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Research Article

EFFICIENCY OF SOME SALYSALIC ACID CONCENTRATIONS ON MORTALITY OF TWO-SPOTTED SPIDER MITES *Tetranychus urticae* KOCH ON EGGPLANT

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Abstract

The current study was conducted to determine efficiency of Salysalic acid concentrations 0, 0.5, 0.75 and 1mM in the corrected mortality ratios of adult females and nymphs of the two-spotted spider mites *Tetranychus urticae* raised on leaves of Eggplant varieties Bowie, Barcelona and Jawaher over four time intervals of 12, 24, 36 and 48 hours. The results showed the significant effect of treatments and the interactions among them on the corrected mortality ratios for both stages adult females and nymphal. As for the interaction of the treatments of variety, concentration and time periods. with concentrations of Salicylic acid, eggplant varieties, time periods and overlap between the treatments in of the two-spotted spider mite *Tetranychus urticae*. The results showed that a gradual rise in the corrected mortality ratios for females by increasing the treatment time periods for all salicylic acid concentrations especially 48 hour on the three varieties, as it reached 100 %, as for the treatments effect on the corrected mortality ratios of the nymphs, the results showed that the lowest acid concentration (0.5 mM concentration) in the advanced time periods (36 and 48 hours) achieved the highest corrected mortality ratios of nymphs on the three varieties, which amounted to 100 %.

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1. Introduction

The two-spotted spider mites *Tetranychus urticae* Koch is affiliated to Class: Arachnidae, Sub class: Acari, Order: Acariforms, Sub order: Actinedida (Prostigmata), Family: Tetranychidae and Sub family: Tetranychinae (Krantz and Walter, 2009).The two-spotted spider mite species *Tetranychus urticae* is a common multi-host (polyphagous) member of the common red mite family Tetranychidae, additionally, it has the capacity to infest more than 1100 different plant species across more than 140 different plant families, including more than 300 species of plants with large economic value, such as vegetables and fruit (Islam, 2019; Ricardo *et al.*, 2019).

The Solanaceae family of crops are affected by the *T. urticae* species of mite, which Sharma and Pati (2011) observed also greatly damages greenhouse crops in India. Indicated by Srinivasan (2009), the mite infected the eggplant crop in the most of Asian and African countries.

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Metabolic defensive compounds are naturally present in plant tissues at low concentrations and inactive forms, but pest infestations cause them to become more focused (War *et al.*, 2012), Salysalic acid is one of these substances, which are plant hormones (War *et al.*, 2013; Siciliano *et al.*, 2015). Studies shown that applying these hormones to plants exogenously, such as by spraying them on their shoots, may increase their defense mechanisms and help the plants develop pest resistance (War *et al.*, 2011).

Bakr *et al.* (2020) confirmed that spraying 50 and 100 mg/L concentrations of Salicylic acid on the bean seedling *Phaseolus vulgaris* L. was a clear effect with controlling of *Tetranychus urticae* population after 9 and 18 days of treatment, 100 mg/L concentration was the most efficient with reducing of mite population and the treatment with Salicylic acid with the right concentration and time can increase the resistance of bean plant to mite.

As a result of the efficiency of salysalic acid in influencing of all life stages of two-spotted spider mite *Tetranychus urticae*, so this study aimed to determine the toxicity of several concentrations of it in the adult stage (female) and nymphal stage of spider mites raised on the leaves of the varieties of an eggplant Bowie, Barcelona and Jawaher in the laboratory.

2. Materials and Methods

Preparation of the laboratory culture of two-spotted Spider mites

Eggplant seedlings of Bowie, Barcelona and Jawaher were obtained from the Palm Land Company produced by Vito-Spanish Company in 1/11/2020 and planted in plastic pots with a capacity of 2 kg containing mixed soil and Bitmos inside the laboratory and it was infected with aspider mite artificial infection to ensure colonies free of effects of chemical pesticides and natural enemies, so that the resulting individuals could be used in subsequent experiments.

Laboratory raising of the two-spotted Spider mites

The Leaf Disc method was adopted according to Kondo and Takafuji (1985), modified by AL-Jboory (1999) and described in Mehdi and Omran (2006) and Fhaid (2013) in raising spider mites in the laboratory, including that placed 5 cm^2 eggplant leaf, inside a plastic dish (diameter of 9 cm and a height of 1.5 cm) that contains a layer of cotton (previously moistened with water and it is moistened whenever needed), by thin forceps were used to lift the cotton and cover the leaf edges so as not to leave any opening between the leaf edges and the cotton and to ensure that the leaf remains for the longest period in order to preserve its vitality, in addition, the leaf edges were surrounded by Tangl foot substance (a mixture of vaseline, canada balsam and citronella oil) to prevent the spider mites from escaping to the bottom. The dish opening was closed by an organza cloth piece which was fixed on the dish by a rubber band.

Evaluation of the effectiveness of several concentrations of Jasmonic acid in mortality adult females of *Tetranychus urticae* on the eggplant leaves

Salysalic acid concentrations was obtained of 0 (water only), 0.5, 0.75 and 1mM. It was used 60 plastic petri dishes, placed at the base of each dish a layer of medical cotton moistened with water, brought eggplant leaves of eggplant varieties grown in the plastic house and washed with distilled water, it dried up by exposing it to the atmosphere of laboratory and it was confirmed that there was no any Spider mite stage on it or any other pests, natural enemies or dust and the flat areas of leaf blade were cut while avoiding midrib into disk shapes with a diameter of 5cm by a Cork borer (at the rate of one disk per dish).

The dishes were divided into three groups of 20 dishes each, the first group placed disks of eggplant leaves of Bowie variety, the second group put disks of eggplant leaves of Barcelona variety and put in the dishes of the third group disks of eggplant leaves a variety of Jawaher.

Each of three groups was divided into four groups, each with 5 dishes (each group was treated with concentration of salysalic acid). 10 adult females were transported by a special brush consisting of one hair to the eggplant disks in each plastic dish and should be one age approximately 24 hours (by raising sufficient numbers of nymphs obtained from colony rearing and monitoring them until adults appearing).

Salysalic acid concentrations are sprayed with a 5 ml hand spray that gives a constant volume of solution of 0.5 ml at a time, take into account use of a special Sprinkler for each concentration to avoid the impact of possible residues if repeated use.

The dishes were examined using a dissecting anatomy microscope after 12, 24, 36 and 48 hours, number of live individuals and dead individuals at a time were recorded. Mortality rates were adopted for individuals after the treatment was made (Amiri, 2008; Mehdi *et al.*, 2017). Mortality rates were corrected according to Orell and Schneider equations (Püntener, 1981):

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

Evaluation of the effectiveness of several concentrations of Salysalic acid in Mortality nymphal stage of *Tetranychus urticae* on the eggplant leaves

Same method was adopted in the previous paragraph, taking into account that nymphs are transported in each plastic dish, it is at same age about 24 hours, was obtained from colony rearing by raising sufficient individuals of larvae and monitoring them until nymphs appeared.

Statistical Analysis

The experiments are designed using Complete Randomized Design (CRD) for Factorial Experiments with three factors, it adopted a test with Least Significant Design (LSD) to compare averages at probability level of 0.05 and the Statistical program used Statistical Package for the Social Science (SPSS) Version 21 in data analysis after converting corrected mortality ratio values to angular conversion to normalize heterogeneous value variations.

3. Results and Discussion

Evaluation of the effectiveness of Salysalic acid concentrations in adult females of a two-spotted Spider mite *Tetranychus urticae* on the leaves of eggplant varieties

The results showed that the significant effect of all single treatments (Table - 1), namely the variety, concentration and time periods, as for salicylic acid concentrations, the highest mortality ratio was 97.5 % when treated with 0.75 mM concentration, and the concentration of 0.5 mM achieved the lowest mortality ratio at 93.51 %, the effect of Barcelona variety was positive in achieving of the highest of mortality ratio of 97.22 % while the lowest of mortality ratio in adult females that it was fed on Jawaher variety, which amounted to 93.51 %. As for the time periods, the highest mortality ratio of mite female was 100 % after 48 hours and the lowest mortality ratio was 87.41 % after 12 hours.

As for the bilateral interference treatments, their effect was significant for all, 0.5 mM concentration with Bowie and Barcelona varieties and 0.75 mM concentration with Jawaher variety out performed the highest mortality ratio at 100 %, while 0.5 mM concentration achieved the lowest mortality ratio of female fed on the Jawaher variety leaves at 80.53 %. As for the overlap between concentration and time, the results indicated that a direct increase in mortality ratio with increasing concentration and time periods, the highest mortality ratio reached 100 % when treated 0.75 and 1 mM concentrations after 36 and 48 hours, 0.5 mM concentration after 48 hours and The lowest was 83.33 % when treated with 1 mM concentration after 12 hours, as for the effect of the variety with time periods, the highest time period of 48 hours corresponded with the highest mortality ratio for the three varieties. At a time that Barcelona variety in the three highest time periods 24, 36, and 48. Bowie variety at 36 and 48 hours, and the Jawaher variety at 48 hours

succeeded in achieving the highest mortality ratio as it reached 100 %, Jawaher variety failed and achieved the lowest mortality ratio after 12 hours, which reached 85.56 %. As for the effect of the triple overlap among treatments, the results of the statistical analysis showed that an obvious significant effect of 48 hour time period with three varieties and concentrations in achieving the highest mortality ratio in adult females, which amounted to 100 %, as for the other the time periods with the varieties, their effect was uneven in achieving the highest mortality ratio while Jawaher variety with 12 hours' time period and 0.5 mM concentration the lowest mortality ratio, which amounted to 56.67 %.

Table - 1: Effect of Salysalic acid concentrations in adult females of *T. urticae* on leaves of eggplant varieties at different time intervals

% Corrected mortality ratios (averages)							
var.	Conc. (mM)	Time (hour) 12	24	36	48	var.×Conc.	Var.
Bowie	0.5	100	100	100	100	100	95.28
	0.75	86.67	90	100	100	94.17	
	1	76.67	90	100	100	91.67	
var.×Time		87.78	93.33	100	100		
Barcelona	0.5	100	100	100	100	100	97.22
	0.75	93.33	100	100	100	98.33	
	1	73.33	100	100	100	93.33	
var.×Time		88.89	100	100	100		
Jawaher	0.5	56.67	76.67	100	100	80.53	93.51
	0.75	100.00	100.	100	100	100	
	1	100.00	100.0	100	100	100	
var.×Time		85.56	92.22	96.27	100		
Conc. \ Time		Conc.×Time 12	24	36	48	Conc.	
0.5		85.56	92.22	96.27	100	93.51	
0.75		93.33	96.67	100	100	97.50	
1		83.33	96.67	100	100	95	
Time		87.41	95.19	98.76	100		
LSD (P≤0.05)		Var.(A)=1.09*		Conc. (B)= 1.09*		Time (C)=1.26*	
		A×B=1.88*				B×C=2.17*	
		A×C=2.17*				A×B×C= 3.76*	

Evaluate the effectiveness of Salysalic acid concentrations in the nymphal stage of a two-spotted Spider mite *Tetranychus urticae* on the leaves of eggplant varieties

The Table - 2 showed that the 0.5 mM concentration took the lead in achieving the highest mortality ratio of nymphs at 96.79 % and a significant difference from the other treatment and the lowest mortality ratio achieved when treated at 0.75 mM concentration that it was 55.16, as for the treatment variety was a significant effect on the mortality ratios of nymphs, and the highest corrected mortality ratio was 76.11 % on Barcelona variety, and unlike the Bowie variety, which had the lowest of nymph mortality ratio at 64.51 %, with regard to the time periods treatment, it had a significant effect, and the highest mortality ratio was after 48 hours of treatment, amounted to 83.33 %, while the lowest mortality ratio was after 12 hours and amounted to 57.54 %. For binary overlap treatments, their effect was significant for all, with regard to the variety concentration treatment, the 0.5 mM concentration treatment for nymphs that it fed on the leaves of the Bowie variety achieved the

highest mortality ratio of at 98.15 %, while the lowest mortality ratio was 27.66 % using the 0.75 mM concentration on nymphs that it fed on Jawaher variety leaves. The 0.5 mM concentration (lowest acid concentration) succeeded in that achieving the highest mortality ratio of nymphs at 100 % after 36 and 48 hours of treatment while the 1 mM concentration (highest acid concentration) failed and that achieved the lowest mortality ratio of nymphs at 30.25 % after 12 hours of treatment. As for the overlap between the variety and time, the Bowie variety distinguished in achieving the highest and lowest mortality ratio of nymphs after 48 and 12 hours of treatment, amounting to 88.89 and 50.77 % respectively. In relation to triple interference treatments, the results of statistical analysis showed that the 0.5 mM concentration with the time periods of 36 and 48 hours exceeded in achieving the highest mortality ratio that it was 100 % for nymphs fed on the three varieties, also the 1 mM concentration achieved that ratio and at the same time intervals of nymphs fed on the Jawaher variety with a significant difference from other treatments, as for the lowest mortality ratio was for nymphs fed on the Bowie variety with the 1 mM concentration of acid after 12 hours of treatment.

In general, it is noted from the above results that there is a positive proportionality between the time periods and mortality ratios of individuals, this result is consistent with previous studies carried out by many researchers, one such study was that of Fhaid (2013) in his lab study to show the effect of four salicylic acid concentrations 100, 200, 300 and 400 mg/L with three time intervals 24, 48 and 72 hours on the average mortality of larvae, nymphs and adults of the two-spotted spider mite *Tetranychus urticae* fed on the leaves of the eggplant plant, the concentrations used and time periods was a clear significant effect on nymphal and adult stages mortality ratios also it was observed that there was a positive proportion among the concentrations and time periods with mortality ratios, the mortality ratio increased by increasing the concentration and the time period, the highest mortality ratio of nymphs and adults using the 400 mg/L concentration was 40.89, 39.67 and 45.67 % for the first and second nymphs instar and adult females, respectively, while the lowest mortality ratio was recorded using the 100 mg/L concentration, which amounted to 22.84 and 24.67 and 27.44 % for the first, second and adult nymphs, respectively, the 72 hour period was a positive effect in increasing the mortality ratio of nymphs and adults, as it reached 32.20, 34.67 and 33.40 %, the lowest time period 24 hours achieved the lowest mortality ratio which was 18.40, 17.66, for the first and second nymphs instar and the adult stage respectively. Park *et al*. (2016) confirmed also that methyl salicylate (MeSA) is known for its efficiency as an insecticide fumigant against *Callosobruchus chinensis* (Coleoptera: Bruchidae) adults. From the salicylic acid applications as a natural product with low environmental impact in pest control is what was found by Fazam and Shimizu (2021) in their lab study in Londrina State in Brazil to show the efficiency of direct treatment with five powders chemical compounds (salicylic acid, sulfur, boric acid, zinc oxide and inert talcum powder (Quimidrol®) as a control treatment) on two species of leaf-cutter ants *Atta sexdensrubropilosa* and *Acromyrmex crassispinus*, salicylic acid powder achieved the highest killing rate of workers of both species, reaching 100 % with a significant difference from the rest of the compounds used in the study. These results may provide opportunities for its use in integrated control programs for nest ants and as a low environmental impact product, that salicylic acid powder achieved the highest mortality ratio of both species workers, reaching 100 %, with a significant difference from the other compounds used in the study, and the researchers stated that these results may provide opportunities for using it in integrated control programs in ants nests and as a low-impact product. As indicated by De Carvalho *et al*. (2019) that a 100 mg/L concentration of salicylic acid isolated from cashew nut shell *Anacardium occidentale* used as larvicidal against *Aedes aegypti* and *Culex quinquefasciatus*, it achieved 39 and 48 % mortality ratio for both species of mosquito larvae respectively.

Table - 2: Effect of Salysalic acid concentrations in nymphal stage of *T. urticae* on leaves of eggplant varieties at different time intervals

% Corrected mortality ratios (averages)							
var.	Conc. (mM)	Time (hour) 12	24	36	48	var.×Conc.	Var.
Bowie	0.5	92.59	100	100	100	98.15	64.51
	0.75	59.72	65.28	62.50	83.33	67.71	
	1	0	14.81	12.50	83.33	27.66	
var.×Time		50.77	60.03	58.33	88.89		
Barcelona	0.5	90	90	100	100	95	76.11
	0.75	70	70	70	70	70	
	1	50	60	63.33	80	63.33	
var.×Time		70	73.33	77.78	83.33		
Jawaher	0.5	88.89	100	100	100	97.22	70.06
	0.75	25.93	25.93	25.93	33.33	27.78	
	1	40.74	100	100	100	85.19	
var.×Time		51.85	75.31	75.31	77.78		
Conc. \ Time		Conc.×Time 12	24	36	48	Conc.	
0.5		90.49	96.67	100	100	96.79	
0.75		51.88	53.73	52.81	62.22	55.16	
1		30.25	58.27	58.61	87.78	58.73	
Time		57.54	69.56	70.47	83.33		
LSD (P≤0.05)		Var.(A)=2.50*		Conc. (B)= 2.50*		Time (C)= 2.88*	
		A×B=4.32*				B×C=4.99*	
		A×C=4.99*				A×B×C= 8.65*	

Some studies was stated that salicylic acid derivatives play an important role in insects control, including what Karatolos and Hatcher (2009) was proven that after direct use of acetylsalicylic acid on plant buds they observed an increase in mortality ratio of *Myzus persicae* insects. Salicylic acid is a phenolic compound with an aromatic benzene ring that carries one or more hydroxyl (OH) groups (Van Hung, 2016), these compounds and their derivatives have an important role in insects controlling by several mechanisms, like oviposition suppression, antifeedant, growth inhibitor and induce both direct and indirect defenses such as induced responses in plan (Van Poecke and Dicke, 2002; Peng *et al*., 2004; Conrath *et al*., 2015; Molinari, 2016) noted that the Acibenzolar-S-methyl nematicidal (ASM) (derived from salicylic acid, known commercially in Europe as Bion) was effective at 0.5 mM concentration on root-knot nematodes *Meloidogyne incognita* individuals, that it was achieved 30%mortality ratio after 24 hours of treatment.

The phenolic compounds had negative effects on some life aspects of some insect species. When comparing the relative efficiency of phenolic compounds including (Salicylic acid, Benzoic acid, Acetylsalicylic acid and sodium benzoate) on certain biological aspects of the Mediterranean fruit fly (MFF), *Ceratitis capitata* and the peach fruit fly (PFF) *Bactrocera zonata* under laboratory conditions, it was found that artificial nutrient with salicylic acid and acetylsalicylic acid showed efficiency in influencing on some life aspects of MFF, as lowest record of % pupation of 70.5 and 75.8 %, the lowest ratio of % adult emerged flies, reached 97 and 94 %, the lowest Oviposition period, which amounted to 16 and 18.3 days, the lowest rate of the total of deposited eggs, which amounted to 252 and 631.7 eggs, the lowest ratio of

hatchability, which amounted to 74.7 and 78.7 %, the shortest longevity of adult males was 13 and 18.3 days, and the shortest longevity of adult females was 20.4 and 23.6 days for both acids respectively (El-Khayat *et al*., 2021).

In addition, phenolic compounds had toxic effects by contact, benzoic acid had been used as a contact pesticide when applied directly against the copra mite *Tyrophagus putrescentiae* (Schrank) (Tak *et al*., 2006). The reason for the individuals mortality of mite using phenolic compounds was attributed to possibility of these substances ability to damage the cells protoplasm by affecting the cells protein by precipitation it, especially in the cells of the epithelial layer lining the middle gut (in stomach toxins case) and these protoplasmic toxins often form heavy salts (Gerges and Amin, 1987).

Also It was found that some salicylic acid derivatives was inhibited oviposition process in some insects species as confirmed, by Ulland *et al*. (2008) in their study of the use of methyl salicylate on cabbage moth adults *Mamestra brassicae* and Movva and Pathipati (2017) confirmed the toxic nature of phenolic compounds such as sinapic acid and chlorogenic acid on larval stage development and oviposition determination in *Spodoptera litturalis* F. when exposed to nutrient treated with these compounds. In a similar study, Risen (1999) was found that the phenolic compound of *Melia azedarach* (Family: Meliaceae) plant achieved a mortality ratio of 52.8 and 53.2 % within 72 hours of adult and nymph stage of two-spotted spider mite (*Tetranychus urticae*) respectively.

Despite of spider mite natural movement, it does not feed on eggplant leaves treated with salicylic acid concentrations correctly, because the acid possesses a nutrient inhibitor and in the nutrition case, the accumulation of acid inside spider mites bodies may lead to toxic effects, this was what Jassim and Masudy (2011) confirmed in their study to show the effect of the use of five salicylic acid concentrations 100, 200, 300, 400 and 500 mg/L water on the cotton leaf worm insect stages *Spodoptera littoralis*, they mentioned that the ratio of adult emerged was 81.1 % at 300 mg/L concentration and they attributed the reason of ratio decrease to Probability of salicylic acid accumulation larvae bodies and its effected on the physiological changes development stages, which led to mortality of larvae percentage and the failure to enter nymphal stage and the adults emerged, and they stated that salicylic acid was highly effective as growth inhibitor and antifeedant of all insect stages especially in high concentrations.

Salicylic acid may had a repellent effect on mite individuals and this was what Pulga *et al*. (2020) observed in their study in Brazil using salicylic acid, they were carried out in three commercial genotypes (‘Redenção’, ‘Giuliana’, and ‘Alambra’) and one wild genotype (*Solanum habrochaites* var. *hirsutum*, accession PI-127826), they found that the two-spotted spider mite *T. urticae* traveled a shorter distance on the leaves of plants treated with acid.

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