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LABORATORY BASED BIOEFFICACY OF SELECTED BIOPESTICIDES AGAINST THE ADULTS AND NYMPHS OF TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

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Abstract

Spider mites are highly mobile and destructive phytophagous pest that require more frequent use of chemical pesticides for successful control. Pesticides are major weapons used to control crop pests and disease vectors, chemical management is commonly practiced by farmers because their efficacy in a short time and ease of application and also was used to increase productivity and reduce losses. The present study is directed to evaluate the toxic effect of commercial chemical pesticide (Abamectin) and botanical pesticide (Neem oil) against adults and nymphs *of Tetranychus urticae Koch* (Acari: Tetranychidae) using leaf-disc spray technique under laboratory conditions. For evaluated adulticidal and nymphicidal effect, Abamectin had been used at five different concentrations (0.50, 0.25, 0.13, 0.06, 0.03) ml/l and neem oil at three different concentrations (1%, 3%, 5%) ml/l. The result showed that all concentrations of abamectin caused toxic effect to adults and nymphs with different mortality rate and the concentration 0.50 ml/l recorded highest mortality rate in adults of mite which was 94.0% during 72 h after application , also nymphicidal effect of abamectin recorded maximum effect at the concentration 0.50 ml/l which was 94.0 during 72h after application , In other hand, the results of the toxic effect of neem oil against adults and nymphs of mite showed that the mortality rate of adults was 100% at all tested concentrations after 72h of application, while the results of nymphicidal effect of neem oil revealed that the rates of mortality were 78%, 100% and 100% at concentration 1%, 3%, 5% respectively after 72h of application.
***Keywords*:** Abamectin, Neem oil, *Tetranychus urticae koch*, Nymphicidal, Adulticidal

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most destructive invasive pest species that attacks more than 1,000 plant species belonging to more than 140 plant families (Grbic *et al.*, 2011; Siddhapara, 2015; Kanika *et al.*, 2016; Paramjit & Frank, 2017; Nabi *et al.*, 2017; Flore *et al.*, 2019; Mohamed *et al.*, 2019). It is a global pest of greenhouse production and field crops, and is parasitic on many annual and perennial crops such as tomatoes, peppers, cucumber, strawberries, apples, grapes and citrus (Wekesa *et al.*, 2011; Kanika *et al.*, 2016 Tarikul *et al.*, 2017).

The two-spotted spider mite *Tetranychus urticae* is one of the most important citrus pests in Mediterranean citrus and among the different citrus varieties, clementines (*Citrus clementina*) are the most heavily affected (Alberto *et al.*, 2020). Mites is a serious pests because they occur several generations each season, phytophagus, high fertility and short life cycle, rapid development and contributed to the development of rapid resistance to many acaricides often after several applications(Huzefa, 2014; Manal and Hany, 2019; Karen *et al.*, 2019; Yasin *et al.*, 2020). This pest can alter the physiological processes of plants, reducing the area of photosynthetic activity and causing severe shedding of leaves (Kumari *et al.*, 2017; Ricardo *et al.*, 2019).

Due to the rapid development rate, short life cycle and high reproductive rate of *T. urticae*, it can reach huge population levels very quickly, resulting in a rapid decrease in host plant quality and plant crop yields, it causes direct damage (ie, defoliation and leaf burning, and excessive plant killing), but also reduces both photosynthesis and transpiration when growth conditions is appropriate to pest (Vassilis and Pavlos, 2013; Khalis and Omar, 2018).

Pesticides are major weapons used to control crop pests and disease vectors, despite major threats to food safety and the environment (Bourguet and Guillemaud, 2016; Flore *et al.*, 2019). Furthermore, the prevalence and rapid spread of pesticide resistance in many taxa has a serious impact on their efficiency. Therefore, alternative control strategies to control the disease are being sought and prevalence and outbreaks of agricultural crop pests, including spider mites (Zindel *et al.*, 2011; Parolin *et al.*, 2012; Attia *et al.*, 2013).

IPM concentrate on long-term protection from pests and their damage during combining techniques such as biological control, modification of cultural practices, habitat manipulation, use of resistant varieties, and minimization of pesticide use (Kos *et al.* , 2009; Hooman, 2017).

Abamectin is one of the active ingredients generally used to combat *T. urticae* which was introduced to the market in the early 1990s. Initially, the biological efficacy of this acaricide in controlling two-spotted spider mites was high (> 95%), for this reason, it was frequently used to control this pest (Karen *et al.*, 2019). Because of its high toxic effect and high toxicity index compared to various acaricides, therefore, abamectin has a special place in chemical control of mite (Keratum *et al.*, 2010). In addition, Vertimec, was more effective than Biofly and Actelic against TSSM (Manal and Hany, 2019).

Plant-derived pesticides are environmentally safe and harmless to humans, fish and wildlife. Many plant-derived essential oils have both acaricidal and insecticidal properties against various types of mollusc pests. As anti-feeding, growth regulating, repellent and toxic to many insect pests and provide new options for controlling TSSM (Tarikul *et al.*, 2017; Ricardo *et al.*, 2019).

Over 60 different types of biochemical products including, Nimbolide, Margolone, Mahoodin, Margolonone have been purified from neem. Several active chemical compounds at least 100 compounds are present in the plant, including glycosides, dihydrochalcone, coumarin, tannins, zadirachtin, nimbin, nimbidine, diterpenoids, triterpenoids, proteins, carbohydrates, sulphurous compounds, polyphenolics, among others (Agbo *et al.*, 2019). The purpose of this study is to determine the toxicity of abamectin and neem oil against *Tetranychus urticae*.

Materials and Method

Location of the study

This study was conducted in the laboratories of the collage of Science \ Wasit University \ Department of Biology in cooperation with the Agricultural Research Directorate \ Ministry of Science and Technology, from march to July in 2018/2019.

Source of pest (mites)

The *Tetranychus urticae* population was obtained from infected cucumber leaves with *T. urticae* in plastic house in Medical Technical Institute\ Kut in March\2019.

Host plant (Cucumber)

Cucumber plants (Italy species) were reared in plastic pots (13 cm diameter, 10 cm height) in a climate-controlled room (25±1°C, 60–70% RH, 16:8 h (L: D). After maturity(three week old age) plants were polluted with infected cucumber leaves that obtained from the plastic house at the Medical Technical Institute\ Kut for one year to obtain a permanent colony(sensitive strain without using pesticide) the same culture has been used for mass multiplication of mites and used in subsequent experiments (Siddhapara, 2015; Sabrine *et al.*, 2015).

Preparation of pesticide (Abamectin) spray

Pesticide was purchased from the local market. The Information of this pesticide used in the experiment include active ingredient, trade name, formulation , chemical group and recommended doses of the acaricide as well as the doses used (ppm), effect range are shown in (Table 1). Spray solutions of abamectin used in biological tests where prepared by dilution different concentrations of acaricide with distilled water to obtain the required concentrations (0.50, 0.25, 0.13, 0.06, 0.03) ml\l respectively according to the concentrations recommended by the manufacturers and according to the quantity required to carry out the experiment where the solutions were prepared and kept in special spraying machine and used directly (Ibrahim *et al.*, 2019).

Table 1 : Acaricide (abamectin) used in the study

Trade name	Active ingredient	Formulation	Recommended dose	Chemical group	MC	Effect range	ppm
Vertimec 18	Abamectin	CE	Ml in 100L35-50	Avermectin	54%	Pesticide Acaricide	540

***MC** : Mean concentration of commercial product for application in 100 liters of water ***CE**: concentrate emulsion

Preparation of Neem oil spray

Commercial neem oil was obtained from local market. It is an Indian original. Since neem oil does not readily mix with water, it must be to use emulsifier like (a mild liquid soap or tween 20). So solutions were prepared by mixing 5ml, 3ml and 1ml of neem oil with emulsifier tween 20(0.0 2%) as a surfactant. Then distilled water was added to obtain the concentrations 1%, 3% and 5%, all spray solutions were stirred well to ensure that the oil and water can mixed well and put in special spraying machine to be ready to use (Vijayalakshmi, 2002).

Leaf disc technique

Before using the leaves for different experiments, the healthy thin green leaves of cucumber selected from potted plants were thoroughly washed with tap water, dried and examined under the microscope to remove or kill any insect or mite stages found on it .Cucumber leafs were cut by Circular cutter into 2.5 cm diameter discs and these discs kept upside down on wet filter paper (7 cm x 5 cm) overlaying a wet cotton swab in petri dish (diam 9 cm) to ensure the leaf remained hydrated. The cotton swabs were kept saturated with water from time to time. Leaves were inoculated with mites. The development of two-spotted spider mite was studied at 27±2°C temperature maintained in biological oxygen demand (B.O.D.) incubator. The old leaf-discs were replaced periodically (every week) with fresh ones so as to ensure their good quality. After the spray, Petri dishes are kept uncovered for around 30 min, which allows for the drying of the leaf disc surface. They are then covered and placed under controlled conditions. Generally, mites that cannot walk a distance equivalent to their body length are considered dead (Manal and Hany, 2019; Flore *et al.*, 2019).

Bioassay procedure

Toxicity of pesticides (abamectin & neem oil) to nymphs and adults

To conduct this test 10 moving individuals from each of stages (nymphs and adults) were placed separately on ventral surface of healthy cucumber leaves placed in petri dish (diam 9 cm) surrounded by tangle foot substance and treated with 1 ml of each pesticide. Control Petri dish was treated with distilled water only. Hand held sprayer size 2.5 ml was used for spraying. The dishes were placed in incubator with a temperature 25±2 C and humidity 65±5%, then the death individuals were calculated after (24,48 and 72) h of spraying (AL- Jubouri, *et al.*, 2000).

Statistical analysis

All experiments were designed according to The Randomized Complete Block Design (RCBD) and complete randomization Design (CRD) and results were analyzed using Spss version 20 program which includes Duncan's Multiple Range Test (DMRT) to compare rates in all coefficients and determine the significant differences at the probability level 0.05 also used T test and Probity analysis to extract the median lethal concentration LC_{50} and median lethal time LT_{50}.

Results and Discussion

Toxicity of abamectin to *Tetranychus urtica*

Effect of abamectin on adults

The data that presented in table (2) indicated that the abamectin showed high toxicity against *T.urticae* adults and the effect of pesticide increased with the increasing of the concentration and period of exposure. The percentages of mortality of adults were 43.3%, 40.0% , 36.7% , 33.3% and 23.3% at the concentrations 0.50, 0.25, 0.13, 0.06 and 0.03 ml/l respectively after 24 h of treatment.In addition 94% mortality of *T.urticae* adults achieved after 72 h of treatment at the concentrations 0.50% and 0.25% ml/l with no significant differences. On the other side the results also revealed that the LC_{50} values were decreased with the increasing of the period of exposure which were 1.05, 0.03 and 0.02 after 24, 48 and 72 h after treatment respectively. The LT_{50} values were decreased with the increasing of the concentration of pesticide which were 25.8, 26.9, 29.4, 36.6 and 45.4 h at the concentrations 0.50, 0.25, 0.13, 0.06 and 0.03 ml/l respectively.

Table 2 : Susceptibility *Tetranychus urticae* adults to abamectin

Abamectin concentrations ML/L	Mortality rates%			LT_{50} hour	X^2
	24 h	48h	72h		
0.50	43.3D	88.0d	94.0c	25.8	6
0.25	40.0cd	84.5d	94.0c	26.9	5.2
0.13	36.7bc	73.9c	79.8b	29.4	9.4
0.06	33.3b	56.4b	65.6a	36.6	3.9
0.03	23.3a	45.9a	62.1a	45.4	2.2
LC50	1.05	0.03	0.02		
X^2	10	9.1	37.2		

Note : Similar letters in same column indicate that there is no significant difference and different letters in same column indicate that there is significant difference at P=0.05

Effect of abamectin on nymphs

The response pattern of the two-spotted spider mite nymphs of *T.urticae* to the five abamectin concentrations showed significant differences in susceptibility Table 3. LC_{50} values in all the treatments decreased gradually with increased time duration. The results revealed that the mortality rates were 56.7%, 53.3%, 36.7%, 33.3% and 23.3% at the concentrations 0.50, 0.25, 0.13, 0.06 and 0.03 ml/l respectively after 24 hours of treatment. The effect of abamectin increased with the increasing of the concentration and time duration and the highest mortality was recorded at the concentration 0.50 ml/l after 72 hours of treatment which was 94%. Moreover the results also showed that the LC_{50} values were decreased with the increasing of the time duration which were 0.22, 0.012 and 0.003 ml/l after 24, 48, and 72h of treatment respectively. The LT_{50} values were decreased with the increasing of the concentration of pesticide and the lowest value was 22.5 h at the concentration 0.50%.

Table 3 : Susceptibility of *Tetranychus urticae* nymphs to abamectin

Abamectin concentrations ML/L	Mortality rates%			LT_{50} hour	X^2
	24 h	48h	72h		
0.50	56.7c	81.7d	94.0d	22.5	10.4
0.25	53.3c	78.1c	86.9c	22.6	7.3
0.13	36.7b	74.5c	83.4bc	24.7	7.3
0.06	33.3b	60.1b	79.8ab	33	1.4
0.03	23.3a	52.9a	76.3a	35	10
LC50	0.22	0.012	0.003		
X^2	11	13	22		

Note : Similar letters in same column indicate that there is no significant difference and different letters in same column indicate that there is significant difference at P=0.05

Due to its high toxic effect and its high toxicity index, in addition, it has a special impact on *T. urticae* compared to various acaricides, therefore abamectin still the best compound has a special position and special importance in mite chemical control or in integrated management of mite (Kumari *et al.,* 2019). They referred that the abamectin superiority in reducing the mite population compared with other insecticides used in his study during two years of labrotary experiments (2011-2013) with mortality rate 72.06% after first spray and mortality rate 25.65% after second spray on grape plant.

Our results showed that abamectin was highly effective in killing adults of the two-spotted spider mite (*T. urticae*) on Cucumber plants and this was in agreement with previous studies (Ji *et al.*, 2013; Niu *et al.*, 2014).

Abamectin has become the predominant acaricide applied to control *Tetranychus urticae* outbreaks worldwide because of the very short residual effectiveness (Omar and Khalis, 2019).

Lagziri and El-Amrani (2009) had been reported that pesticide (Abamectin) exhibited high efficacy on *T. urticae* in laboratory test and 100% mortality was obtained when the recommended dose of it was applied and they recommended that the abamectin could be used as a selective acaricide in IPM programs because of its strong efficacy on pests, its persistence and its limited toxicity on predatory mites. Similar study was conducted by Duchovskienė (2007) who reported that the abamectin reduced the number of *T. urticae* and is highly efficient 3-14 days after application and he showed that the persistence of abamectin efficacy depends on the dose applied.

Our results also agree with the findings of Cobanoğlu and Alzoubi (2013) who found that the miticidal effect of acaricides (abamectin) against *Tetranychus urticae*(TSSM) were time dependent.

Also our results are similar with the findings of Youssef and Faiod (2013) found that abamectin achieve mortality rates reached 100% for both susceptible strain and resistant strain of adults of *T. urticae* after direct treatments.

Similar results were obtained by Abd El- Rahman and El-keblawy (2016) and they found that among the five compounds used against adults of *Tetranychus urticae*, the abamectin was the most toxic pesticide against this pest. França *et al.*(2018) reported that abamectin presented higher toxicity to *Steneotarsonemus concavuscutum* (another type of mite).

From the mortality responses of *T. urticae* adult females to different acaricidal molecules (LC_{50} values) it is evident that abamectin was most toxic to adults with the lowest LC_{50} value of 0.01 ppm followed by fenpyroximate (1.91 ppm), fenpropathrin (2.14 ppm) and fenazaquin (7.17 ppm) and among the other synthetics spiromesifen was least toxic with the LC_{50} value of 298.79 ppm (Kavya, 2018)

Recent study had been revealed that the concentration range of nano-abamectin (10-250 mg/L) induced mortality rates ranged from 25 to 96% in a femal adults of *T.urticae* (Halim and Kalmosh, 2019). Tarikul, (2019) recorded that the abamectin provided a high toxic effect against *T. urticae* population compared with other acaricides such as azadirachtin, emamectin benzoate, spinosad and hexythiazox.

The results of our study revealed that the highest mortality of the nymph was 94% after 72h of treatment and this result was in agreement with results of Wu_ and Liu (1996) who found that the mortality of nymphs of *Tetranytrus cinnabarinus* by abamectin was 52.8% after 24h and 90.0% after 72h of treatment. Chi-Yang *et al.* (2006) found that the abamectin recorded 100% mortality in *Tetranychus urticae* nymphs. Akashe *et al*. (2006) reported that the abamectin 0.0025 percent as most effective in checking *T. urticae* population on rose. Keratum *et al.* (2010) indicated that abamectin and cypermethrin have a especial effect on *T. urtica* and considered the best compounds that have a special importance in integrated mite management.

Present study revealed that the cypermethrin was the most effective in reducing the population density of motile stages of mite *T. urticae*, followed by abamectin benzoate while chlorpyrifos was of moderate effect in reducing the population density of motile stages of *T. urticae* (Abd El-Rahman and El-keblawy, 2016). Kumari *et al.*, 2017 found that the Abamectin resulted in highest nymphal mortality (96.05%) followed by dicofol (94.51%), hexythiazox (90.24) propargite (90.00), chlorfenapyr (89.33) and fenpyroximate (86.84%).

Toxicity of neem oil to *Tetranychus urticae*

Effect on adults

The results of this test were presented in table (4). The data revealed that the neem oil exhibited various levels of toxicity to adults of *T. urticae* and caused mortality in adult ranging from 40 to 100% depending on the concentrations and exposure time. The percentages of mortality of adults were 40%, 40% and 50% at the concentrations 1%, 3% and 5% ml/l respectively during 24 hours of application.

All tested concentrations existed 100% mortality after 72h of treatment. LC_{50} values decreased gradually over time and the LT_{50} of female adults of the *T. urticae* were 29, 28.3 and 24 h at the concentrations 1%, 3% and 5% ml/l respectively.

Table 4 : Susceptibility of *Tetranychus urticae* adults to neem oil

Neem oil concentrations ML/L	Mortality rates%			LT_{50} hour	X^2
	24 h	48h	72h		
1%	40a	73a	100a	29	37
3%	40a	78.5a	100a	28.3	34
5%	50b	97 b	100a	24	5.4
LC_{50}	5.65	0.9	--		
X^2	8.15	28	--		

Note : Similar letters in same column indicate that there is no significant difference and different letters in same column indicate that there is significant difference at P=0.05

Effect on nymphs

The neem oil tested against nymph of two spotted spider mite, *T. urticae* under laboratory condition showed a varied responses on their acaricidal property, which is presented and discussed below (Table 5). The results of this test showed good efficacy of neem oil in controlling the two-spotted spider mite population. The mortality rates of nymph were 33, 33.33 and 57% at the concentrations 1%, 3% and 5% respectively after 24h of treatment and the highest mortality (100%) was recorded at the concentrations 3% and 5% after 72h of treatment.

In addition the LC_{50} of nymphs of the *T. urticae* was decrease with increasing of time and the lowest was 0.063 ml/l after 72h. LT_{50} values also were decrease with increasing of concentration which were 39.7, 29 and 22.23h at the concentrations 1, 3, 5% respectively.

Table 5 : Susceptibility of *Tetranychus urticae* nymphs to neem oil.

Neem oil concentrations ML/L	Mortality rates%			LT_{50} hour	X^2
	24 h	48h	72h		
1%	33.00a	50.50a	78a	39.7	16
3%	33.33a	86.00b	100b	29	14
5%	57.00b	92.00b	100b	22.23	6.4
LC_{50}	5.1	1.27	0.063		
X^2	2.5	17.7	10.77		

Note : Similar letters in same column indicate that there is no significant difference and different letters in same column indicate that there is significant difference at P=0.05

Our result were in conformity with the finding of Premalatha and Chinniah (2017) who referred that the treatment with neem oil at concentration 3% ml/l contributed in reduction population of nymphs and adults of *T.urtica* on tomato at rate 74,88%. In other hand, our results are incompatible with report carried out by Tarikul, *et al* (2017), who concluded that the neem oil exist highest LC_{50} as comparing with mahogany and karanja oil were found significantly better than neem oil and this may be due to the environment or the biotype of the pest.

Ramaraju (2004) has published fool proof evidence that neem oil caused as high as 70.56 to 91.85% mortality of *T. urticae*. Similar study was conducted to evaluate the toxicity of selected commercial formulations of neem on *Tetranychus urticae* and two predatory mites and indicated that the toxicity of Neem on eggs and adults was greater for *T. urticae* compared to the toxicity observed for the predatory mites. So the neem that exhibited relatively low impact against the predatory mites can be used in integrated management of *T. urticae* (Hilda *et al.*, 2006).

Bernardi *et al*. (2012) in study to evaluate the effects of azadirachtin on *T. urticae* and its compatibility with the predatory mites *N. californicus* indicated that the Azadirachtin was efficient against *T. urticae*, with a mortality rate similar to that of abamectin and did not cause significant mortality of adult predatory mites *N. californicus* and they refer that the use of azadirachtin and predatory mites is a valuable tool for controlling *T. urticae*.

Premalatha and Chinniah (2017) concluded that the neem oil 3% and rosemary oil 3% can be well fit in as a viable component in the IPM package against two spotted spider mites on tomato which are certainly cost effective and eco-friendly. Botanical pesticides arise as a complementary alternative in the control of *T. urticae* Koch. Among the most studied botanical families for this purpose are plants from Lamiaceae, Asteraceae, Myrtaceae, and Apiaceae taxons. These are particularly abundant and exhibit several results at different levels; therefore, many of them can be considered as promising elements to be included into integrated pest management for controlling *T. urticae* (Rincón, et al. 2019).

Conclusions

The obtained data indicated that the abamectin was more toxic to adults and nymphs of *Tetranychus urticae* and it is crystal clear that neem oil have promising effects on mite pests at same time very safe / alternative to chemical acaricides, therefore, neem oils may be a useful part of IPM programs for management of *T. urticae* populations. Additional laboratory trials with another acaricides and different dose rates should be conducted to provide adequate control.

References

Abd El-Rahman, H.A. and El-keblawy, M.S. (2016). Toxicological Studies of Some Compounds on Two-Spotted Spider Mite *Tetranychus urticae* on Different Host Plants. J. Plant Prot. and Path., Mansoura Univ., 7(7): 519– 524.

Agbo, B.E.; Nta, A.I. and Ajaba, M.O. (2019). Bio-pesticidal properties of neem (*Azadirachta indica*) Advances and Trends in Agricultural Sciences Vol. 1.

Akashe, V.B.; Indi, D.V.; Patil, A.J.; Gud, M.A. and Ghadge, S.M. (2006). Persistence and toxicity of some miticides against *Tetranychus urticae* Koch on rose foliage. J. Maharashtra Agric.Univ., 31(3): 318-320.

Al-Jubouri, I.J.; Abdul Sattar A.A. and Al-Anbaki, N.N. (2000). Cotton pests and her control methods, National Program for the Development of Cotton Cultivation in Iraq, Indicative Bulletin, 60.

Alberto, F.; Cruz, G.; Alejandro, T. and Patricia, C. (2020). Citrus Vol Validation for the Adjustment of Spray Volume in Treatments against *Tetranychus urticae* in Clementines. Agronomy, 10(32): 2-24.

Attia, S.; Grissa, K.L.; Lognay, G.; Bitume, E.; Hance, T. and Mailleux, A.C. (2013). A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae) with special reference to natural pesticides. J. Pest Sci. 86(3): 361–386.

Bernardi, D.; Botton, M.; da Cunha, U.S.; Bernardi, O.; Malausa, T.; Garcia, M.S.; and Nava, D.E. (2012). Effects of azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and its compatibility with predatory mites (Acari: Phytoseiidae) on strawberry. Pest Management Science, 69(1): 75–80.

Bourguet, D. and Guillemaud, T. (2016). The hidden and external costs of pesticide use. In: Sustainable Agriculture Reviews. Springer, 35-120.

Chi-Yang, L.; Kang-Chen, L. and Me-Chi, Y. (2006). Effects of Household Soap Solutions on the Mortality of the Two-spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).Formosan Entomol., 26 : 379-390.

Cobanoğlu, S. and Alzoubi, S. (2013). Effects of soft soap and abamectin on the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and predatorymite *Phytoseiulus persimilis* AH (Acari: Phytoseiidae) under laboratory conditions. Turkish Journal of Entomology. 37(1): 31-38.

Duchovskienė, L. (2007). Effects of Abamectin on the two-spotted spider mite *(Tetranychus urticae* Koch.*)* in greenhouse cucumbers. Sodininkystė ir daržininkystė,. 26(1): 166-175.

Flore, Z.; Mustafa, A.; Inês, S.; Ibrahim, C. and Sara, M. (2019). Inter-and intra-specific variation of spider mite susceptibility to fungal infections: implications for the long term success of biological, control. CC-BY-NC-ND4.0 International license It pp 1-19.

França, G.V.; Monteiro, V.B.; Debora, B.L. and Manoel, G.C. (2018). Toxicity of acaricides to and the behavioural response of *Steneotarsonemus concavuscutum* (Acari: Tarsonemidae). Crop Protection, 112(2018): 83–89

Grbic, M.; Van Leeuwen, T.; Clark, R.; Rombauts, S.; Rouze, P.; Grbic, V.; Osborne, E.J.; Dermauw, W.; Cao, P. and Ngoc, T. (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature, 479: 487-492

Halim, K.Y.A. and Kalmosh, F.S. (2019). Acaricidal Activity of Nano-Abamectin Against the Two-Spotted Spider Mite; *Tetranychus urticae* Koch (Acari: Tetranychidae). Academic Journal of Life Sciences, 5(10): 81-86.

Hilda, M.B.; Manoel, G.C.G.; José, V.D.O. and Cláudio, A.G.D.C. (2006). Toxicidade de formulações de nim (*Azadirachta indica* A. Juss.) ao ácaro-rajado e a euseius alatus de leon e phytoseiulus macropilis (banks) (acari: phytoseiidae). Neotropical Entomology 35(4): 500-505.

Hooman, H.N. (2017). Characterization of acaricide resistance, plantmediated RNAi against two-spotted spider mites (*Tetranychus urticae* Koch), and assessing off and non-target effects. Ph.D. Thesis, University of Western Ontario.

Huzefa, R. (2014). Two spotted spider mite (*Tetranychus urticae*) selection to *Arabidopsis thaliana*. Thesis The University of Western Ontario, 12-13.

Ibrahem, A.S.; Majeda, M.M. and Randa, A.S. (2019). Controlling the population of *Tetranychus urticae* Koch on tomato under the greenhouse conditions using some chemical and biological treatments.

Ji, X.X.; Qiao, K. and Qiu, S.F. (2013). Evaluation of abamectin 18 g/L EC in controlling *Tetranychus cinnabarinus* (Boisduval). Biological Disaster Science, 36(4): 417-419.

Kanika, T.; Rachna G. and Monika G. (2016). Host Plant Responses to *Tetranychus urticae* Koch Mediated Biotic Stress and Management Strategies. Dynamics of Crop Protection and Climate Change, chapter 4, Studera Press, 93.

Karen, V.D.A.; Rodriguez-Maciel, J.C.; Lagunes-Tejeda, A.; Aguilar-Medel, S.; Tejeda-Reyes, M.A. *et al*. (2019). Resistance to Abamectin in Field Population of *Tetranychus urticae* Koch (Acari: Tetranychidae) Associated with Cut Rose from State of Mexico, Mexico. Florida Entomologist, 102(2).

Kavya, M.K. (2018). Toxicity of newer acaricides to two spotted spider mite, *Tetranychus urticae* koch (acari: tetranychidae) infesting brinjal, *Solanum melongenalinn*. Ph.D. Thesis, University of Agricultural Sciences, Bengaluru.

Keratum, A.Y.; Hosny, A.H. and Hasan, N.E. (2010). Comparative efficiency of pesticides and some predators to control spider mites: 1-Toxicological studies of some environmentally safe chemicals against the two spotted spider mite, *Tetranychus urticae* and their predators *Amblyseius gossipi*, *Phytoseiulus macropili* and *Stethorus gilvifrons*. J. Plant Protect. Pathol. Mansoura Univ., 1: 1049-1063.

Khalis, A.H.A. and Omar, O.H. (2018). The Relationship between Temperature, *Tetranychus urticae* and Cucumber Hybrids, ZANCO. Journal of Pure and Applied Sciences, 30(5) : 114-122.

Kos, M.; van Loon, J.J.; Dicke, M. and Vet, L.E. (2009). Transgenic plants as vital components of integrated pest management. Trends in Biotechnology, 27: 621-627.

Kumari, S.; Chauhan, U.; Kumari, A. and Nadda, G. (2017). Comparative toxicities of novel and conventional acaricides against different stages of *Tetranychus urticae* Koch (Acarina: Tetranychidae) J. Saudi Soc. Agric. Sci.; 16: 191–196.

Kumari, D.A.; Reddy, G.R. and Vijaya, D. (2019). Efficacy of Insecticides on Mites in Grape. International Journal of Current Microbiology and Applied Sciences. ISSN: 2319-7706, 8(1): 3000-3006.

Lagziri, M. and El-Amrani, A. (2009). Effect of a microbial-based acaricidal product on spotted and predatory spider mites. African Crop Science Journal, 17(3).

Manal, A.R.A.M.A.R. and Hany, M.H. (2019). Toxicity of Some Pesticides and Plant Extracts on *Tetranychus urticae* and its Predator, *Phytoseiulus persimilis*. International Journal of Zoological Research, 15(1): 28-37.

Mohamed, E.O.; Amany, A.A.; Mohamed, A.N. and Gohyza, A.H. (2019). Myco-metabolites as biological control agents against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Egyptian Journal of Biological Pest Control 29:64.

Nabi, A.K.; Pınar, H.G.; Elif, A.A.K. (2017). Biological parameters and population development of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) on different pepper cultivars. Turk. Entomol. Derg., 41(3): 263-273.

Niu, Z.M.; Xie, P.; Yu, L. and Bi, J.L. (2014). Efficacy of selected acaricides against the two-spotted spider mite *Tetranychus urticae* on strawberries in greenhouse production.

Omar, O.H. and Khalis, A.H.A. (2019). Population dynamic of *Tetranychus urticae* on cucumber in Erbil region with study of the effects three different miticides on T. *urticae*. IOP Conf. Series: Journal of Physics: Conf. Series, 1294.

Paramjit, K. and Frank, G.Z. (2017). Effect of temperature on the development of *Tetranychus urticae* and *Eotetranychus lewisi* on strawberry, Journal of Entomology and Zoology Studies, 5(4): 441-444.

Parolin, P.; Bresch, C.; Desneux, N.; Brun, R.; Bout, A. and Bollr, P.C. (2012). Secondary plants used in biological control: A review. International Journal of Pest Management, 58: 91-100.

Premalatha, K. and Chinniah, C. (2017). Evolving an integrated management strategy for effective suppression of mite pests infesting tomato. Journal of Entomology and Zoology Studies, 5(6): 38-42.

Ramaraju, K. (2004). Evaluation of acaricides and TNAU neem oils against spider mite, *Tetranychus urticae* (Koch) on *bhindi* and brinjal. Madras Agricultural Journal. 2004; 91(7-12): 425-429.

Ricardo, A.R.; Daniel, R. and Ericsson, C. (2019). Botanicals against *Tetranychus urticae* Koch under laboratory conditions: a survey of alternatives for controlling pest mites. Plants (Basel). Aug.; 8(8): 272.

Rincón, R.A.; Rodríguez, D. and Coy-Barrera, E. (2019). Botanicals Against *Tetranychus urticae* Koch Under Laboratory Conditions: A Survey of Alternatives for Controlling Pest Mites. Plants, 8(8): 272.

Sabrine, A.; Kaouthar, G.L.; Stéphanie, H.; Georges, L. and Thierry, H. (2015). An analysis of potential resistance of the phytophagous mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) to four botanical pesticides. Biotechnol. Agron. Soc. Environ., 19(3): 232-238.

Siddhapara, M.R. (2015). Biology, seasonal incidence and management of red spider mite, *Tetranychus urticae* koch in okra. PhD thesis, College of Agricutlure, Junagadh Agricutlural University, Junagadh.

Tarikul, I. (2019). Host Plant-Induced Susceptibility of Two-Spotted Spider Mite *Tetranychus urticae* (Acari: Tetranychidae) to Some Reduced-Risk Acaricides. American Journal of Agricultural and Biological Sciences, 14: 11-15.

Tarikul, I.; Md. Jamil, H.B.; Mohammad, T.H.H. and Mohammad, S.U. (2017). Laboratory evaluation of *Beauveria bassiana*, some plant oils and insect growth regulators against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), Persian J. Acarol., 6(3): 203–211.

Vassilis, A.V. and Pavlos, K. (2013). Acaricide Resistance in *Tetranychus urticae* (Acari: Tetranychidae) Populations From Cyprus. Journal of economic entomology, 106(4): 1849-1854.

Vijayalakshmi, K. (2002) Preparation of neem biopesticides at farm level, appropriate technology, part 111: 169.

Wekesa, V.W.; Vital, S.; Silva, R.A.; Ortega, E.M.M.; Klingen, I. and Delalibera, I. (2011). The effect of host plants on *Tetranychus evansi*, *Tetranychus urticae* (Acari: Tetranychidae) and on their fungal pathogen *Neozygites floridana* (Entomophthorales: Neozygitaceae). Journal of Invertebrate Pathology, 107(2): 139–145.

Wu, Y. and Liu, X. (1996). Toxicity and biological effect of abamectin on *Tetranychus cinnabarinus* in lab. Acta Agriculturae Boreali-sinica, 12(1): 108-111.

Yasin, N.A.; Emre, I. and Selçuk, U. (2020). Acaricide resistance and mechanisms in *Tetranychus urticae* populations from greenhouses in Turkey, Systematic and Applied Acarology, 25(1): 155-168.

Youssef, R.M. and Faiod, D.M. (2013). Primary study about the effect some practical procedures in management of resistant two spotted red spider mite of *Tetranychus urticae* koch to dimethoate 14(4). 2224-9796.

Zindel, R.; Gottlieb, Y. and Aebi, A. (2011). Arthropod symbioses: a neglected parameter in pest-and disease-control programmes. Journal of Applied Ecology, 48: 864–872.