

**Keywords:** Egg Plant, Jasmonic acid, Median lethal concentration, Salicylic acid, two spotted spider mite.

### I. Introduction:

The Tetranychidae family, particularly the *Tetranychus urticae* species, is a prevalent polyphagous pest that infests agricultural crops worldwide, including both open-field and protected cultivation environments (Kamaluddin *et al.*, 2020).

The two spotted spider mite *Tetranychus urticae* feeds on the lower surface of the leaves by perforating the host plant tissue through epidermal cells, spongy mesophyll tissue and lowest parenchyma of the leaves lower surface by inserting their pointed chelicerae into the plant leaf tissue to a depth of 70-120  $\mu$ m (Wang *et al.*, 2016). The cell contents are then absorbed and thus the chlorophyll content, photosynthesis, CO<sub>2</sub> consumption are reduced transpiration (Fasulo and Denmark, 2000). The metabolic disorder leads to reduced growth and flowering and thus reduced yield and this represents the mite direct damage to the plant (Yu *et al.*, 2019).

Mothes and Seitz (2004) stated that the mite's digestive system has well-developed salivary glands and that the secreted saliva has a role in nutrition and consists of mucous material, glycoprotein, liquid materials and analyzed enzymes. Moreover, many members of the genus *Tetranychus*, including the type

*T. urticae*, inject part of their saliva into the plant tissue during their feeding, causing severe damage to the plant because this saliva contains toxic substances toxin (Steinkraus *et al.*, 2005).

As for its indirect damage due to its weaving of spider mite webs by means of ventral flirtations connected to silk production glands, all species of the genus *Tetranychus* can silk tissue secretion, in addition to some species, such as *T. urticae*, which makes several layers of dense silvery tissue on the leaves lower surface, making the leaf veins supports on which the tissue rests. Furthermore, the silk tissue is a protective cover for aspider mite colonies that are under it from their natural enemies, rain and pesticides, and uses it to move from one place to another when severe infection, as well as, it's important in the individuals reproduction, by its used as an attraction for males by *T. urticae* second phase (Murase *et al.*, 2017).

The two spotted spider mite *Tetranychus urticae* population dynamics changes from year to year Due to several factors, including environmental factors such as temperature, food quality, soil type, proximity to plant hosts, natural enemies and the used chemical pesticides (Jokar, 2022; Tiftikçi *et al.*, 2022). Therefore, recent studies have turned to the search for more effective control methods, such as the phytohormones use, as salicylic and jasmonic acids (Kumari and Singh, 2022).

Fazam *et al.* (2021) confirmed that salicylic acid have an important role in pest control as a low-impact product, when the direct acid treatment on the leaf-cutting ants workers *Atta sexdens rubropilosa* and *Acromyrmex crassispinus* have highest mortality ratio, which reached 100%, this result are providing opportunities for use in integrated ant control programs in nests.

Methyl Jasmonate (MeJA) was effective after 48 hours treatment second-stage juveniles (J2) individuals of root-knot nematode *Meloidogyne incognita* (Schouteden *et al.*, 2016).

## II. Material and method:

### 1- Preparation of the two-spotted Spider mites laboratory culture:

Barcelona eggplant seedlings were obtained and planted in 2 kg plastic pots filled with a mixture of soil and Bitmos. The pots were infected with a spider mite artificial infection to ensure colonies free of effects of chemical pesticides and natural enemies, so that the resulting individuals could be used in further studies.

### 2- Laboratory incubation of two-spotted spider mites:

According to Kondo and Takafuji (1985), the Leaf Disc method was used to raise spider mites in a lab setting. This involved placing a 5-cm-square eggplant leaf inside a 9-cm-diameter, 1.5-cm-high plastic dish that contained a layer of cotton that had been previously moistened with water and was kept moist as needed. To ensure that the cotton remained covered and that there were no gaps between the cotton and the leaf, thin forceps were used to lift the cotton and cover the leaf edges. In order to ensure that the leaf stays alive for as long as possible, Tangl foot substance—a concoction of vaseline, Canada balsam, and citronella oil—was applied around the edges of the leaf to keep spider mites from sinking to the bottom. A piece of organza cloth that was secured to the dish with a rubber band sealed the entrance.

### 3- Examination of the amounts of salicylic and jasmonic acids that are effective on eggplant leaves during the life stages of the two-spotted spider mite *Tetranychus urticae* in a laboratory setting:

The salicylic acid concentrations were seen to be 0 (distilled water alone), 0.5, 0.75, and 1 mM, whereas concentrations of jasmonic acid were observed to be 0 (distilled water only), 5, 50, and 100µM. Eighty plastic petri dishes were used, and at the bottom of each was a layer of moistened medical cotton. The leaves of the Barcelona eggplant variety, which was grown in the plastic house, were brought in and cleaned with distilled water. The leaves were then allowed to dry in the laboratory's atmosphere, and it was verified that the leaves were free of dust, natural enemies, and pests. According to paragraph 2, the acork borer cut the flat portions of the leaf blade, avoiding the midrib, and formed the flat parts into disc shapes with a diameter of five centimeters (one disc per dish).

The plates were separated into eight groups of ten dishes each, and salicylic and jasmonic acid concentrations were applied to each group. A total of ten adults, both male and female, were placed to the eggplant discs in each plastic dish using a unique brush made of one hair. They were supposed to be one day old (as per paragraph 1), then they were released to get married and lay eggs. The adults were



removed after there were enough eggs to observe. 10 eggs leaved in every dish (more eggs are removed by micro needle). The dishes were examined by dissecting anatomy microscope, below periods are recorded:

- Egg stage (from egg laying to before egg hatching).
- Larval stage (from egg hatching to before nymph enhancing).
- Nymphal stage (from nymph enhancing to before adult enhancing).
- Longivity (from adult enhancing to adult mortality).
- Life cycle (from egg laying to started adult enhancing).

#### 4- Examination of the lethal concentrations of salicylic and jasmonic acids on adult *Tetranychus urticae*, two-spotted spider mites, on Eggplant leaves in a laboratory setting :

Salicylic acid concentrations were attended of 0 (distilled water only), 0.5, 1, 1.5, 2 and 2.5 mM, jasmonic acid concentrations were attended of 0 (distilled water only), 5, 50, 100, 200 and 250  $\mu$ M.

According to paragraph 2, sixty plastic petri dishes were used. Ten adult individuals were transferred to the eggplant discs in each plastic dish using a unique brush made of one hair, and they were expected to age in about twenty-four hours (by rearing a sufficient number of nymphs from colony rearing and watching them until adults appear). The meals were split up into twelve groups, each of which included five dishes. The Salicylic and jasmonic acid concentrations were applied to each group.

After a day, the dishes were inspected under a dissecting anatomy microscope, and the number of living and dead individuals at each time was noted. Following the course of therapy, mortality rates were assigned to specific people (Amiri, 2008; Mehdi *et al.*, 2017). The Abbott equations were used to adjust the mortality rates (Püntener, 1981):

$$\% \text{ Corrected mortality} = \frac{(\% \text{ mortality in treatment} - \% \text{ mortality in control})}{100 - \% \text{ mortality in control}} \times 100$$

Then converted corrected mortality ratio values to angular conversion and finding Probit values with log of concentration to maked toxicity line and finding LC50 according to Finney (1971).

#### 5- Statistical Analysis:

The experiment (in paragraph 3) are designed using Complete Randomized Design (C.R.D.), it adopted a test with Least Significant Design (L.S.D.) to compare averages at probability level of 0.01 and the statistical program used Statistical Package for the Social Science (SPSS) Version 21 in data analysis. The experiment (in paragraph 4) are designed using Complete Randomized Design (C.R.D.) additional to making Probit Analysis by Ldp Line application after converting mortality ratio values to corrected mortality ratio values to angular conversion to normalize heterogeneous value variations and finding Probit values.

### III. Result and Discussion:

#### I: Examination of the concentration of salicylic and jasmonic acids effective on *T. urticae* life stages on eggplant leaves in a laboratory setting:

##### 1- Salicylic acid concentrations' effects on *T. urticae* life stages:

The results shown in table 1 showed that the effect of salicylic acid concentrations on the duration of the lifespan and life cycle of the mite *T. urticae* on eggplant leaves in the laboratory. As the results of the statistical analysis, there is a significant effect of concentrations of salicylic acid on the duration of both the lifespan of the adult and the life cycle of the mite *T. urticae*. The highest duration of adult lifespan and life cycle was 10.33 and 18.67 days, respectively. In the control treatment, the lowest average adult lifespan was 7 days when treated with a concentration of 0.75 mM, and the lowest average life cycle duration was 12.67 days when treated with a concentration of 0.5 mM of acid, having a notable distinction from the remaining concentrations of acid. Salicylate, noting that lower concentrations of the acid were more efficient in reducing the life span of adult animals and the life cycle. Salicylic acid concentrations did not record any significant effect on the duration of the larval and nymphal stages and the incubation of eggs.

Table1 : Effect of Salicylic acid concentrations on Eggplant leaves during different <i>T. urticae</i> 's life stages					
parameters	Period (day)				
	Longevity	Larval stage	Nymphal stage	Egg stage	Life cycle
Concen.(mM)					
0.5	7.33	1.33	2.67	1.33	12.67
0.75	7.00	1.33	3.00	2.33	13.67
1	7.67	2.33	3.33	2.67	16.00
Control	10.33	2.67	3.33	2.67	18.67
LSD(0.05)	0.94 *	1.09 <sup>NS</sup>	0.94 <sup>NS</sup>	1.09 <sup>NS</sup>	2.31*

These results were in agreement with the study of (Ahmed, 2018). They mentioned that the using of Potassium humates, Potassium silicates, Salicylic acid, and methyl jasmonate) can improve the resistance of two varieties of tomato plants. Supper-gekal and Salymia (65010) confirmed that the duration of the total immature stages and the life cycle of *T. urticae* females was longer and the longevity of the adult female was shorter when reared on the leaves of both tomato plants treated with Salicylic acid and methyl jasmonate compounds compared to those reared on the leaves of untreated tomato plants. **Fhaid (2013)** verified that different doses of salicylic acid impact the life roles of the two-spotted mite *Tetranychus urticae* (Koch) on eggplant plants. The acid concentration of 400 mg/L was confirmed to be the most effective in affecting the mite's behavior, resulting in the lowest percentage of egg hatching. The female Adults had the highest mortality rate. The individual mortality due to these substances potentially damaging cell protoplasm by altering cell proteins and inducing deposition.

Protein precipitation, particularly in the middle gut's epithelial cells, and these protoplasmic toxins frequently combine to produce heavy salts that cause fatalities (Gerges and Amin, 1987). Another study illustrates that the salicylic acid may have a repellent effect on mite individuals (Pulga et al., 2020). Phenolic compounds and their derivatives, for example, salicylic acid and acetylsalicylic acid, negatively impact certain aspects of the life of certain insect species by suppressing oviposition, acting as an antifeedant, and inhibiting growth (Conrath et al., 2015).

The artificial food for the larvae of the peach fruit fly (PFF) *Bactrocera zonata* and the Mediterranean fruit fly (MFF) *Ceratitis capitata* had various effects on the life cycle of the Mediterranean fruit fly. This included a decrease in pupation rates to 70.5% and 75.8%, emergence rates to 97% and 94%, egg-laying period to 16 and 18.3 days, male adult longevity to 13 and 18.3 days, and female adult longevity to 20.4 and 23.6 days for broodfish, respectively (El-khayat et al., 2021).

## 2- Effect of jasmonic acid concentrations on life stages of *T. urticae*:

Table 2 shows the impact of different doses of jasmonic acid on the duration of life stages and the life cycle of *T. urticae* mites on eggplant leaves in a laboratory setting. The results of statistical analysis confirmed that jasmonic acid concentrations had significantly affected of the longevity of adult, eggs stage and life cycle periods of *T. urticae*. The control group had the longest average longevity and the shortest eggs stage period, at 10.33 and 1 day, respectively. However jasmonic acid application lead up to decrease of adult longevity, with individuals rearing on leaves treated with 100µM of jasmonic acid having adult longevity as low as 6.33 days. The concentrations of jasmonic acid were most effective in eggs stage period for the individuals rearing grown on leaves treated with 50 and 100µM of jasmonic acid had the longest eggs stage period was 2.67 days, with a significant difference than the other acid concentrations.

The control treatment had the longest life cycle periods at 10.33, 2.67 and 18.33 days respectively, whereas the 100µM acid concentration had the shortest longevity at 6.33, 1 and 13 days respectively. Jasmonic acid concentrations had no significant effect in the periods of the larval and nymphal stages of *T. urticae*.

**Table2 : Effect of Jasmonic acid concentrations on Eggplant leaves during different *T. urticae*'s life stages**

parameters	Period (day)				
	Longivity	Larval stage	Nymphal stage	Egg stage	Life cycle
Concen.( μM)					
5	9.67	1.67	3.33	2.33	17.00
50	8.00	1.00	3.00	2.67	14.67
100	6.33	1.67	2.33	1.00	13
Control	10.33	2.00	3.33	2.67	18.33
LSD(0.05)	0.94*	0.77 <sup>NS</sup>	1.33 <sup>NS</sup>	0.94*	1.631*

This study's results were similar with some studies that confirmed the negative effect of jasmonic acid treatment on insect and mite populations.

Warabieda and Olszak (2010); Rohwer and Erwin (2010); Miyazaki *et al.*, (2014) found that the treatment with jasmonic acid reduced the two spotted spider mite *T. urticae* reproduction rate by reducing the eggs laid average number for the female. Furthermore, the jasmonic acid increased accumulation in the tomato leaves infected causes to delay nymphs whitefly development *Bemesia tabaci* (Zhang *et al.*, 2018).

The jasmonic acid efficiency may be return to the inhibition of the activity of the protease enzyme in the insects' and mites' digestive systems by protease inhibitors (PIs).

The protease inhibitors are a factors that, reducing or inhibiting necessary protein synthesis for their growth, development and reproduction (Meriño-Cabrera *et al.*, 2018).

**II: The LC50 estimation for Salicylic acid and Jasmonic acid on *T. urticae* on the Egg Plant under laboratory Conditions.**

**1- Determination of the LC50 estimation of Salicylic acid:**

The results in table 3 and figure 1 revealed that the LC<sub>50</sub> level for the Salicylic acid against the red mite *T. urticae* adults. The value is estimated at 1.53mM. This LC<sub>50</sub> means that 50% of the red mite has died using 1.5 mM. This low concentration indicates that the Salicylic acid have a high-toxicity level.

**Table 3 : Results of the lethal concentration of Salicylic acid for the determination of LC<sub>50</sub> after treated adults of two spotted spider mite *Tetranychus urticae* (n=10)**

	Conc. (mM)	Conc. (*10)	Log. (Conc.*10)	Treated	% Mortality	% Corrected	Probits	Slope	LC <sub>50</sub> (mM)
1	0.5	5	0.70	10	77.71	66.99	5.44	-0.91 ± 0.23	1.53
2	1	10	1	10	59.01	56.64	5.17		
3	1.5	15	1.18	10	28.78	50.31	5.01		
4	2	20	1.30	10	6.15	45.81	4.89		
5	2.5	25	1.40	10	90	42.35	4.81		





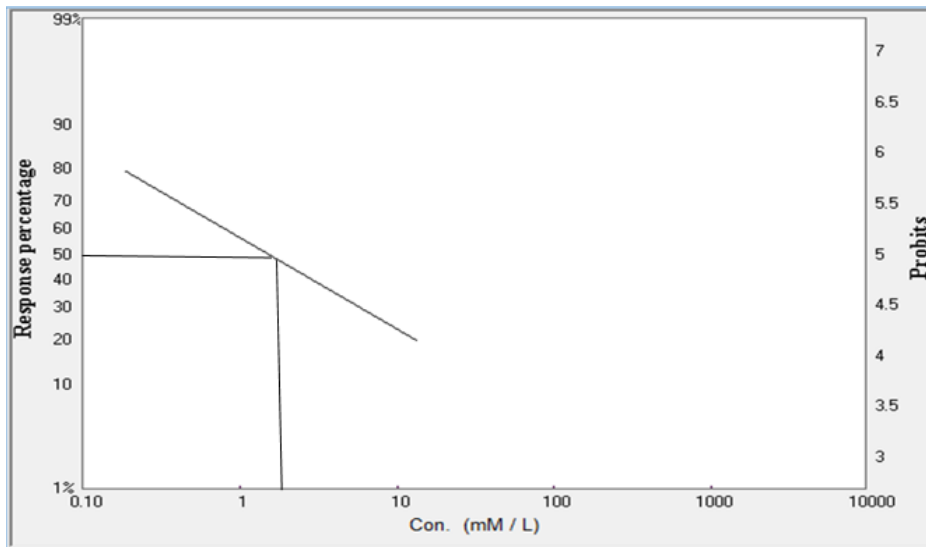


Figure 1: Plot of Log Concentration versus probits for calculation of LC<sub>50</sub> of Salicylic acid.

Salicylic acid, ethylene, and Jasmonic acid are all crucial to the activation in the plant defense mechanism via the single transduction pathways (Shivaji *et al.* 2010). However, jasmines cause plant defensive responses by inducing the secretion of extra floral nectar (EFN), volatile organic compounds (VOCs), richome formation, proteinase inhibitors (PIs), antioxidative enzymes, and v, alkaloid production (Mao *et al.* 2007). In contrast, the using high concentrations in salicylic acid (SA) (0 mg·L<sup>-1</sup>, 25 mg·L<sup>-1</sup>, 50 mg·L<sup>-1</sup>, 75 mg·L<sup>-1</sup> and 100 mg·L<sup>-1</sup>) resulted in the induce plant to biotic and abiotic stress, resulting in more resistance to the red mite (De Resende *et al.*, 2021). The results obtained are consistent with the study of Hassan *et al* (2020), they noticed that the SA caused a general reduced 26.71%.

## 2- Determination of the LC<sub>50</sub> of Jasmonic acid

The results of the Jasmonis's LC<sub>50</sub> analysis can be seen in table 4 and figure 2. It is apparent from this figure that the value of LC<sub>50</sub> is low to cause the mite mortality, this low concentration indicates that the jasmonic acid have a high-toxicity level. It is scored 43.75 μM. According to Pieterse *et al.* (2012), Jasmonic acid generally stimulates plant defenses against herbivores, such as spider mites (Salehipourshirazi *et al.*, 2021). Additionally, it was linked to priming defence responses generated by Jasmonic acid plants (Sakr, 2017).

	Conc. (μM)	Log. Conc.	Treated	% Mortality	% Corrected	Probits	Slope	LC <sub>50</sub> (μM)
1	5	0.70	10	36.93	27.56	4.40	<b>0.63 ± 0.09</b>	<b>43.75</b>
2	50	1.70	10	28.78	51.46	5.04		
3	100	2	10	59.22	58.99	5.23		
4	200	2.30	10	56.79	66.19	5.42		
5	250	2.40	10	90	68.40	5.48		

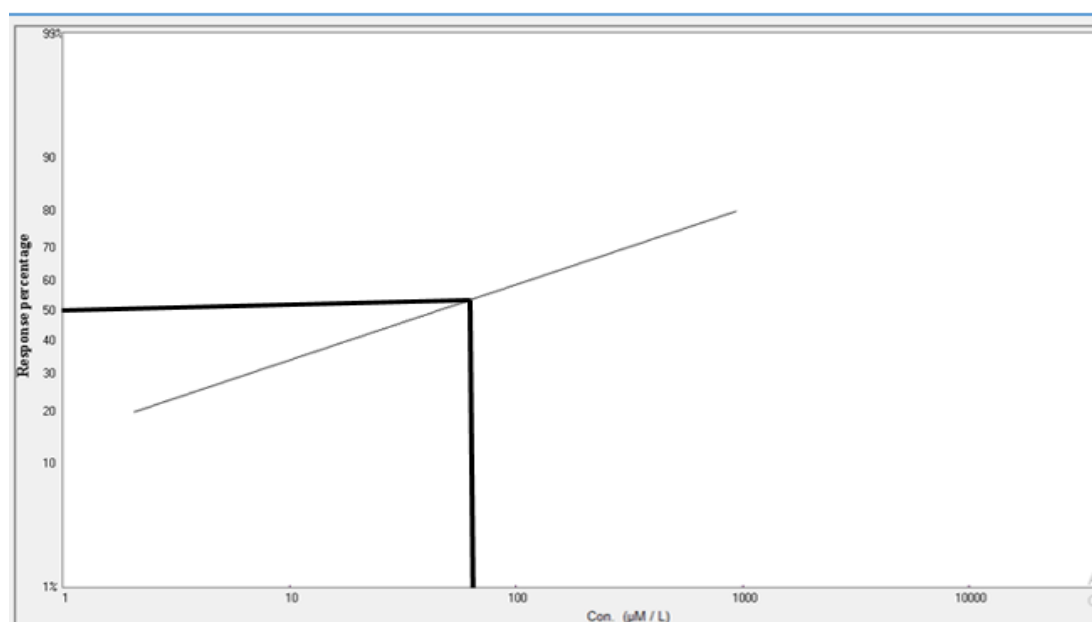


Figure 2: Plot of Log Concentration versus probits for calculation of  $LC_{50}$  of Jasmonic acid.

#### IV. Conclusions

This study contacted to that the immature stages of *T. urticae* females was longer, and the female's adult lifespan was shorter when reared on the leaves of eggplant plants treated with concentrations of salicylic acid and jasmonic acid compared to those reared on the leaves of untreated eggplant plants. Furthermore, under laboratory settings, the investigation was conducted to find the median lethal concentration of two regulator growth, salicylic and jasmonic acid, against the two spotted spider mite, *Tetranychus urticae* Koch. The results showed that both acids were harmful to adult *T. urticae* at low concentrations: 1.5 mM for salicylic acid and 43.75  $\mu$ M for jasmonic acid.

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