J. Plant Prot. and Path., Mansoura Univ., Vol.9 (10): 645 - 648 , 2018

Foliar Spray of Zinc, Manganese and Iron for *Tetranychus urticae* Control in *Phaseolus vulgaris* Plant

Walaa R. Abou Zaid* ; Doaa A. Abou El- Atta and Amira E. Mesbah
Plant protection research Institute, Agriculture research center, Egypt.
***Corresponding author: dr_walaaroushdy@yahoo.com**

ABSTRACT

Mites control via micronutrients spray represents a good alternative for acaricides in terms of cost, efficiency and preservation of the environment. In this study, two concentrations (65 & 130 ppm) of zinc, manganese and chelates of iron have been used to control the population of *Tetranychus urticae* in *Phaseolus vulgaris* plants. The highest conc. of each mineral led to higher reduction % that reached to 39.56, 44.93 and 42.72 % respectively in response to the third spraying and the treatments in general did not show instantaneous effect. The positive effect of these parameters on the plant has been confirmed by the observed increase in the contents of chlorophyll, soluble carbohydrates and protein compared with that of the control that have been evaluated after the third spraying. These results support the use of foliar spray of minerals to replace acaricides. The benefits behind that extended to coast-reduction, efficient alternative that would be supportive to plant nutrition that would increase the yield and at the same time environmentally- friendly.

Keywords: Chelates of iron, foliar spray, manganese, *Tetranychus urticae*, and zinc

INTRODUCTION

Cultivated crops across the world are usually threatened by pests such as mites, insects, nematodes, parasitic weeds, bacteria and fungi. The intensive use of pesticides helped the farmers for some extent to control; however, Long-term damage has become more of its benefits as the use of pesticides affect human health (Senthil-Nathan, 2015) as well as the biodiversity in the environment(Pavela, 2015).Spider mites such as *Tetranychus urticae* are among the pests threating crop production (20-45% of the yield might be lost depending on season)as growth, chlorophyll contents and fruit size as well as quality are greatly affected in case of severe mites infection(Rhodes *et al.*, 2006; Premalatha *et al.*, 2018).The danger of this pest was heightened since World War II. *T. urticae* has a high rate of fecundity and a short developmental time that can be as brief as one week at high temperatures of about 32°C.*T. urticae* greatly affect strawberries, hops, mint, citrus and apple (Van de Vrie *et al.*, 1972; Rhodes *et al.*, 2006).The long term intensive use of acaricides leads to the dominance of resistant population that could not be affected by chemical pesticides and that would be reflected on plant yield (Fraulo and Liburd, 2007).

Plant nutritional status and structural features would afford a favorable microenvironment for mites propagation (Mworia *et al.*, 2017). Drought stress has been suggested to decrease susbtability to mites, although, *Panonychus ulmi* population has been retarded on apple trees under this stress (Specht, 1965). Recently it has been found that high level of nitrogen fertilization (300 kg N/ha/year) led to a high infestation level.

Mineral elements whether macro or micro nutrients as a part of plant nutrition are also in the play. Essential micronutrients supplied mainly by soil are important for almost all metabolic and cellular functions of plants. Generally iron, manganese, zinc, copper, boron, chloride, molybdenum and nickel are regarded as essential micronutrients for almost all higher plants.

Iron plays a vital role in several components of the redox system such as cytochrome and Fe-S clusters. Additionally, it acts as a cofactor for several oxidoreductases beside its involvement in chlorophyll formation and chloroplast biogenesis (Said-Al Ahl and Mahmoud, 2010; Marschner, 2012).Manganese acts as a cofactor for several proteins such as acid phosphatise, superoxide dismutase, allantoateamidohydratase, arginase, RNA-polymerase and protein-photosystem II (Marschner, 2012). In higher plants, zinc activates several dehydrogenases, aldolases and isomerases (Marschner, 2012). In this study, the aim was to evaluate the effect of foliar application of iron, manganese and zinc on *T.urticae* infestation of common bean plant.

MATERIALS AND METHODS

Materials

Oto manganese (13%) and oto zinc (13%) were obtained from Misr El Dawlia for Agricultural & Industrial Development Company and use as sources for the corresponding minerals. The iron containing nutrient used in this study was obtained from Pioneers Fertilizers Production Company, Jordan. Max fert (13 % Fe) was used as a source of iron.

***Tetranychus urticae* culture**

The culture of *T. urticae* (TSSM) was obtained from a laboratory pure colony that was maintained on common bean leaves incubated in petri-dishes at 25 ± 2^{o}C.

Experimental design

Pure strains of apparent uniform *Phaseolus vulgaris* seeds were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The experiment has been started in April 2017 in the experimental field of Faculty of Agriculture, Mansoura University. The used soil was a mixture of clay and sand (2:1 v/v) and the pots used in this study were filled with equal amounts of this soil. Before cultivation, the soil was supplied with super phosphate fertilizer (1g/ each pot). In the age of 14 days, plants were infected with TSSM obtained from large sensitive laboratory colony as mentioned previously. The required number of TSSM was transferred from the colony to a 1.5 cm diameter common bean leaf disc that was then placed onto a leaf of each experimental plant.

The pots were divided into 8 groups including control one as shown in Table 1. Each group was sprayed with its represented treatment three times during the course of the experiment. The control plants were subjected to water spray at the same time other groups received their spraying treatments. Additionally and to act as a positive control, another group was sprayed three times too with

Abmectin (Vertimec® 1.8% EC) with 40 cm^3/100 liter water at the same time of spraying other groups with the designed treatments.

Table 1.The materials used and their concentrations.

Treatment	Used concentration
Oto zinc (13%)	65 ppm 130 ppm
Oto manganese (13%)	65 ppm 130 ppm
Max fert (13 % Fe)	65 ppm 130 ppm
Abmectin (Vertimec® 1.8% EC)	40 cm^3/100 liter water.
Control	Water spraying

The first spraying treatment was committed for plants in the age of 20 days, followed by another treatment after 2 weeks (34 days old plant) and finally the last treatment was given for 48 days old plants. From each group, 20 leaves were collected randomly and the number of TSSM was counted by using stereo-binocular microscope. The leaves were collected 3, 7 and 14 days after each spraying treatment. The number of moving stages, corrected mortality and the reduction percentages of mites were calculated by using the Henderson -Tilton formula (Henderson and TILTON, 1955).

$$\text{Corrected }\% = 1 - \left(\frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}\right) \times 100$$

- Estimation of photosynthetic pigments:

Chlorophyll a and chlorophyll b were determined at the late flowering stages "after the third spraying" of plant growth using the spectrophotometric method as recommended by (Dye, 1962) for pigments as adopted by (Taylor and Achanzar, 1972). A known fresh weight of plant leaves was cut and ground with 80 % acetone. After centrifugation, the supernatant absorbance was measured at 644 and 663 nm.

- Estimation of total soluble carbohydrates:

For plants at the late flowering stage "after the third spraying", a known volume of the dry leaf powdered tissue was submerged overnight in 10 ml 80 % (v/v) ethanol at 25°C with periodic shaking. After one day, the obtained ethanol mixture was filtered, made up to 20 ml and kept in the refrigerator (Vedder, 1915).Total soluble sugars (TSS) content was determined using the procedures described previously (Hansen and Møller, 1975). An aliquot of 0.1 ml of the alcoholic extract was added to 3 ml of freshly prepared anthrone and incubated in a boiling water bath for 10 min and the absorbance was obtained at 625 nm. The amounts of TSS in plant extracts were obtained using the standard curve of glucose.

- Estimation of total proteins:

The method of protein extraction was adopted by (Scarponi and Perucci, 1986). For plants at the late flowering stage "after the third spraying", a known weight of fresh plant tissue was cut into small pieces and homogenized in five volumes of chilled acetone using a homogenizer for one minute followed by sonication. The crude homogenate was filtered and the residue was used for determination of protein content after re-suspension in 50 mM tris-HCl buffer pH 9. Protein content was determined spectrophotometrically according to the method adopted by (Bradford, 1976). Bovin serum albumin was used as standard in this experiment.

- Estimation of mineral elements:

For plants at the late flowering stage "after the third spraying" and after washing with de-ionized water and drying at 90°C, 0-5 gm of the dry ash of each plant sample was digested in 10 ml HNO_3 for 10 h. After filtration, the supernatant volume was raised by de-ionized water to 20 ml and then Fe, Mn and Zn were measured by Atomic Absorption Spectrometry (Mazumdar and Das, 2015).

RESULTS

Generally, TSSM number has been reduced in response to all nutrients spraying and the reduction percent increased in response to higher concentrations of nutrients (Table 2). Expectedly, the reduction increased by time passing after each spray and the highest reduction percent was attained after the third spray. It is observable that 130 ppm Mn gave the highest reduction % after the first and the third spraying (28.69& 44.93 % respectively); however, Fe treatment gave the highest reduction percent, 40.13%, after the second spraying.

Table 2. % Reduction of TSSM individuals/ plant leaf in response to nutrients foliar spray.

Treatment	% reduction after 1st spray			Total reduction	% reduction after 2nd spray			Total reduction	% reduction after 3rd spray			Total reduction
	3 days	7 days	14 days	%	3 days	7 days	14 days	%	3 days	7 days	14 days	%
Zn 65ppm	4.20	14.85	16	11.68	12.5	18.33	25.76	18.86	22.07	28.57	43.61	31.41
Zn 130ppm	3.8	22.36	36.38	20.86	20	35.48	45.45	33.64	18.18	40	60.53	39.56
Mn 65ppm	4.76	26.92	39.29	23.65	18.3	32.26	36.36	28.98	23.07	40.66	51.42	38.38
Mn130ppm	9.52	34.23	42.32	28.69	21.02	30.52	41.26	30.93	30.07	47.25	57.49	44.93
Fe 65ppm	4.49	30.98	35.91	23.79	21.40	33.45	41.95	32.26	21.21	37.14	47.37	35.24
Fe130ppm	3.86	31.49	40.63	25.32	28.62	44.8	46.97	40.13	24.24	50	53.95	42.72
Abamactin	91.38	89.56	87.07	89.33	88.33	84.95	90.02	87.76	87.01	82.86	80.26	83.37

As regard to photosynthetic pigments, proteins and total carbohydrates there was a general non-significant increase in response to different elements treatments. In response to nutrients spraying, these minerals contents has increased in their corresponding treatments as shown in table 3. However, Mn treatments did not show any synergetic effect to other elements. That means, in case of Mn spraying, neither Zn nor Fe increased in the plant tissue compared with that of control or Abmectin. Zn did not show any synergism to the other elements and Fe treatments increased Mn significantly.

Table 3. Effect of nutrients foliar spray on plant chlorophyll contents, total soluble carbohydrates, total protein , Zinc, Manganese and Iron content.

	Chlorophyll a	Chlorophyll b	T. chlorophylls	T. carbohydrates %	Protein %	Zn mg/100g	Mn mg/100g	Fe mg/100g
Zn 65ppm	0.74 [a]	0.52 [a]	1.27 [a]	39.98 [ab]	15.68 [a]	7.71 [a]	1.65 [d]	13.18 [c]
Zn130ppm	0.78 [a]	0.55 [a]	1.34 [a]	40.84 [ab]	16.6 [a]	8.98 [a]	1.79 [c]	13.53 [c]
Mn 65ppm	0.73 [a]	0.51 [a]	1.25 [a]	39.11 [b]	15.4 [a]	5.07 [b]	3.71 [b]	12.36 [c]
Mn130ppm	0.77 [a]	0.54 [a]	1.32 [a]	40.55 [ab]	16.29 [a]	5.26 [b]	3.87 [a]	12.74 [c]
Fe 65ppm	0.76 [a]	0.53 [a]	1.29 [a]	40.27 [ab]	16.02 [a]	5.48 [b]	1.34 [f]	16.42 [b]
Fe130ppm	0.79 [a]	0.56 [a]	1.36 [a]	41.17 [a]	16.89 [a]	5.66 [b]	1.47 [e]	19.65 [a]
Abamactin	0.73 [a]	0.55 [a]	1.28 [a]	40.24 [ab]	15.67 [a]	4.9 [b]	1.2 [g]	12.05 [c]
control	0.72 [a]	0.5 [a]	1.23 [a]	39.43 [ab]	15.09 [a]	4.88 [b]	1.19 [g]	11.95 [c]
LSD	0.17	0.17	0.17	1.73	1.73	1.73	0.107	1.73

DISCUSSION

There is no doubt that *Tetranychus urticae (Koch)* is one of the most polyphagous species with a long list of hosting plants including vegetables, fruits and weeds. Acaricides was and still the most prominent way to control *T. urticae* although their side effects on the environment as well as coast. It has become necessary to find alternative ways to resist this pest in an environmentally friendly way and it is desirable to supply plant with beneficial nutrients to achieve duel benefits. An earlier observation of the ability of foliar spraying of Fe, Mg or Zn led to the complete absence of spider mites from the treated leaves was the start to use such nutrients as an alternative control way (Terriere and Rajadhyaksha, 1964).

In this study, the foliar spraying of Zn, Mn and Fe led to a reduction in the population of mites that reached 39.56, 44.93 and 42.72 % respectively in response to the third spraying, a result that is more or less similar to that conduced using bean as experimental plant in which 33.8, 46.6 and 53.4 reduction percent was recorded for the aforementioned elements respectively (Terriere and Rajadhyaksha, 1964). It has been shown that foliar spray of Zn, Mn and Fe chelates led to low egg production of *T. urticae* on apple , peach and pear seedlings (Harries, 1961). It has also been found that chelates of iron, manganese, zinc, and magnesium led to 90% reduction in the fecundity of *T. urticae* (Terriere and Rajadhyaksha, 1964). On the other hand, the number of *T. urticae* eggs was not affected by spying bean leaves with Zn, Mn or Fe (Cannon Jr and Terriere, 1966). Most recently in 2018 it has been shown that the foliar spray with Calcium, Magnesium, Zinc, Copper, Iron, Manganese, and Boron led to a reduction in the population of *Panonychus citri* and *Phyllocoptruta oleivora* on citrus (Chávez-Dulanto *et al.*, 2018).

The treatments of this study did not show an instantaneous effect as the reduction % gradually and slowly increased and often the maximum value was attained in the third observation after two weeks. This might be attributed to the slow absorption of the nutrients and then their effect on metabolism slowly appeared (Terriere and Rajadhyaksha, 1964). Using the chelates of iron (EDTA chelated) may led to a duel effect due to EDTA and due to Iron (Terriere and Rajadhyaksha, 1964) although iron itself was reported as effective agent against citrus mites (Chávez-Dulanto *et al.*, 2018). Basically the nutrients used in this study are playing a vital role in several metabolic processes particularly as activators for several enzymes. The availability of such nutrients via foliar spray may lead to strengthen the reinforcement of these metabolic processes that might be extended to produce secondary metabolites of antibiosis activity against mites (Chávez-Dulanto *et al.*, 2018). The general increase in chlorophyll, proteins and soluble carbohydrates in response to our treatments may support this assumption although these increases did not extend to the significant level.

Summering up, these results support that the foliar spray of minerals represent a potential acaricides alternatives that could control mites population on different crops. The benefits behind that extended to coast-reduction, efficient alternative that would be supportive to plant nutrition that would increase the yield and at the same time environmentally- friendly.

REFERENCES

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72(1-2): 248-254.

Cannon Jr, W. and L. Terriere (1966). Egg production of the two-spotted spider mite on bean plants supplied nutrient solutions containing various concentrations of iron, manganese, zinc, and cobalt. Journal of Economic Entomology 59(1): 89-93.

Chávez-Dulanto, P. N., B. Rey, C. Ubillús, V. Rázuri, R. Bazán and J. Sarmiento (2018). Foliar application of macro-and micronutrients for pest-mites control in citrus crops. Food and Energy Security 7(2): e00132.

Dye, D. (1962). The inadequacy of the usual determinative tests for the identification of Xanthomonas spp. New Zealand Journal of Science 5(4).

Fraulo, A. B. and O. E. Liburd (2007). Biological control of twospotted spider mite, Tetranychus urticae, with predatory mite, Neoseiulus californicus, in strawberries. Experimental and Applied Acarology 43(2): 109.

Hansen, J. and I. Møller (1975). Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical biochemistry 68(1): 87-94.

Harries, F. (1961). Effect of certain antibiotics and other compounds on the two-spotted spider mite. Journal of Economic Entomology 54(1): 122-124.

Henderson, C. F. and E. W. TILTON (1955). Tests with acaricides against the brown wheat mite. Journal of Economic Entomology 48(2): 157-161.

Marschner, H. (2012). Marschner's Mineral Nutrition of Higher Plants.(Ed.): Marschner, P, Academic press.

Mazumdar, K. and S. Das (2015). Phytoremediation of Pb, Zn, Fe, and Mg with 25 wetland plant species from a paper mill contaminated site in North East India. Environmental Science and Pollution Research 22(1): 701-710.

Mworia, J. K., L. K. Murungi, T. Losenge and R. MeyhÃ (2017). Plant Nutrition Impacts Host Selection in Red Spider Mites. African Journal of Horticultural Science 11: 35-46.

Pavela, R. (2015). Acaricidal properties of extracts of some medicinal and culinary plants against Tetranychus urticae Koch. Plant Protection Science 52(1): 54-63.

Premalatha, K., S. Nelson, R. Vishnupriya, S. Balakrishnan and V. Santhana Krishnan (2018). Acaricidal activity of plant extracts on two spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae).

Rhodes, E. M., O. E. Liburd, C. Kelts, S. I. Rondon and R. R. Francis (2006). Comparison of single and combination treatments of Phytoseiulus persimilis, Neoseiulus californicus, and Acramite (bifenazate) for control of twospotted spider mites in strawberries. Experimental & applied acarology 39(3-4): 213-225.

Said-Al Ahl, H. and A. A. Mahmoud (2010). Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (Ocimum basilicum L.) under salt stress. Ozean Journal of Applied Sciences 3(1): 97-111.

Scarponi, L. and P. Perucci (1986). Effect of Some s-Triazine Herbicides on Phosphatases from Corn (Zea mays) Roots. Weed Science 34(6): 807-810.

Senthil-Nathan, S. (2015). A review of biopesticides and their mode of action against insect pests. Environmental sustainability, Springer: 49-63.

Specht, H. (1965). Effect of water-stress on the reproduction of European red mite Panonychus ulmi (Koch) on young apple trees. The Canadian Entomologist 97(1): 82-85.

Taylor, W. I. and D. Achanzar (1972). Catalase test as an aid to the identification of Enterobacteriaceae. Applied microbiology 24(1): 58-61.

Terriere, L. and N. Rajadhyaksha (1964). Reduced fecundity of the two-spotted spider mite on metal-chelate-treated leaves. Journal of Economic Entomology 57(1): 95-99.

Van de Vrie, M., J. McMurtry and C. Huffaker (1972). Ecology of tetranychid mites and their natural enemies: A review: III. Biology, ecology, and pest status, and host-plant relations of tetranychids. Hilgardia 41(13): 343-432.

Vedder, E. B. (1915). Starch agar, a useful culture medium. The Journal of Infectious Diseases: 385-388.

الزنك والمنغنيزوالحديد لمكافحة العنكبوت الاحمر *Tetranychus urticae* في نبات الفاصوليا

ولاء رشدي ابو زيد ، دعاء عبد المقصود أبو العطا و أميرة الدسوقى مصباح
معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، مصر.

تمثل مكافحة العنكبوت الاحمر عن طريق المغذيات الصغري بديلا جيدا للمبيدات من حيث التكلفة والكفاءة والمحافظة على البيئة. في هذه الدراسة تم استخدام اثنين من تركيزات (٦٥ و ١٣٠ جزء في المليون) لكل من المنغنيز والزنك والحديد للسيطرة على الاصابة ب *Tetranychus urticae* في النباتات الفاصوليا.لقد أظهرت النتائج أن أعلى تركيز من كل من المعادن سالفة الذكر أدى إلى الانخفاض الأكبرلتعداد الافه بنسبة تصل إلى ٣٩.٥٦ و ٤٤.٩٣ و ٤٢.٧٢٪ للعناصر سالفة الذكرعلى التوالي كنتيجة للرش ثلاثة مرات بيد ان هذه المعاملات لم تظهر تأثيراً فورياً. وقد تأكد التأثير الإيجابي لهذه المعاملات على النبات من خلال الزيادة في محتويات الكلوروفيل والكربوهيدرات الذائبة والبروتين بالمقارنة مع العينة الضابطة. هذه النتائج تدعم استخدام الرش الورقي للمعادن ليحل محل المبيدات. وقد امتدت الفوائد الكامنة وراء ذلك إلى تخفيض التكلفة فضلا عن فعالية هذا البديل الذي من شأنه أن يدعم التغذية النباتية ويزيد من إنتاجه بطريقة صديقة للبيئة.