Cairo University
Faculty of Agriculture
Zoology and Agricultural Nematology Department

New Approaches in Controlling Red Spider Mite *Tetranychus urticae* Koch on Tomato Plants with Reference to Genetic Mutations Associated with Pesticides

By

MAHMOUD MONIR AHMED ABD-ELMOHSIN
B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2006
M.Sc. Agric. Sci. (Agric. Zoology), Fac. Agric., Cairo Univ., 2011

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INTRODUCTION

Tomato *Solanum lycopersicum* L. (Family: Solanaceae) is considered one of the most important vegetable crops. Egypt is the fifth greatest producer of tomatoes in the world, 531,115 Fa. of the crop grown annually, and an average productivity of 8105260 tons (FAO 2013). Tomato production for fresh consumption is the most important source of income for small producers in several region of country. However, this vegetable crop is often severally damaged by many phytophagous pests (insects and mites). In Egypt, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is become one of the most important pests on many greenhouse and filed vegetable corps, especially Solanaceae and Cucurbitaceae which can build to high population densities and cause serious damage to tomato plants. The two-spotted spider mite *T. urticae* is highly polyphagous and has been recorded to feed from over 1100 plant species, among them tomato (Dermauw *et al.* 2012).

The application of synthetic acaricides are the most widely methods used for controlling *T. urticae*. One of the major problems in the control of *T. urticae* is their ability to rapidly develop resistance to many important acaricides after only a few applications because of the frequent use of pesticide, (Van Pottelberge *et al.* 2008). Several groups of insecticides have been used to control *T. urticae*, such as organophosphates (OPs) and carbamates, targeting acetylcholinesterase, pyrethroids and avermectins, targeting the voltage-gated sodium channel and glutamate-gated chloride channels, etoxazole and bifenazate targeting chitin synthase 1 and cytochrome b, respectively. (Helle 1962 and Van Leeuwen *et al.* 2009).

Many resistance mutations associated with target site of pesticides include, G119S, A201S, T280A, G328A and F331W in acetylcholinesterase gene (*ace*); L1024V, A1215D, F1538I in the voltage-gated sodium channel gene (*VGSC*); G314D and G326E in glutamate-gated chloride channel genes (*GluCls*); G126S, I136T, S141F, D161G, P262T in the cytochrome b (*cytb*) and I1017F in the chitin synthase 1 gene (*CHS1*), (Van Leeuwen *et al.* 2008, Tsagkarakou *et al.* 2009, Van Nieuwenhuyse *et al.* 2009, Khajehali *et al.* 2010, Kwon *et al.* 2010a, b and c, Dermauw *et al.* 2012, Van Leeuwen *et al.* 2012 and Ilias *et al.* 2014).

To reduce these problems, it is necessary to minimize the chemical control. Synthetic pesticides have been used less frequently, consumption rates dropped 61% by (FAO 2001), but the market share remains high. In contrast, the consumption of botanical and biological products had increased 69% by (FAO 2001). In recent years, the use of plant-derived chemical compounds has increased, reaching 7.6% of the world market (FAO 2001 and Yu 2008).

Induced response in plants is one of the important components of pest control in agriculture, and has been exploited for regulation of pest herbivore population, (Howe and Jander 2008, Sharma *et al.* 2009 and Agrawal 2011). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores, (Hanley *et al.* 2007). Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores, (Arimura *et al.* 2009).

Induced resistance could be exploited as an important tool for the pest management to minimize the use insecticides in pest control (War *et al.* 2012). Plants respond to herbivory through various morphological, biochemicals, and molecular mechanisms to counter/offset the effects of herbivore attack (War *et al.* 2012). Most of the plant defense responses against insects are activated by signal transduction pathways mediated by Jasmonic acid, Salicylic acid, and ethylene (Gill *et al.* 2010 and Shivaji *et al.* 2010). A broad spectrum of defensive responses are induced by jasmonates that include antioxidative enzymes, proteinase inhibitors (PIs), volatile organic compounds (VOCs), alkaloid production, trichome formation, and secretion of extra floral nectar (EFN) plays an important role as plant indirect defence (Mao *et al.* 2007, Dickens 2006 and Pauwels *et al.* 2009).

Most studies on conservation biological control of *T. urticae* focus on predators (Fejt and Jarosik 2000, Heikal and Fawzy 2003, Heikal *et al.* 2003, El-Saiedy *et al.* 2008, Sarwar *et al.* 2011 and Afifi *et al.* 2013a). These predators are frequently responsible for maintaining spider mite populations below damaging levels and play an important role in their natural control (Croft and Luh 2004). The compatibility of two entomopathogenic fungi, *Beauveria bassiana* and *Paecilomyces fumosoroseus*, in combination with plant extracts, garlic-pepper, along with the predatory mites. *Phytoseiulus persimilis* and *Neoseiulus californicus* were evaluated by (Numa-Vergel *et al.* 2011). It provided safe environment resulted in reduction in pesticides application for about 60% for tomatoes and 70% for cucumbers, (Granges and Leger 1998).

This study covered the following points in a trial to reach an integrated control for red spider mite, *T. urticae* on tomato plants by means of decreasing the effects of genetic mutations associated with the frequent use of synthetic pesticides.

1. Identified resistance mutations associated with target site of pesticides in field populations of *T. urticae* using quantitative sequencing.
2. Evaluation the susceptibility of five tomato hybrids to *T. urticae* infestation during two seasons and the effect of the morphological, histological leaf characteristics and its phytochemical components on the infestation rate.
3. Evaluate the acaricidal activity of seventeen-plant extracts and six essential oils compared with three recommended acaricides against *T. urticae* and its predators; *Phytoseiulus persimilis* (A.-H) and *Orius albidipennis* (Reuter).
4. Enhancement of resistance in tomato plants using different compounds against the two-spotted spider mites *T. urticae*.
5. Compatibility and integration between some control methods in controlling *T. urticae* infesting tomato plants in open field.

Materials and methods

Part (1). The genetic mutations associated with pesticides resistance in field populations of *Tetranychus urticae* using quantitative sequencing.

Samples collection

samples of mites *Tetranychus urticae* Koch were collected from tomato plants (*Solanum lycopersicum* L.) grown in open field, Barnasht village, El-Ayat, Giza Governorate. Approximately 120 adult mites (females and males) of *T. urticae* were transferred to plastic tube, and *T. urticae* were stored in 95% ethanol until use.

DNA extraction and PCR reaction

DNA extraction of mass mites Genomic DNA was prepared by crushing approximately 120 adult mites (females and males) with a plastic pestle in a 1.5-mL micro centrifuge tube, using GeneJET Genomic DNA Purification Kit, (Thermo scientific#K0721). PCR reaction (25 µl) contained 2 µl genomic DNA, 2 µL primers (Primer forward and Primer reverse), 1µL (dNTP Mix, 2 mM each (#R0241)), 2.5 µl of 10X DreamTaq buffer, 0.25 µl DreamTaq DNA Polymerase and Water, nuclease-free (#R0581) 17.25 µL. The thermal conditions were: 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, (Annealing °C of gene) for 30 s, 72 °C for 60 s, and final extension at 72 °C for 10 min. DNA sequences were aligned and compared

online with the published protein by similarity search engines such as BLAST in the NCBI Web.

Part (2). Evaluate the acaricidal activity of seventeen-plant extracts and six essential oils compared with three recommended acaricides against *Tetranychus urticae* Koch and its predators; *Phytoseiulus persimilis* (A.-H) and *Orius albidipennis* (Reuter).

The study included testing the toxicity of seventeen crude plant extracts compared with acaricide; Maccomite 10% and six essential oils compared with two acaricides; Milbeknock 1% EC and Nimbecidine 0.03 % to *Tetranychus urticae* Koch and its predators *Phytoseiulus persimilis* (A.-H) & *Orius albidipennis* (Reuter) under laboratory conditions by using spraying technique. The essential oil compounds were also determined by GC-MS.

This study covered seventeen plant species in Table (1) which tested as an alternative miticidal.

Table 1. Ornamental & medicinal plants evaluated against the two-spotted spider mite *Tetranychus urticae* Koch and its predators, *Phytoseiulus persimilis* (A.-H) and *Orius albidipennis* (Reuter).

English name	Scientific name	Family name	Plant part used	Obtained from
Chili peppers	*Capsicum annuum* L.	Solanaceae	Fruits	Local market
Lemongrass	*Cymbopogon citrates* L.	Graminae (Poaceae)	Herbal	*
Fennel	*Foe niculum vulgare* L.	Umbelliferae (Apiaceae)	Herbal and seeds	*
Coriander	*Coriandrum sativum* L.	Umbelliferae (Apiaceae)	herbal	*
Dill	*Anethum graveolens* L.	Umbelliferae (Apiaceae)	herbal	*
Cypress lemon	*Cupressus sempervirens*	Cupressaceae	herbal	*
Khilla Baladi	*Ammi visnaga* L.	Umbelliferae (Apiaceae)	Seeds	Local market
ambrosia	*Ambrosia maritima* L.	Asteraceae	Leaves	*
Lemon balm	*Melissa officinalis* L.	Lamiaceae (Labiatae)	Leaves	*
Lupine	*Lupinus albus* L.	Fabacea	Seeds	Local market
Black pepper	*Piper nigrum L.*	Piperaceae	Fruits	Local market
Jojoba	*Simmondsia chinensis* Link.	Buxaceae	Seeds	Local market
Capsicum	*Capsicum minimum*	Solanaceae	Fruits	Local market
oleander	*Nerium oleander*	Apocynaceae	Leaves	*
Rosemary	*Rosmarinus officinalis* L.	Labiatae (Lamiaceae)	herbal	*
Thevetia	*Cascabela thevetia* L.	Apocynaceae	Leaves	*
acokanthera	*Acokanthera spectabilis*	Apocynaceae	Leaves	*

* Cairo University Faculty of Agriculture Ornamental horticulture Department

Essential oils

Six of pure essential oils obtained from Kato aromatic company, Egypt were used in this study, table (2).

Table 2. The essential oils evaluated against *T. urticae* and its predators. *P. persimilis* and *O. albidipennis*.

English name	Scientific name	Family name
lemongrass	*Cymbopogon citrates* L.	Graminae (Poaceae)
Fennel	*Foeniculum vulgare* L.	Umbelliferae (Apiaceae)
Basil	*Ocimum basilicum* L.	Labiatae (Lamiaceae)
Peppermint	*Mentha piperita* L.	Labiatae (Lamiaceae)
Rosemary	*Rosmarinus officinalis* L.	Labiatae (Lamiaceae)
Cinnamon	*Cinnamomum verum*	Labiatae (Lamiaceae)

Acaricides

The three recommended acaricides for controlling the two-spotted spider mite *T. urticae* were Maccomite 10% WP; Milbeknock 1% EC and Nimbecidine 0.03 % in Table (3).

Table 3. Trade name, Active ingredient and rat of application of the three tested acaricides against the two-spotted spider mite *T. urticae*.

Trade name	Active ingredient (s)	Mode of action	Usage	Rate of application	Obtained from	Producted by
Maccomite 10% WP	Hexythiazox	Non- Systemic with contact and stomach	Acaricide	20g/hl water	Cairo chemical company	Nippon Soda Co., LTD. Japan
Milbeknock 1% EC	Milbemectin	Acts on the nervous system mediated by the inhibitory neurotransmitter, GABA	Acaricide & Insecticide	50cm^3/hl water		Sankyo Agro. Co.,LTD. Japan
Nimbecidine 0.03 %EC	Azadirachtin	- Antifeedant - Repellent - Ovi-position Deterrent - Insect Growth Regulator - Sterilant	Insecticide & Acaricide	500cm^3/hl Add water to Nimbecidine	Gaara Establishment for Import and Export In Egypt	T.Stanes & Company Limited India

Mortality percentages were calculated for the *T. urticae* adult, *P. persimilis* movable stages and *O. albidipennis* 2nd Nymphs after 24 h of spraying and estimated by Abbot's formula (1925). The numbers of hatching and non-hatching eggs were recorded, and un-hatchability of *T. urticae* eggs was corrected by Abbot's formula (1925). LC50, LC90 and slope values were computed according to Finney (1971), and using Ldp line soft were according to Bakr (2000).

Part (3). Induce resistance in tomato plants using ten different compounds against the two-spotted spider mites *Tetranychus urticae* Koch.

Tomato hybrid: Tomato hybrid super strain B was chosen for this study. It was obtained from Agrimatco For Agriculture Company, Egypt.

Experimental procedure: these experiments were conducted in Acarology greenhouse, Faculty of Agriculture, Cairo University, Giza Governorate during season 2013. The experimental area was divided into 11 treatments according to complete randomized block design including three replicates for each treatment. Soil was well prepared before the tomato seedlings planted; Tomato plants received all normal agricultural processes without any pesticides application. Tomato hybrid seeds were sown at the first week of January in the nursery then seedlings were planted in the greenhouse at the first week of February and after about one week. The infestation occurred naturally by *T. urticae* on tomato hybrid. The different compounds with their concentration and produced company were tableted in Table (4).

Table 4. Treatments of ten different compounds used against *T. urticae* on tomato plants.

Treat. no.	Compound name	Concentration	Produced company
(T1)	Methyl Jasmonate 97%	0.1ml /L	Duchefa Biochemie B.V. Netherlands
(T2)	Salicylic acid 99%	100 mg/l	Piochem, In Egypt
(T3)	Gibbereillc acid 10%	0.2 g/l	shoura chemicals,In Egypt
(T4)	Pot. Humates 80-85%	1g/l	Humintech GmbH,Germany
(T5)	Propolis	15g in 10 ml acetone + 0.3 ml triton x-100/l water	Imtenan Health Shop company,In Egypt
(T6)	Vital power calico (CaO) 15.28%	12 ml/l	Zoberbac company,In spain nutricion y proteccion de cultivos
(T7)	Universal Ergofito	0.5 ml/l	BEA - Biotecnologie per l'Ecologia e l'Agricoltura, Italiy
(T8)	Potassium silicate	1g/l	PQ Corporation company
(T9)	Legmongrass oil	2 ml+0.3 triton x-100/l	Kato aromatic company In Egypt
(T10)	Pot.humates + Salicylic acid 99%	50 mg salicylic acid 99%+0.5gPot.humates	Mentioned before
(T11)	Control (Water only)		

The application of spray was started after one weak of cultivation. Check treatment was sprayed with water only. A compressor spryer (5 liters capacity) was used. Samples (30 leaves) were randomly collected from treatments (10 / each replicate), just before spraying then weekly afterwards. Additional sprays were conducted in the fourth, seventh and tenth weeks of seedling cultivation. Leaf samples were kept into perforated polyethylene bags, closed with rubber bands, and kept in an ice box then transferred to the laboratory for

examination using a stereomicroscope. Different stages of *T. urticae* were counted and recorded. The essential oil compounds and defense enzymes activity in tomato leaves were also determined.

The morphological leaf characteristics of tomato after treated by ten different compounds against *T. urticae*

Morphological characteristics, samples of tomato leaves were taken from treatment plots and imaged the upper and lower surface of tomato leaves using the Analytical a Scanning Electron Microscopic Technique (SEM) (Joel jsm.6390LA) at the Central Laboratory of Water Station Fustat, Greater Cairo Water Company.

Histological characteristics, samples of tomato leaves were taken from treatment plots and imaged the different cells behind the midrib of tomato leaves using the Transmission Electron Microscopy (TEM) in TEM lab FA-CURP, Faculty of Agriculture, Cairo University - Research Park CURP).

Part (4). Evaluation the susceptibility of five tomato hybrids to *T. urticae* infestation during two seasons and the effect of the morphological, histological leaf characteristics and its phytochemical components on the infestation rate.

Tomato hybrids

Five tomato hybrids were chosen for this study. They were (Supper-gekal and F1Gs-12), (Marwa and El-pasha 1077) and (010), the seeds of five tomato hybrids were obtained from Syngenta, Agrimatco for Agriculture and Tecnogreen Companies Egypt, respectively.

Experimental procedure

Barnasht village, El-Ayat, Giza Governorate was chosen to conduct these experiments during the two successive seasons 2013 and 2014. Five tomato hybrids seeds were sown at the last week of June in the nursery then seedlings were planted in the field at the third week of July and after about one week, samples were taken weekly. The cultivated tomato hybrids received all normal agricultural processes without using pesticides. Weekly samples each of 30 leaves were randomly collected from each tomato hybrid. Samples were collected from the fourth week of July till the last week of October during the two successive seasons (2013 and 2014). Leaf samples were examined using a stereomicroscope and hand lens. Movable stages and eggs of *T. urticae* were counted and recorded.

Part (5). The efficiency of releasing two predators, spraying four products entomopathogenic fungi, three acaricides and lemongrass oil (single or combined treatments) against *T. urticae* infesting tomato plants during two seasons.

For controlling the two spotted spider mite, *T. urticae* on F1Gs-12 hybrid tomato plants; by predatory mites *P. persimilis* and predatory insects *O. albidipennis* were released as biological control agents, and four product (entomopathogenic fungi) a biological insecticide; Bio-Power, Bio-Magic, Bio-Catch and Priority contains spores and mycelial fragments $1x10^9$ CFU's/ml of *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Paecilomyces fumosoroseus*, , respectively, they were obtained from T.Stanes & Company Limited India by Gaara Establishment for Import and Export In Egypt. The rate of application was $5cm^3$/L water. In addition to three acaricides; Maccomite 10% WP; Milbeknock 1% EC and Nimbecidine 0.03 % and lemongrass oil were used in control of *T. urticae*; they were recommended as acaricides for controlling the red spider mite. The rate of application of three acaricides and lemongrass oil was 20g/hl water, $50cm^3$/hl water, $500cm^3$/hl water and $1000cm^3$/hl water, respectively.

The application of release and spray started on 10^{th} August during season (2013), and on 9^{th} August during season (2014). Experiment was contained sixteen treatments and check treatment, each treatment was contained four replicate. Check treatments were sprayed with water only. A compressor spryer (20 liters capacity) was used. Samples (10 leaves of tomato) were taken randomly from each replicate, just before releasing or spraying then weekly afterwards. Samples were carefully examined and the number of alive moving stages and eggs of *T. urticae* were recorded. An additional spray was conducted during the third and fifth, seventh and ninth weeks of the first spray and an additional release were conducted during the fifth and ninth weeks of the first treatment because of the increase of *T. urticae* population.

Statistical analyses of data in all experiments showed that One-way analysis of variance and mean comparison at 5% level of significance using Fisher's least significant difference (LSD). Using the Statistical Analysis System (SAS software 1988).Reduction percentages of the *T. urticae* were calculated according to Henderson and Tilton (1955).

$$\text{Reduction \%} \equiv (1 - \frac{\text{Treatment after} \times \text{Control before}}{\text{Treatment before} \times \text{Control after}}) \times 100$$

RESULTS

The results revealed the following:-

1. **Genetic mutations in spider mites associated with pesticides**

Some of common mutations associated with different groups of pesticides identified in samples of *T. urticae* collected from Tomato plants grown in open field, Bernasht village, El-Ayat, Giza Governorate.

a. Comparison of *T. urticae acetylcholinestrerase* gene amino acid polymorphism between Eg-Bernasht field strain and reference GenBank strains.

1. Three from the five common mutations associated with OPs and Carbamates pesticides identified in Eg-Bernasht strain; a threonine residue is replaced by an alanine (T280A), a glycine is replaced by an alanine (G328A) and a phenylalanine is replaced by a tryptophan (F331W).

b. Comparison of *T. urticae VGSC, GluCl1, GluCl3* and *CHS1* genes amino acid polymorphism between Eg-Bernasht field strain and reference GenBank strains

1. One of the three common mutations associated with Pyrethroids pesticides identified (A1215D) an alanine replaced by an aspartic acid, also identified two substitution of amino acids a leucine to a valine (L988V) and an aspartic acid to a glutamic acid (D1569E).
2. The common mutations associated with target site of avermectins (Glutamate-gated chloride channels, *GluCl1, GluCl3*) and etoxazole pesticides (Chitin synthase, I *CHS1*) were not present in Eg-Bernasht strain.
3. Also, with *Glutamate-gated chloride channels* genes identified in one clones of Eg-Bernasht strain two substitutions of amino acids an alanine to a leucine (A308L) and a valine to a leucine (V309L).

2. **Ecological studies**

Five tomato hybrids were chosen for this study. They were Supper-gekal, F1Gs-12, Marwa, El-basha 1077 and Salymia (65010). These experiments were conducted during the two successive seasons 2013 and 2014.

a. Population dynamics of the two spotted spider mite, *T. urticae* during 2013 and 2014 seasons

1. The infestation of five tomato hybrids with *T. urticae* started on the 4th week of July then it gradually increased to reach its peaks during September and October in seasons 2013 and 2014, respectively.The highest population density of *T. urticae*

found during 2013 than in 2014 may be due to environmental conditions.

2. The number of *T. urticae* movable stages and eggs in peaks of infestation averaged (53.20 & 45.17; 48.40 & 42.17; 46.17 & 37.53; 40.80&32.70 and 36.10 & 29.43) for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia (65010) hybrids during 2013 season respectively.
3. While during 2014 season it averaged (42.87 & 39.33; 36.83 & 30.73; 33.17 & 30.67; 30.50&27.27 and 25.87 & 22.73) and (42.87 & 31.53; 40.40 & 30.67; 35.40 & 27.33; 29.73&26.10 and 27.63 & 22.17) in two peaks of infestation (the 2nd week of September and the 1st week of October) for aforementioned tomato hybrids, respectively.

b. Host plant resistance

1. **Susceptibility of five tomato hybrids to *T. urticae* infestation during 2013&2014 seasons**

a. Supper-gekal and F1Gs-12 hybrids were the most highly significant susceptible to infestation. It recorded 31.16 (25.10%**)** & 24.61 **(**24.82%) and 28.10 (22.63%) & 22.09 (22.28%) moving mite stages / leaf during the two successive seasons, respectively.

b. The moderately infestation was observed on hybrids (El-basha 1077 and Marwa), being 25.20 **(**20.29%) & 19.80 (19.96%) for the former; 21.32 (17.17%) & 17.67 (17.82%) for the later hybrid during the two seasons, respectively.

c. The lowest infestation was observed on Salymia (65010) hybrid which recorded, 18.38 (14.81%) and 15.00 (15.13%) during the two successive seasons, respectively.

2. **Effect of the morphological leaf characteristics of five tomato hybrids on *T. urticae* infestation**

Susceptibility of tomato hybrids to infestation with *T. urticae* may be affected by plant leaf morphological structure; the shape of epidermal cells, Length, thickness and density of leaf trichomes. The average number of upper surfaces trichomes /cm2 was 2606, 3939, 5788, 7030 and 10970, while the average number of lower surfaces trichomes /cm2 was 8606, 10557, 9000, 13870 and 16818 for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia (65010) respectively.

3. **Effect of the histological leaf characteristics of five tomato hybrids on *T. urticae* infestation**

Tomato hybrids leaf histological structure may be affected on *T. urticae* infestation. The Mean thicnths µm of lower epidermis was 19.61, 18.85, 27.57, 22.63 and 26.55, while the upper epidermis was 25.64, 28.58, 27.77, 26.78 and 30.50 for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia (65010) respectively.

4. **Effect of some phytochemical components of five tomato hybrids on the infestation rates of *T. urticae***

The high mean number of *T. urticae* movable stages occurred on the leaves of Supper-gekal and F1Gs-12 hybrids were associated with higher levels of total carbohydrates and this indicates a positive correlation with the population densities, while the reverse was true with alkaloids, total phenolic compounds and total flavonoids indicated negative correlation with the population densities as the resultant throughout the growing season.

3. Toxicological studies

The toxicity of seventeen crude plant extracts, and six essential oils and three acaricides to *T. urticae* and its predators; *Phytoseiulus persimilis* (A.-H) and *Orius albidipennis* (Reuter) was conducted under laboratory conditions using spraying technique and four essential oils analysed by GC-MS to determined their volatile components.

a. Toxicity of seventeen crude plant extracts and Maccomite 10% to *T. urticae* and its predators

1. According to their values of LC_{50} and LC_{90}, the best acaricidal activity of tested seventeen crude plant extracts against *T. urticae* adults recorded with Chilli pepper, lemongrass, Fennel, Coriander and Dill, (3.36, 3.89, 4.03, 4.80 and 4.88 mg/ml) and (11.68, 8.53, 12.97, 15.37 and 14.07 mg/ml), respectively, while against *T. urticae* eggs were Chilli pepper and Fennel, (3.11& 3.31) and (10.30 & 15.93 mg/ml), respectively.
2. The lowest toxicity of these extracts on two predators, was Cypress lemon on *P. persimilis* (LC_{50}=16.59 and LC_{90} = 168.08 mg/ml) and Coriander on *O. albidipennis* (LC_{50}=72.38 and LC_{90} = 1347.33 mg/ml).
3. The Maccomite 10% was high toxic on *T. urticae* adults (LC_{50}=0.03 & LC_{90} = 0.11) and eggs (LC50=0.02 and LC_{90} = 0.08 mg/ml) than on two natural enemies; *P. persimilis* (LC_{50}=0.04 and LC_{90} = 0.21 mg/ml) & *O. albidipennis* (LC_{50}=0.07 and LC_{90} = 0.26 mg/ml).

b. Toxicity of six essential oils compared and two acaricides; Milbeknock 1% EC & Nimbecidine 0.03 % to *T. urticae* and its predators; *P. persimilis and O. albidipennis.*

1. According to their values of LC_{50} and LC_{90}, the best acaricidal activity of

essential oils against *T. urticae* adults recorded with Lemongrass oil, peppermint oil, fennel oil and basil oil, (2.88, 4.55, 4.62 and 4.75 ml/L) and (6.63, 11.08, 10.26 and 10.78 ml/ L), respectively, while against *T. urticae* eggs were fennel oil, peppermint oil and Lemongrass oil, (3.72, 4.51and 5.03) and (9.67, 20.30 and 10.64 ml/ L), respectively.

2. The lowest toxicity of essential oils on two predators, was Rosemary oil on *P. persimilis* (LC_{50}=7.658 and LC_{90} = 17.617 ml/L) and on *O. albidipennis* (LC_{50}=7.496 and LC_{90} = 63.602 ml/L).
3. The two acaricides, Nimbecidine 0.03 % and Milbeknock 1% EC recorded higher toxicity on *T. urticae* adults (LC_{50}=0.0001 & LC_{90} = 0.002) and (LC_{50}=0.002 & LC_{90} = 0.005) and on eggs (LC_{50}=0.0005 & LC_{90} = 0.002) and (LC50=0.003 & LC_{90} = 0.015 mg/ml) than on two natural enemies; on *P. persimilis* (LC_{50}=0.001 & LC_{90} = 0.003) (LC_{50}=0.009 & LC_{90} = 0.046) and on *O. albidipennis* (LC_{50}=0.002 and LC_{90} = 0.063) and (LC_{50}=0.013 and LC_{90} = 0.05 mg/ml), respectively.

4. The major volatile components in lemongrass oil were D-Limonene, cis-citral and trans-citral as (45.06, 10.30 and 9.90 conc. % respectively), in basil oil were Estragole, Methyl eugenol and Linalool as (42.08, 8.15 and 8.09 conc. % respectively), in fennel oil were cis-Anethol and Fenchon as (44.97 and 12.91 conc. % respectively), and in peppermint oil were Levomenthol, 1-Menthone and trans-p-Menthone as (32.03, 30.18 and 11.53 conc. % respectively).

4. **Control Studies**

a. Enhancement of resistance in tomato plants against red spider mite *T. urticae*

To induce resistance of tomato plants against *T. urticae* nine different compounds were used. The effect of these compunds on *T. urticae*, the essential oil components and defense enzymes activity in tomato leaves were determined.

1. The effect of different compounds application in tomato plants against *T. urticae* infestation.

a. The highest reduction percentages of *T. urticae* movable stages were recorded with Potassium humates, (55.38%) followed by Salicylic acid, (Potassium humates + Salicylic acid), Methyl jasmonate and Potassium silicate (50.76, 47.34, 46.37and 37.81%) respectively.While in *T. urticae* eggs, it recorded with Potassium humates, (57.24%), followed by (Potassium humates +

Salicylic acid), Potassium silicate and Methyl jasmonate 49.78, 46.66 and 35.64), respectively.

2. **The effect of different compounds application on phytochemical components and defense enzymes activity in tomato plants against *T. urticae* infestation.**
    a. The treated tomato plants by compounds; Potassium humates, Salicylic acid, Methyl jasmonate, Potassium humates + Salicylic acid and Potassium silicate increased some phytochemical components in tomato leaves which affected on *T. urticae* infestation such as alkaloids, phenolic compounds and flavonoids.
    b. The application of different elicitors, particularly Potassium humates, Salicylic acid, (Potassium humates + Salicylic acid), Methyl jasmonate and Potassium silicate enhanced concentrations of essential oil components and enzymatic and non-enzymatic antioxidants in tomato leaves infested by *T. urticae*, thus suppressing invasion-induced oxidative damage and enhancing tolerance.
3. **The effect of different compounds application on trichome density in tomato plant leaves against *T. urticae* infestation**
    a. Most of the used compounds increased trichomes / cm^2 except Propolis, Salicylic acid and Lemongrass oil decreased the number of trichomes compared with untreated plants (control).
    b. The highest increase number of lower surfaces trichomes / cm^2 recorded with treatments; Gibbereillc acid, Potassium humates, Potassium humates + Salicylic acid, Methyl jasmonate and Vital Power Calcio Cao averaged 10090.91, 9696.97, 7360.91, 7248.48 and 6212.12 trichomes / cm^2, respectively.
    c. The treatments; Methyl jasmonate, Potassium silicate, Potassium humates + Salicylic acid, Vital Power Calcio Cao and Potassium humates recorded the highest increase number of upper surfaces trichomes / cm^2 averaged 7151.51, 3444.44, 3428.57, 2984.13 and 2484.85, respectively, compared with untreated plants (control) averaged 2206.35 trichomes / cm^2.

b. Filed applied control

An experiment was conducted to estimate the efficiency of the two predators; *P. persimilis* and *O. albidipennis* , four products of entomopathogenic fungi; (Bio-Power, Bio-Magic, Bio-Catch and Priority contains spores & mycelial fragments 1x109 CFU's/ml of

Beauveria bassiana, *Metarhizium anisopliae*, *Verticillium lecanii* and *Paecilomyces fumosoroseus*, respectively), three recommended acaricides; (Maccomite 10% WP, Milbeknock 1% EC and Nimbecidine 0.03 % EC) and Lemongrass oil for controlling *T. urticae* on tomato plants during the two successive seasons 2013and 2014 in open field at Barnasht village, El-Ayat, Giza Governorate. The results revealed the following:-

1. The highest reduction percentages (≤ 70 %) of *T. urticae* recorded with treatments; (*P. persimilis* + Milbeknock 1% EC), (*P. persimilis* + Nimbecidine 0.03%EC), (*P. persimilis* + Priority + Nimbecidine 0.03% EC) and (*P. persimilis* + Priority + Milbeknock 1% EC), it averaged 76.76, 74.89, 73.91 and 73.31%, respectively.
2. The moderately reduction percentages (≤50 to70> %) recorded with treatments; Milbeknock 1% EC, *P. persimilis*, Maccomite 10% WP, Nimbecidine 0.03% EC, (*P. persimilis* + Priority) and *O. albidipennis*, it averaged 60.88, 60.33, 59.07, 58.76, 58.41and 50.22%, respectively.
3. The lowest reduction percentages (> 50%) of *T. urticae* recorded with treatments; Lemongrass oil, (*P. persimilis* + *O. albidipennis*), Bio-Power, Bio-Magic, Priority and Bio-Catch, it averaged 46.10, 38.34, 33.89, 32.22, 28.65 and 25.95 %), respectively.
4. The combination of (Milbeknock 1% EC or Nimbecidine 0.03% EC) with *P. persimilis* releases had highly effective for management of the TSSM on tomato plants in open field. The combination of the predatory mite with the predatory insect had less effective than single treatment of each.