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Effect of Antioxidants on *Tetranychus urticae* (Antioxidants as a Chemical Defense on Controlling *T. urticae*)

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Abstract: The antioxidant salsylic acid, humic acid, and ascorbic acid were evaluated against two spotted spider mites *Tetranychus urticae (*Koch). Determination of total phenol content in leaves of eggplant treated with these antioxidants at different intervals was performed. The results clearly revealed that these caused reduction in mite number. Such reduction in mites were time – dependent over 28- day exposure period to reach 88 % in case of salsylic acid – treated mites whereas the percent reduction 91 % was recorded for each of ascorbic acid – and humic acid – treated mites after 28- day exposure period. A potential elevation of total phenol content was found in leaves of eggplant- treated with salsylic acid through the last 14 – day exposure. Such increases of phenol content were found to be (1.9, 13.44 and 18.7) mg/ 100 gm after 14, 21 and 28 day of treatment compared with control value to be 5.37, 10.86 and 13.44 mg/ 100 gm at parallel intervals. The percent reduction in mites could be related to the elevation of phenol content as natural defense substances.

Keywords: *Tetranychus urticae*, Antioxidants, Humic acids, Ascorbic acid, Salsylic acid, total phenol

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is one of the most important pests, causing yield losses to many horticultural, ornamental and agronomic crops. Defoliation, leaf burning, and even plant death can occur due to direct feeding damage. Indirect effects of feeding may include decreases in photosynthesis, transpiration (Le Goff *et al*., 2009) a major problem in controlling *T. urticae* is the resistance to many acaricides (Puinean *et al.,* 2010) and to the hazard effect of using pesticides. So, using new method like antioxidants, using it as a chemical defense. Ascorbic acid is an essential element of plant and animal antioxidant systems, which can be defined as complex redox networks, including metabolites and enzymes (Paciolla *et al*., 2016).Humic Acids is a main component of humic substances, which are the major soil organic constituents. Its substances are commercially products which consist of some organic molecules that originate from decomposition, microbial activity of dead biological material and plant tissues (Ekin, 2019). Salsylic acid is a plant phenolic and considered to be a hormone-like endogenous regulator, has been proposed as an endogenous signal associated with regulating oxidant levels in response to biotic stress. Recent studies have shown that its plays an important role in provoking plant resistance to various biotic and abiotic stresses (Farouk *et al.* 2008).

The aim of the present study is to clarify the effect of antioxidant on population of *Tetranychus urticae,* and to estimate the total phenol on plant.

MATERIALS AND METHODS

1. Antioxidants:
The following antioxidants were used throughout the course of this study (at concentration 0.5 %)

1.1. Humic acids:
Chemical name: Humic acid
IUPAC name: 3-nitrobicyclo [2.2.1] hept-5-ene-2,3-dicarboxylic acid
Molecular formula: $C_9H_9NO_6$
Molecular weight: 227.172 g/ mol.

1.2. Ascorbic acids
Chemical name: Ascorbic acids
IUPAC name: (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one
Molecular formula: $C_6H_8O_6$
Molecular weight: 176.124 g/mol

1.3. Salsylic acid
Chemical name: salsylic acid
IUPAC name: 2-Hydroxybenzoic acid; 69-72-7;
O-hydroxy benzoic acid; 2-Carboxyphenol; O-Carboxyphenol
Molecular formula: $C_7H_6O_3$.
Molecular weight: 138.122 g/ mol.

2. Maintenance of the colony of *T. urticae*:

For establishing a colony of the two spotted spider mites *Tetranychus urticae* Koch in the laboratory, the technique of (Guirguis *et al.,* 1977) was followed. The mites were collected from infested leaves of the castor bean, *Ricinus communis* trees grown at the Experimental Farm of Ismailia Agricultural Research Station, Ismailia, Egypt. One hundred adult females of *T. urticae* were transferred with a fine brush (Pelikan brush No. 000) to sweet potato leaves. Sweet potato cutting, each holding about 8 leaves, were washed under running water and then placed in tap water in 250 ml glass jar. Each jar contained three sweet potato cuttings. The colony was established with three jars. The sweet potato cuttings were changed twice a week in summer, and once a week in winter or when it was necessity. The colony was kept in cheese cloth cage (60 x 60 x 60) cm under laboratory conditions of 25±2ºC; 65±5% relative humidity and 12 hrs daily illuminations by using fluorescent tubes of 40–60 watt. The colony was kept away from any pesticide contamination for six months before used in experiment.

Determination of total phenolic contents in the eggplant leaves:

Total phenols were assayed using the folin-Denis method according to (William *et al*., 1965). 1 ml of the ethanolic extracts was added to 1 ml of 2 N folin-Denis reagent (1 ml of cons. folin reagent added to 2 ml of distilled water), 1 ml of 14% Na_2co_3 and 17 ml distilled water. The mixtures were heated to about 70ºC in a water bath, and then left for cooling at room temperature. The optical density of these samples was measured by a spectrophotometer at 650 nm. The concentration of the total phenols in the samples was calculated as mg/100g by the application of a standard curve of solutions of pure Gallic acid at concentrations of 10, 20, 30, 40, and 50 ppm.

Abbot's formula (1925) was used to get correction for natural mortality. Lines of toxicity were statistically analyzed according to the method described by Finney (1952).

Henderson and Tilton equation (1955) was used to calculate the reduction percentage in mite infestation due to field pesticide application as follows:

Reduction percentage = $100 \times [1 - \frac{Ta \times Cb}{Tb \times Ca}]$

Tb ⟶ number of mite before treatment
Ta ⟶ number of mite after treatment
Cb ⟶ number collected from control before treatment
Ca ⟶ number collected from control after treatment

RESULTS AND DISCUSSION

Reduction percentages of *T. urticae* after treatment by antioxidant:

Data in Table (1) showed that reduction percentage after 3 days of treatment with S.A and A.A was 20 % while H.A was 50% after 7 days of treatment. After 14 days of treatment the reduction percentage was 60, 60, and 67% for S.A, H.A and A.A. While after 28 day of treatment the reduction percentage was 88 for S.A, and it was 91 for H.A and A.A.

Results showed that the effect of H.A was latent effect after 3 days of treatment, and reached high effect after 28 day of treatment.

Result showed that H.A has the most effect then A.A, then S.A has the least effect.

Table (1): Reduction percentages of *T. urticae* after treatment by antioxidants

Time (days)	% Reduction		
	A.A	S.A	H.A
3	20	20	0
7	42	42	50
14	67	60	60
21	80	80	85
28	91	88	91

A.A Ascorbic acid S.A Salsylic acid H.A Humic acids

Determination the total phenol of eggplant:

Data in Table (2) showed that the value of total phenol in leaves of eggplant treatment by antioxidants comparing with control. In ascorbic acid, value of total phenol after 3 day after treatment (5.27) mg/100 g while it was (7.64) mg/100g after 14 days of treatment and it was (7.46) mg/100 g after 28 days of treatment. Salsylic acid the value of total phenol was (6.5, 6.03, 11.9, 13.44, and 18.7) mg/100g after 3, 7, 14, 21, and 28 days of treatment.

In humic acids, it was observed that the total phenol was 12.05 mg/100 g after 3 days of treatment, (9.36) mg/100 g after 21 days of treatment.

Comparing the total phenol value in the antioxidant treatment with control, it observed that the total phenol was 7.25 mg/100 g after 3 days of treatment, after 21 days of treatment it was (10.86) mg/100g and it was (13.44) mg/100 g after 28 days of treatment.

Results showed that the antioxidant effect on the phenol and it was increased in the leaves of plant, so the plant becomes more resistance to *Tetranychus urticae*.

Table (2): Value of total phenol in the leaves of eggplant after 3, 7, 14, 21 and 28 days :(mg/100g)

	Time (days)				
Chemical used	**3**	**7**	**14**	**21**	**28**
Control	7.25	7.80	5.37	10.86	13.44
A.A	5.27	7.80	7.64	7.35	7.46
S.A	6.5	6.03	11.9	13.44	18.7
H.A	12.05	9.65	9.57	9.36	8.49

Data in Table (3) showed that the relation between the reduction percentages and the value of the total phenol. In salsylic acid, percent reduction was (88%) with the phenol (18.7) mg/100 g after 28 days of treatment. In ascorbic acid after 28 days of treatment, the reduction percent reduction was (91%) with (7.46) mg/100 g total phenol after 28 days of treatment. While the percent reduction was (91%) after 28 days of treatment with (8.49) mg/100 g value of phenol.

Results showed that it was relation between the percent reduction and the total phenol in leaves.

Table (3): Relations between reduction percentages of mites and value of total phenol in leaves of eggplant

Time (days)	***S.A***		**A.A**		**H.A**		
	% Reduction	**Total phenol**	**% Reduction**	**Total phenol**	**% Reduction**	**Total phenol**	**Control**
3	20	6.5	20	5.27	0	12.05	7.25
7	42	6.03	42	7.80	50	9.65	7.80
14	60	11.9	67	7.64	60	9.57	5.37
21	80	13.44	80	7.35	85	9.36	10.86
28	88	18.7	91	7.46	91	8.49	13.44

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تأثير مضادات الأكسدة علي الإصابة بالعنكبوت الأحمر ذو البقعتين
(مضادات الأكسدة كمواد كيميائيه دفاعيه لمكافحه العنكبوت الأحمر ذو البقعتين)

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تم استخدام مضادات الأكسدة كماده دفاعيه لمكافحه الإصابة بالعنكبوت الأحمر ذو البقعتين، حيث استخدام ثلاثة أنواع من مضادات الأكسدة وهم (حامض السالسيلك ، حامض الهيوميك، حامض الاسكوربك) وتم تسجيل النتائج بعد ٣، ٧، ١٤، ٢١، ٢٨ يوم من المعاملة. وقد أظهرت النتائج انه عند استخدام حامض السالسيلك أعطي نسب خفض في تعداد العنكبوت الأحمر ذو البقعتين بنسبه ٤٢% بعد ٦ أيام من المعاملة، وقد وصلت ٨٨% بعد ٢٨ يوم من المعاملة. بينما أعطت المعاملة حامض الهيوميك ٦٠% بعد ٢١ يوم من المعاملة بينما وصلت النسبة لـ ٩١% بعد ٢٨ يوم من المعاملة. وعند المعاملة بحامض الاسكوربك كانت نسبه الخفض ٦٧ % بعد ١٤ يوم من المعاملة، وكانت نسبه الخفض ٩١ % بعد ٢٨ يوم من المعاملة. وعند قياس نسبه الفينول في الأوراق النباتية المعاملة بمضادات الأكسدة المختلفة نجد أن في حامض السالسيلك كانت قيم الفينول في الأوراق كالآتي (٦.٥ ، ٦.٠٣، ١١.٩، ١٣.٤٤، ١٨.٧) مليجرام/١٠٠ جرام بعد (٣، ٧، ١٤، ٢١، ٢٨) يوم من المعاملة ، أما عند المعاملة بحامض الهيويك فكانت قيم الفينول (١٢.٠٥، ٩.٦٥، ٩.٥٧، ٩.٣٦، ٨.٤٩) مليجرام /١٠٠جرام بعد (٣، ٧، ١٤، ٢١، ٢٨) يوم من المعاملة. وعند المعاملة بحامض الاسكوربك كانت قيمه الفينول في الأوراق (٥.٢٧، ٧.٨٠، ٧.٦٤، ٧.٣٥، ٧.٤٦) مليجرام /١٠٠جرام بعد (٣، ٧، ١٤، ٢١، ٢٨) يوم من المعاملة.

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