J. Agr. Sci. Tech. (2021) Vol. 23(6): 1281-1295

Developing a Vegetable Oil Formulation as a Safe Acaricide against *Tetranychus urticae*

M. Bashiri[1], S. Moharramipour[1*], and M. Negahban[2]

ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch, is a highly polyphagous pest that is considered a serious pest worldwide. Due to problems associated with chemical pesticides such as resistance to pesticides and environmental contamination, plant oils have been considered for use against mites' control. However, their low solubility in water and phytotoxicity are the major constraints that limit their application. In this research, a developmental screening process was carried out on some commercial emulsifiers and stabilizers to obtain a safe acaricide from suitable vegetable oils including castor and soybean. Among eight emulsifiers, Nonylphenol Ethoxylate 6M (NPE6) had far more excellent emulsification ability and less phytotoxicity with some level of mite toxicity. Among vegetable oils, castor oil had greater toxicity in comparison with soybean oil. Also, Polyethylene Glycol 400 (PEG 400) had more thermal stability in the formulation. In the last step, the best ratios of NPE6, castor oil, and PEG 400 were evaluated for their toxicity, stability, and phytotoxicity. Finally, the ratios of 1:8:1 or 2:6:2 (NPE6: Castor oil: PEG 400) were found as the best end product that could be potent for use in a large scale rose greenhouse. Also, the efficacy of emulsifier-oil-stabilizer mixtures was investigated against *T. urticae* by two different methods. The results indicated that the petri dish test method caused overestimating in mortality rates compared to the standing leaf test method. New methods such as polymerization can show a new insight for pest control without chemical pesticides.

Keywords: Emulsions, Nonylphenol ethoxylate 6, Phytotoxicity, Polymerization, Spider mite.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae), is considered a serious pest worldwide, particularly in cut rose greenhouses. Spider mites are active mainly on the underside of rose leaves. They suck out the vegetable sap and cause the qualitative and quantitative yield loss of ornamental plants (Khodayari *et al*., 2008; Motazedian *et al*., 2012). Given the high reproduction and short life cycle, it has a high ability to develop resistance to many chemical pesticides (Lee *et al*., 2003; Sedaratian *et al*., 2009). Due to resistance, the number of effective acaricides has decreased, and this in turn has encouraged research on new alternative safe pesticides. It has been demonstrated that botanical pesticides, such as plant essential oils, plant extracts, and vegetable oils are effective for controlling mites of the family Tetranychidae (Lancaster *et al*., 2002; Tsolakis *et al*., 2008; Ismail *et al*., 2011; Attia *et al*., 2013; Bashiri *et al*., 2016; Mohammadi *et al*., 2016; Seifi et al., 2018).

Vegetable oils in combination with surfactants and some polymers are considered

[1]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[2] Department of Pesticide Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization, Tehran, Islamic Republic of Iran.
*Corresponding author; e-mail: moharami@modares.ac.ir

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

as cheap, safe, and applicable alternatives to chemical pesticides (Butler Jr *et al.*, 1993; Yang *et al.*, 2009). Farmers used vegetable oils to control insect pests before the prevalence of chemical pesticides (Davidson, 1991; Puritch, 1981). Oils can suffocate and kill mites by blocking the respiratory system, so that resistance to this mechanism may be impossible (Chapman, 1967). Also, oils help pesticides to penetrate the insects and mites' waxy bodies and eggs. Further studies have focused on the applications of vegetable oils and their commercial formulations on *T. urticae* (Amer *et al.*, 2001; Park *et al.*, 2008; Yu and Chen, 2009). However, oil applications cause phytotoxicity on some plants such as cut roses that have young branches and delicate petals (Chiasson *et al.*, 2004). To overcome this issue, the preparation of suitable formulation techniques such as encapsulation and polymerization can be performed (Bashiri *et al.*, 2016). Vegetable oils are non-polar compounds that can be dispersed in water depending on oil and emulsifier type (Rahate *et al.*, 2007). In this study, castor oil and soybean oil, as active ingredients, are the most widely used oils and ricinoleic and linoleic acids are their major constituents, respectively (Ogunniyi, 2006; Ivanov *et al.*, 2010). Stabilizers in emulsions are mainly polymeric compounds that are placed on the surface of the oil particles and prevent coalescence (Dai *et al.*, 2014).

The goal of this study was to prepare a safe formulation of vegetable oils as an acaricide by screening some commonly used surfactants and polymers against *T. urticae*. In this regard, polymerization is a suitable method that can resolve the limitations of vegetable oils to use in field applications for pest management purposes.

MATERIALS AND METHODS

Chemicals and Reagents

Eight surfactants including Lauryl myristyl Alcohol ethoxylate 3 (LA3), and 7M (LA7), Nonylphenol Ethoxylate 6 (NPE6), and 10M (NPE10), Castor Oil Ethoxylate 36M (COE36), Tallow Amine Ethoxylate 15M (TAE15), Coconut Fatty acid Ethoxylate 10M (CFE10), and Coconut Fatty acid Diethanolamide (CFD) were obtained as a gift from the Kimiagaran Emrooz Ltd, Iran. Glycerol and Polyethylene Glycol 400 (PEG 400) and 600 (PEG 600) were purchased from Dr. Mojallali Chemical Complex, Tehran, Iran.

Vegetable Oils as Active Agents

Castor oil was purchased from Dr. Mojallali Chemical Complex, Tehran, Iran. Soybean oil was obtained from Behshahr Industrial Company, Behshahr, Iran.

Mite Rearing

Tetranychus urticae originated from a research colony without any pesticide exposure for about 10 generations. Mites were reared on *Rosa hybrida* var. Avalanche pots were in a growth chamber set at 25°C, 60–65% R.H., and a photoperiod of 16:8 hour (L:D).

Preparing the Formulation

Emulsion formulation was prepared in three screening steps.

Step 1: All surfactants were mixed with castor and soybean oil separately as an emulsifier-oil mixture at a rate of 1:9 for 10 minutes (10,000 rpm) using the silent crusher homogenizer M (Heidolph, Germany).

Step 2: Glycerol, PEG 400, and 600 were added as a stabilizer to candidate emulsifier-oil mixture at different ratios, and based on physical stability tests (Table 1), the best one (PEG 400) was selected.

Step 3: Finally, formulation indices were investigated on different rates of emulsifier: oil: stabilizer including: 1:1:8, 2:2:6, 3:3:4,

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Table 1. Effects of stabilizers in thermal stability of formulations.[a]

Mixtures	Ratio	4°C	25°C	40°C
NPE6: Castor oil: PEG 600	3:3:4	F	S	S
	4:4:2	F	S	S
NPE6: Castor oil: PEG 400	3:3:4	S	S	S
	4:4:2	S	S	S
NPE6: Castor oil: Glycerol	3:3:4	PhS	PhS	PhS
	4:4:2	PhS	PhS	PhS

[a] Physical condition of compounds: F= Freezing (mixture freeze and melt back again based on temperature), S= Stable (mixture was a clear solution), PhS= Phase Separation (The mixture was divided into two different phases); Mixtures: NPE6= Nonylphenol Ethoxylate 6M, PEG 400 and 600: Polyethylene Glycols 400 and 600.

4:4:2, 1:8:1, 2:6:2, 3:4:3, 4:2:4, 8:1:1, 6:2:2, 4:3:3 and 2:4:4.

Thermal Stability of Formulations

All formulations were poured into 15 mL test tubes and kept in an oven at different temperatures of 4, 25, and 40°C for a month. Then, freezing and phase separation were investigated (Esmaeelian, 2016).

Emulsification Activity and Emulsion Stability

The absorbance of different mixtures of the emulsion was determined using a UV–Vis Spectrophotometer at 400-600 nm, and the appropriate wavelength was selected (Microplate spectrophotometer, Epoch BioTek, USA). The emulsions were prepared at 1 g L^{-1} by distilled water and distilled water was used as the blank (Souza *et al.*, 2006; Nejadmansouri *et al.*, 2016; Li *et al.*, 2020). Since pesticide emulsions were used over the 1 (min) to 24 hours (max), the absorbance of emulsions was measured in 1 and 24 hours after preparation.

For the measurement of emulsion capacity, the method described by Sciarini *et al.* (2009) was used. For this purpose, 5% w/w of emulsifier-oil mixtures were diluted by distilled water and kept at 25°C. Emulsion Capacity (EC) was calculated after 1 and 7 days as follows:

$$EC(\%) = \frac{e_v}{t_v}$$

Where, e_v is the emulsion volume and t_v is the total volume.

Toxicity Tests

Petri dish test:

Acaricidal activity of some treatments was investigated including: (1) Emulsifiers alone (1 g L^{-1}), (2) Emulsifier-oil formulations (1 g L^{-1}), (3) Stabilizers alone (5 g L^{-1}), and (4) Stable mixtures of final emulsifier-oil-stabilizer formulations (1 and 5 g L^{-1}) were investigated against adult females of *T. urticae*.

The acaricidal activity was conducted by the leaf dipping method. Test units were 6 cm diameter plastic Petri dishes without lids for proper ventilation. The leaf discs (2 cm in diameter) were dipped in each solution for 5 seconds and allowed to dry for 15 minutes. Then, the upper surface of rose leaf discs was placed downwards on water-saturated cotton in a petri dish. Fifteen mated adult females (> 3 days old) of *T. urticae* were placed on each leaf disc using a fine soft pointed brush. Four replicates were taken for each treatment. Distilled water was used as a control treatment and no mortality was observed within the experiment. The mortality was assessed 24 hours after treatment. Mites were considered dead when they could not move by fine brush stimulation.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Standing leaf test:

Twenty adult females were placed on each rose leaflet. Leaflets were dipped into the emulsions of the selected emulsifier-oil (5 g L^{-1}) and final emulsifier-oil-stabilizer formulations (5 and 8 g L^{-1}). Afterward, the petiole of leaflets was covered with cotton and placed in a tiny vial filled with distilled water. Mortality was calculated after 24 hours. All the experiments were conducted in the laboratory at 25°C, 60–65% RH., and a photoperiod of 16:8 hour (L:D).

Viscosity Measurements

Kinematic viscosity for emulsions of all final emulsifier-oil-stabilizer mixtures were measured by SVM 300 viscometer (Anton Paar, Austria) at 20°C. Also, for comparison purposes, soybean oil was replaced with castor oil in the final emulsifier-oil-stabilizer formulations and their viscosity was measured.

Phytotoxicity Test

The highest concentrations of selected formulations (5 g L^{-1} for emulsifier-oil and 8 g L^{-1} for emulsifier-oil-stabilizer formulations) were exposed to roses (var. Avalanche) in a commercial glass greenhouse (located in Hashtgerd, Iran) that was equipped with a climate control system (20-24°C, 65–70% RH). Phytotoxicity symptoms such as deformation, spots, etc. were investigated on young and mature leaves within a week and categorized as Slight (S), Low-level (L), Moderate (M), High (H), and No (N) phytotoxicity.

Data Analysis

Normal distribution of data was confirmed using the Kolmogorov–Smirnov test (K–S test) before subjecting to the one-way ANOVA (Tukey's test, $P< 0.05$) and independent t-student test (SPSS, 2011).

RESULTS

Physical Stability of the Formulation

All the emulsifier-oil formulations from step 1 were stable after one month at 4, 25, and 40°C. In step 2, emulsifier-oil-stabilizer formulations that had PEG 400 as stabilizer were stable in all temperatures, but PEG 600 containing formulations were stable in 25 and 40°C, while being frozen at 4°C. Glycerol-containing formulations were divided into separate phases immediately. However, the 4:4:2 formulation was stable just up to 48 h (Table 1). In step 3, all final emulsifier-oil-stabilizer formulations were stable in different temperatures, except 1:1:8 and 2:2:6 formulations that divided into separate phases (Table 2).

Emulsification Activity and Emulsion Stability

Results showed that NPE6 and COE36 caused significantly high absorbance in castor oil emulsions (0.325 and 0.333, respectively). However, after 24 hours, the absorbance of COE36 significantly decreased to 0.255, though it was still in the highest range. Also, in CFD containing emulsion absorbance was in the lowest value and significantly fell after 24 hours (Table 3). In soybean oil emulsions, NPE6 caused significantly higher absorbance than other emulsifiers in 1 and 24 hours after preparation of emulsions (0.462 and 0.407, respectively). The absorbance of each emulsion did not decrease significantly over 24 hours (Table 4).

The emulsion capacity of NPE6 in castor oil emulsifier-oil emulsions was significantly greater than other emulsions after 1 and 7 days (75.67 and 75.33%, respectively). In soybean oil emulsifier-oil emulsions, LA7 (69.66%) and NPE10 (66.67%) caused significantly better emulsion capacity after 1 and 7 days, respectively. Additionally, after 7 days, breaking emulsion and oiling off occurred in both oils emulsions, except for COE36, NPE6, and NPE10 (Table 5).

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Table 2. Thermal stability of emulsifier-oil-stabilizer formulations at different ratios.[a]

Mixture	Ratio	4°C	25°C	40°C
NPE6: Castor oil: PEG 400	1:1:8	PhS	PhS	PhS
	2:2:6	PhS	PhS	PhS
	3:3:4	S	S	S
	4:4:2	S	S	S
	1:8:1	S	S	S
	2:6:2	S	S	S
	3:4:3	S	S	S
	4:2:4	S	S	S
	8:1:1	S	S	S
	6:2:2	S	S	S
	4:3:3	S	S	S
	2:4:4	S	S	S

[a] Physical condition of compounds: F= Freezing (mixture freeze and melt back again based on temperature), S= Stable (mixture was a clear solution), PhS= Phase Separation (The mixture was divided into two different phases); Mixtures: NPE6= Nonylphenol Ethoxylate 6M, PEG 400 and 600: Polyethylene Glycols 400 and 600.

Table 3. Emulsion absorbance values in combination of emulsifier and castor oil at a rate of 1:9.[a]

Emulsifiers [b]	Absorbance at 600 nm (±SE) at 1 g L^{-1}				
	After 1 h	After 24 h	t	df	P-value
COE36	0.333 (0.003)^{a}	0.255 (0.032)^{a}	2.37	4	0.024*
TAE15	0.097 (0.006)^{c}	0.066 (0.004)^{cde}	4.02	4	0.453^{ns}
CFE10	0.116 (0.004)^{bc}	0.106 (0.004)^{bc}	1.68	4	1.000^{ns}
NPE6	0.325 (0.006)^{a}	0.284 (0.006)^{a}	5.01	4	0.909^{ns}
NPE10	0.134 (0.003)^{b}	0.135 (0.004)^{b}	-0.27	4	0.379^{ns}
CFD	0.065 (0.005)^{d}	0.049 (0.004)^{de}	7.55	4	0.002**
LA3	0.119 (0.003)^{b}	0.099 (0.006)^{bcd}	2.80	4	0.335^{ns}
LA7	0.096 (0.004)^{c}	0.092 (0.005)^{bcde}	0.63	4	0.809^{ns}
Control	0.035 (0.001)^{e}	0.037 (0.002)^{e}	-0.93	4	0.101^{ns}
F	700.5	57.75			
df (t, e)	8, 18	8, 18			
P	< 0.001	< 0.001			

[a] Means followed by the same letters in each column are not significantly different (Tukey's test, P< 0.05). ns: Indicates non-significant differences. * and **: Indicate significant differences at 0.05 and 0.01, respectively. [b] Emulsifiers: COE36= Castor Oil Ethoxylate 36M, TAE15= Tallow Amine Ethoxylate 15M, CFE10= Coconut Fatty acid Ethoxylate 10M, NPE6 and 10= Nonylphenol Ethoxylate 6 and 10M, CFD= Coconut Fatty acid Diethanolamide, LA3 and 7= Lauryl myristyl Alcohol ethoxylate 3 and 7M.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Toxicity Tests

In the first test, the acaricidal activity of 8 emulsifiers was investigated at 1 g L^{-1} and NPE6. The mortality percentage (33.96%) was not significantly different from LA3 (22.79%) and LA7 (18.56%). However, it was significantly greater than the other treatments (F= 9.194; df= 8, 22; P=< 0.001) (Figure 1).

In the second test (emulsifier-oil formulations), the acaricidal activity of castor oil and soybean oil significantly varied by different emulsifiers. For castor oil emulsions, the COE36, CFE10, NPE6, and

Table 4. Emulsion absorbance values in combination of emulsifier and soybean oil at a rate of 1:9.[a]

Emulsifiers[b]	Absorbance at 600 nm (±SE) at 1 g L^{-1}		t	df	P-value
	After 1 h	After 24 h			
COE36	0.336 (0.002)[b]	0.214 (0.003)[c]	39.59	4	0.404[ns]
TAE15	0.194 (0.003)[d]	0.112 (0.003)[f]	18.38	4	0.729[ns]
CFE10	0.149 (0.003)[e]	0.140 (0.013)[e]	0.74	4	0.078[ns]
NPE6	0.462 (0.005)[a]	0.407 (0.005)[a]	7.91	4	0.889[ns]
NPE10	0.239 (0.003)[c]	0.251 (0.003)[b]	-2.71	4	1.000[ns]
CFD	0.123 (0.003)[f]	0.103 (0.003)[f]	4.62	4	0.904[ns]
LA3	0.328 (0.003)[b]	0.180 (0.004)[d]	28.07	4	0.726[ns]
LA7	0.228 (0.005)[c]	0.110 (0.005)[f]	17.36	4	0.762[ns]
Control	0.038 (0.002)[g]	0.037 (0.003)[g]	0.18	4	0.259[ns]
F	1327.5	402.8			
df (t, e)	8, 18	8, 18			
P	< 0.001	< 0.001			

[a] Means followed by the same letters in each column are not significantly different (Tukey's test, P< 0.05). ns: Indicates non-significant differences. [b] Emulsifiers: COE36= Castor Oil Ethoxylate 36M, TAE15= Tallow Amine Ethoxylate 15M, CFE10= Coconut Fatty acid Ethoxylate 10M, NPE6 and 10= Nonylphenol Ethoxylate 6 and 10M, CFD= Coconut Fatty acid Diethanolamide, LA3 and 7= Lauryl myristyl Alcohol ethoxylate 3 and 7M.

Table 5. Emulsion capacity of combination of emulsifier and oil (at 1:9 rates) 1 and 7 days after formulation at 50 g L^{-1}.[a]

Emulsifiers[b] (10%)	Emulsion capacity (±SE) (%)			
	Castor oil (90%)		Soybean oil (90%)	
	1 Day	7 Days	1 Day	7 Days
COE36	67.5 (±0.03)[d]	69.0 (±0.58)[c]	65.0 (±0.58)[c]	64.7 (±0.33)[b]
TAE15	Phs	Phs	Phs	Phs
CFE10	75.0 (±0.00)[a]	Phs	62.0 (±0.00)[d]	Phs
NPE6	75.7 (±0.33)[a]	75.3 (±0.3)[a]	62.7 (±0.33)[d]	62.0 (±0.00)[c]
NPE10	72.3 (±0.33)[c]	72.0 (±0.0)[b]	67.0 (±0.0)[b]	66.7 (±0.33)[a]
Kemamide	Phs	Phs	Phs	Phs
LA3	Phs	Phs	67.3 (±0.33)[b]	Phs
LA7	73.7 (±0.33)[b]	Phs	69.7 (±0.33)[a]	Phs
F	156.07	67.75	78.17	74.00
df (t, e)	4, 10	2, 6	5, 12	2, 6
P	< 0.001	< 0.001	< 0.001	< 0.001

[a] Means followed by the same letters in each column are not significantly different (Tukey's test, P< 0.05). Phs: Phase separation. [b] Emulsifiers: COE36= Castor Oil Ethoxylate 36M, TAE15= Tallow Amine Ethoxylate 15M, CFE10= Coconut Fatty acid Ethoxylate 10M, NPE6 and 10= Nonylphenol Ethoxylate 6 and 10M, CFD= Coconut Fatty acid Diethanolamide, LA3 and 7= Lauryl myristyl Alcohol ethoxylate 3 and 7M.

LA3 caused a higher mortality percentage than the others. For soybean oil emulsions, LA3 caused maximum mortality percentage in adult females, but there was no difference between the others, except for CFD and TAE15. Also, soybean oil emulsions produced less mortality percentage than castor oil; however, this reduction was only significant in mortality in COE36, CFE10, and NPE6 (Table 6). In the standing leaf method test, emulsifier-oil emulsions of COE36 caused significantly greater mortality than NPE6 and the mortality percentage of castor oil emulsions was significantly higher than that of soybean oil (F= 30.49; df= 3, 12 ; P< 0.001) (Figure 2).

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

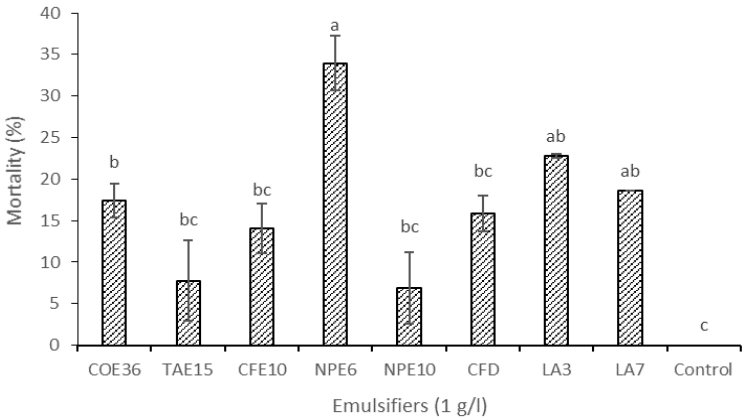

Figure 1. Mortality (±SE) of *T. urticae* adult female exposed to different surfactants in the petri dish method. Means followed by the same letter are not significantly different (Tukey's test, P< 0.05). Symbols are defined under Table 5.

Table 6. Mortality of *T. urticae* adult females treated with combination of emulsifier and oil at a rate of 1:9 in the petri dish method.[a]

Emulsifier[b] (10%)	Mortality (±SE) (%)		t	df	P-value
	Castor oil (90%)	Soybean oil (90%)			
COE36	100 (±0)^{a}	82.16 (±1.96)^{ab}	10.87	5	0.039*
TAE15	61.86 (±2.08)^{c}	28.11 (±1.68)^{c}	11.90	5	0.364^{ns}
CFE10	100 (±0)^{a}	83.41 (±3.04)^{ab}	6.53	5	0.005**
NPE6	96.33 (±2.26)^{a}	76.39 (±1.44)^{ab}	6.25	6	0.022*
NPE10	75.59 (±2.73)^{b}	78.24 (±1.7)^{ab}	-0.82	4	0.496^{ns}
CFD	75.93 (±2.59)^{b}	73.55 (±3.28)^{b}	0.57	5	0.984^{ns}
LA3	97.50 (±2.5)^{a}	88.60 (±2.23)^{a}	2.50	5	0.567^{ns}
LA7	73.05 (±2.04)^{b}	76.90 (±4.64)^{ab}	-0.76	4	0.218^{ns}
Control	0 d	0 d			
F	277.96	141.29			
df (t, e)	8, 26	8, 18			
P	< 0.001	< 0.001			

[a] Means followed by the same letters in each column are not significantly different (Tukey's test, P< 0.05). ns: Indicates non-significant differences, * and **: Indicate significant differences at 0.05 and 0.01, respectively.[b] Symbols as defined under Table 3.

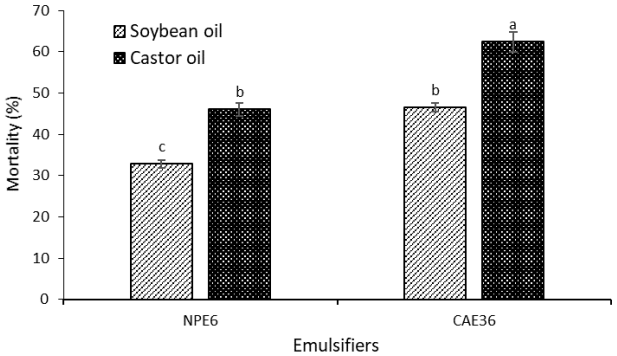

Figure 2. Mortality (±SE) of *T. urticae* adult female exposed to emulsifiers in combination with vegetable oils at a rate of 1:9 in the standing leaf method at 5 g L^{-1}. Means followed by the same letters are not significantly different (Tukey's test, P< 0.05). COE36: Castor Oil Ethoxylate 36M, NPE6: Nonylphenol Ethoxylate 6M.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Stabilizers alone at 5 g L^{-1} caused a low level of mortality and the mortality percentage of PEG 600 was significantly higher than glycerol (F= 7.524; df= 3, 15; P= 0.003). However, there was no difference between PEG 600 and 400 (Figure 3).

In Table 7, the acaricidal toxicity between different combinations of final emulsifier-oil-stabilizer formulations was investigated on *T. urticae* by petri dish and standing leaf methods. Mortality percentage in petri dish tests was significantly less than standing leaf tests, except for 6:2:2 formulation. Moreover, in most cases, 1:8:1 and 2:6:2 mixtures, which had the highest amounts of oil, caused significantly greater mortality than other emulsifier-oil-stabilizer formulations (Table 7).

Viscosity Measurements

According to the viscosity results, increasing NPE6 had a direct relationship

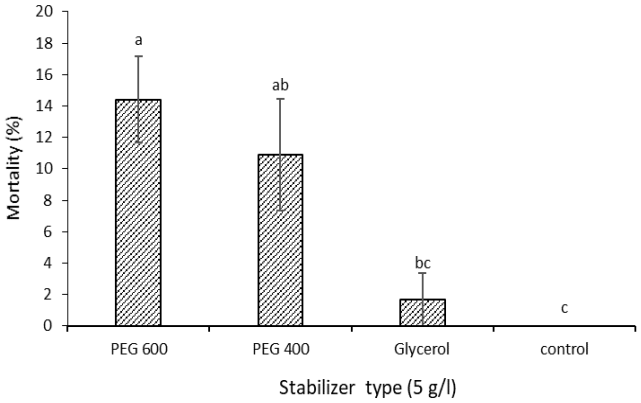

Figure 3. Mortality (±SE) of *T. urticae* adult female exposed to stabilizers in the petri dish method. Means followed by the same letters are not significantly different (Tukey's test, P< 0.05). PEG 400 and 600: Polyethylene Glycol 400 and 600.

Table 7. Percent mortalities of *T. urticae* adult females treated by emulsifier-oil-stabilizer mixture at different ratios.[a]

Formulation (NPE6: Oil: PEG400)	Mortality (±SE) (%)				F	df (t, e)	P
	Petri dish leaf test		Standing leaf test				
	1 g L^{-1}	5 g L^{-1}	5 g L^{-1}	8 g L^{-1}			
3:3:4	51.21 (±2.24)^{b}	92.46 (±4.14)^{a}	31.03 (±1.09)^{ab}	53.87 (±2.46)^{b}	44.21	3, 12	<0.001
4:4:2	21.98 (±1.86)^{cd}	81.29 (±0.81)^{abcd}	26.05 (±1.51)^{bc}	44.78 (±1.90)^{bc}	262.14	3, 12	<0.001
1:8:1	82.77 (±3.57)^{a}	88.88 (±1.44)^{abc}	40.03 (±1.06)^{a}	76.38 (±3.05)^{a}	56.72	3, 12	<0.001
2:6:2	73.24 (±2.66)^{a}	73.80 (±5.12)^{bcde}	34.16 (±2.91)^{ab}	56.55 (±2.96)^{b}	29.83	3, 11	<0.001
3:4:3	31.42(±3.85)^{bc}	90.44 (±3.54)^{ab}	12.88 (±1.93)^{d}	30.48 (±3.03)^{d}	52.38	3, 12	<0.001
4:2:4	28.67 (±3.76)^{cd}	68.83 (±2.43)^{cdef}	27.87 (±3.53)^{ab}	52.25 (±2.96)^{b}	33.97	3, 12	<0.001
8:1:1	9.22 (±3.46)^{e}	43.30 (±1.68)^{fg}	12.01 (±1.64)^{dB}	29.10 (±2.08)^{d}	13.89	3, 11	<0.001
6:2:2	16.89 (±3.04)^{cde}	29.05 (±5.57)^{g}	15.20 (±1.64)^{cd}	24.48 (±2.57)^{d}	3.53	3, 12	0.048
4:3:3	33.24 (±1.96)^{bc}	61.63 (±1.80)^{def}	9.66 (±2.29)^{d}	35.05 (±2.06)^{cd}	84.51	3, 12	<0.001
2:4:4	13.15 (±2.6)^{de}	49.70 (±3.87)^{efg}	27.62 (±3.63)^{ab}	45.05 (±3.18)^{bc}	24.64	3, 11	<0.001
Control	0^{f}	0^{h}	0^{e}	0^{e}			
F	53.62	49.13	41.761	82.48			
df (t, e)	10, 32	10, 28	10, 33	10, 32			
P	< 0.001	< 0.001	< 0.001	< 0.001			

[a] Means followed by the same letters in each column in each row are not significantly different (Tukey's test, P < 0.05). NPE6: Nonylphenol Ethoxylate 6 M, PEG 400: Polyethylene Glycol 400.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

with increasing viscosity. The viscosity of castor oil formulations was greater than soybean oils, and viscosity in 8 g L^{-1} concentrations was greater than 5 g L^{-1} (Table 8).

Phytotoxicity tests

Results showed that formulations in step 1 caused phytotoxicity, but NPE6 containing formulation had less phytotoxicity. In step 3, only 1:8:1 caused slightly phytotoxicity in young leaves (Table 9 and Figure 4). High amounts of oil in formulations caused phytotoxicity, but when the stabilizer was added to the formulations, oil was dispersed all over the leaf surface and could not be concentrated to a single spot. Furthermore,

Table 8. Kinematic viscosity of formulations for castor and soybean oil at different concentrations.

Mixture (NPE6: Oil: PEG400)[a]	Viscosity ($mm^2 s^{-1}$)			
	Castor oil		Soybean oil	
	5 (g L^{-1})	8 (g L^{-1})	5 (g L^{-1})	8 (g L^{-1})
3:3:4	1.0266	1.1436	0.9905	1.0431
4:4:2	1.0379	1.1308	0.9451	1.0487
1:8:1	1.0121	1.1045	0.9832	1.0105
2:6:2	1.0201	1.0912	0.9961	1.0174
3:4:3	1.0316	1.0969	0.9931	1.0187
4:2:4	1.0553	1.1657	1.0123	1.0845
8:1:1	1.1422	1.2335	1.0755	1.1727
6:2:2	1.1148	1.1550	1.0611	1.1638
4:3:3	1.0402	1.1561	1.0346	1.0476
2:4:4	1.0338	1.1343	1.0832	1.0361

[a] NPE6: Nonylphenol Ethoxylate 6 M, PEG 400 and 600: Polyethylene Glycol 400 and 600.

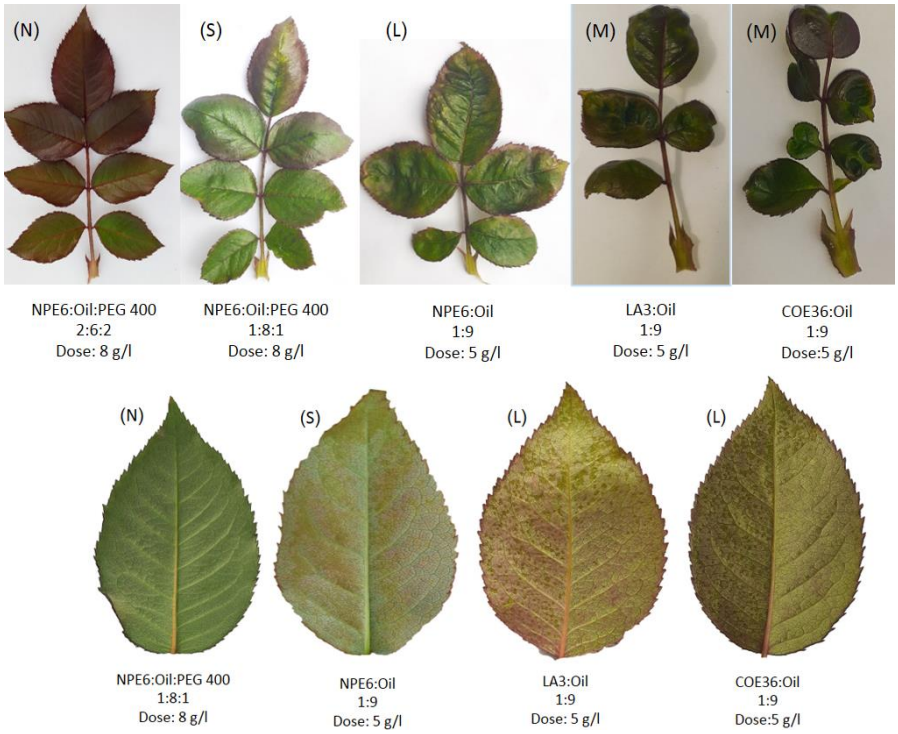

Figure 4. Phytotoxicity of toxic formulations and a non-toxic formulation on young (top row) and mature (bottom row) cut rose leaves after one week. N: No damage; S: Slight, the plant generally can recover over a few weeks; L: Low-level of damage, especially caused deformation in young shootings and slightly affected branch height and bud size; M: Moderate damage, greatly reduced branch height and bud size. COE36, NPE6, LA3, and PEG 400 as defined under Table 3.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Table 9. Phytotoxicity of formulations based on leaf age.[a]

Concentration (g L^{-1})	Mixtures	Ratio	Young leaf	Mature leaf
5	LA3: Oil	1:9	M	L
	NPE6: Oil	1:9	L	S
	COE36: Oil	1:9	M	L
8	NPE6: Oil: PEG 600	3:3:4	N	N
		4:4:2	N	N
	NPE6: Oil: PEG 400	3:3:4	N	N
		4:4:2	N	N
	NPE6: Oil: Glycerol	3:3:4	N	N
		4:4:2	N	N
8	NPE6: Oil: PEG 400	3:3:4	N	N
		4:4:2	N	N
		1:8:1	S	N
		2:6:2	N	N
		3:4:3	N	N
		4:2:4	N	N
		8:1:1	N	N
		6:2:2	N	N
		4:3:3	N	N
		2:4:4	N	N

[a] N: No damage; S: Slight, the plant generally can recover over a few weeks; L: Low-level of damage, especially caused deformation in young shootings that slightly affected branch height and bud size; M: Moderate damage, greatly reduced branch height, and bud size. Symbols as defined under Table 3.

young leaves were more susceptible to phytotoxicity than mature leaves.

DISCUSSION

According to the results, emulsifiers and stabilizers alone had a low level of mortality on *T. urticae*, but, when the oils were added, the mortality increased. Therefore, it can be concluded that oil may be the main factor in *T. urticae* mortality. In step 1, a suitable emulsifier should be selected for the preparation of an appropriate emulsion formulation for controlling *T. urticae*. Due to the bipolar nature of the emulsifiers (Chaurasiya and Hebbar, 2017), all the oil mixtures were stable at different temperatures. Pesticides for agricultural applications should be diluted in water so that the physical and biological properties of the mixtures should be investigated as oil in water emulsions. In spectrophotometry tests, the absorbance has a direct relationship with the number and size of emulsions droplets (Nejadmansouri *et al.*, 2016). High absorbance amounts in NPE6 and COE36 emulsifier-oil emulsions may be due to the high number or greater size of oil particles dispersed in the water, and vice versa in less absorbance in some other emulsifier-oil emulsions. Emulsion capacity test somewhat clarified that oiling off had occurred in emulsifier-oil emulsions with less absorbance. Therefore, in these emulsions, oil had not dispersed well and the number of oil particles was low. According to Iran National Standard No. 14588 (INSO, 2011) oiling off and high creaming are not good properties for our purpose. Uniform distribution of oil in water and absence of oiling off caused more oil exposure with the individual target, increasing the toxicity of emulsion. In agricultural spray systems, the emulsion is drained from the bottom of the spray tank. Therefore, over time, the concentration of the creamy layer increases and causes phytotoxicity. A less creamy layer causes the emulsion to be used at a higher concentration for effective mortality.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

According to our findings, toxicity tests show high mortality of COE36 emulsifier-oil emulsions against *T. urticae* female adults. However, COE36 emulsifier-oil emulsion caused phytotoxicity with economic damages and led to rose's short branches and small buds. Vegetable oils caused deformation of leaves due to changes in membrane permeability, stomatal structure, and leakage of cellular contents into intercellular spaces (Currier, 1951; Santos *et al.*, 2017). Probably, COE36 and LA3 caused the oil to pass through the leaf cuticle into the parenchyma tissue more comfortably (Manthey *et al.*, 1992). According to viscosity results, increase in NPE6 increased viscosity as well and, probably, high viscosity reduced phytotoxicity. Among the oils, the toxicity of castor oil was significantly higher than soybean oil; therefore, castor oil was chosen for the next steps of formulations. Also, the viscosity of castor oil formulations was greater than that of the soybean, and as a result, viscosity reduced movements of oil particles and prevented coalescence and caused emulsion stability (McClements, 2015). High viscosity reduced phytotoxicity because it takes much more time to penetrate leave parenchyma (Knight *et al.*, 1929). Thus, the castor oil was selected as the better oil, and NPE6, due to reducing oil phytotoxicity and high emulsion capacity, wave absorbance, and mortality, was selected for final formulations.

In the second step for stabilizer selection, there were three candidates for liquid water-soluble stabilizer. Mixtures of NPE6, castor oil, and candidate stabilizers were prepared at two 3:3:4 and 4:4:2 rates that were, respectively, more and less than emulsifier and oil rates. None of these mixtures had phytotoxicity at the mentioned concentrations. Glycerol mixtures were divided into two phases in all temperatures because of their high density. Therefore, it was removed from the selection for the next step. The glycerol, which has higher density than castor oil (1.098 and 0.95 g/mL, respectively), settles at the bottom of the test tubes (Koc, 2009; Petrović and Fajnik, 1984). Sarı and Buildings (2014) measured the melting point and frozen temperatures of PEG 600, which were 10.00±0.03 and 6.42±0.04°C, respectively. It is to be noted that, in our study, emulsifier-oil-stabilizer mixture of PEG 600 froze at 4 °C as well. Rose greenhouses are active throughout the year as well as mites. In cold seasons, if this formulation stays out of a warm place, it will not be usable for the consumer until the mixture is warm again. Consequently, PEG 400 was selected as a suitable stabilizer because of its stability in all rates and temperatures.

In the third step, 12 rates of NPE6-castor oil-PEG 400 were investigated for reaching a suitable rate. Rates of 1:1:8 and 2:2:6 that respectively contained 80% and 60% w/w of PEG 400 were divided into the two phases and removed from the treatment list. This phase separation might have been due to the poor solubility of PEG in oils and its higher density than oil and, as a result, more rates of emulsifier are needed to make a stable mixture. PEG has the hydrophobic ethylene units and the hydrophilic oxygen, which can form hydrogen bonds and provide van der Waals interactions (Özdemir and Güner, 2007). Therefore, its good solubility in water is due to H-bonds and poor solubility in oils is due to van der Waals forces and emulsifiers enhanced PEG 400 and oil compatibility through its bipolar nature (Cui *et al.*, 2009).

The other ten mixtures were stable in all temperatures and were investigated together. Toxicity tests showed that 1:8:1 and 2:6:2 that had maximum amounts of oil as an active ingredient caused greater toxicity in each petri dish method and standing leaf method. Cui *et al.* (2009) showed that PEG 400 helps to reduce oil particle size in the emulsion. Therefore, in our study, polymeric stabilizers greatly reduced phytotoxicity. Also, 1:8:1 only caused slight deformation on newborn leaves and plants could recover themselves in a few weeks without yield loss.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Results show that mortality in the petri dish method was significantly more than the standing leaf method. This may be due to the fact that in the petri dish method, underside of leaf discs are wet and there is high relative humidity around the leaf disc. However, in the standing leaf method, there is no such condition, and leaves dried very faster. Musser and Shelton (2005) reported that environmental conditions affect pesticide efficiency. Elshazly (2015) demonstrated that the toxicity of several chemical acaricides increased at high temperature and low relative humidity. It is probably due to the increasing biochemical activities in metabolites cells. However, it seems that in oil emulsions, low temperature and high relative humidity decrease water evaporation on leaves, thereby increasing mites' mortality. This condition caused micelles coagulants to happen fast and oil droplets did not have much time for adsorption on mites' lipophilic cuticle. Liburd *et al.* (2007) demonstrated that acaricidal activities of a commercial acaricide based on castor and sesame seed oil in the laboratory were greater than greenhouse conditions while the bifenazate, which is a neurotoxic chemical, did not show a significant difference between its acaricidal activity in laboratory and greenhouse condition. Therefore, we recommend using oil-based pesticides in the night or early morning with the off ventilation systems.

Although nonylphenols are non-ionic surfactants widely used in numerous industries, they have some harmful effects on organisms (Hu *et al.*, 2014; Lewis, 1991; Naylor, 1995). Miles-Richardson *et al.* (1999) reported that the number and size of Sertoli cells in fathead minnows *Pimephales promelas* Rafinesque changed when exposed to 3.4 µg/L of nonylphenol ethoxylate. Our studied compounds are safe in concentrations used. However, to reduce the environmental hazards, the use of a 1:8:1 mixture that has less surfactant rate is recommended.

CONCLUSIONS

Our results show that oils are the main active ingredients and castor oil toxicity on *T. urticae* was more than soybean oil. Also, NPE6 improved emulsifying and toxicity properties of emulsifier-oil emulsions better than other emulsifiers, and caused the lowest rate of phytotoxicity. Similarly, PEG 400 caused better stability than other stabilizers in emulsifier-oil-stabilizer formulations. Finally, between the various ratios of emulsifier-oil-stabilizer formulations, the 2:6:2 ratio was a potent formulation in terms of toxicity against *T. urticae* and lack of phytotoxicity on the rose. It is noteworthy that overestimation may occur in laboratory toxicity tests of oil-based pesticides due to poor ventilation and brief droplet dripping from leaves. Vegetable oils have a good potential to be a suitable alternative for chemical acaricides if they are well studied and tested in the pilot area before application on a large scale. Also, new formulation methods such as polymerization of emulsions can reduce the phytotoxicity problems of oils.

ACKNOWLEDGEMENTS

This research was partly funded by the Center of Excellence for Integrated Management of Pests and Diseases of Greenhouse Crops in Tarbiat Modares University.

REFERENCES

1. Amer, S., Momen, F. and Saber, S. 2001. A Comparative Study of the Effect of Some Mineral and Plant Oils on the Two Spotted Spider Mite *Tetranychus urticae* koch (Acari: Tetranychidae). *Acta Phytopathol. Entomol. Hung*., **36**: 165-171.
2. 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

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Reference to Natural Pesticides. *J. Pest Sci.*, **86(3):** 361-386.
3. Bashiri, M., Moharramipour, S., Negahban, M. and Mafi-Pashakolaei, Sh. A. 2016. Effects of Nanoencapsulated Formulation of *Cuminum cyminum* Essential Oil on *Panonychus citri* (Acari: Tetranychidae). *J. Entomol. Soc. Iran*, **36:** 151-162.
4. Butler Jr, G. D., Henneberry, T. J., Stansly, P. A. and Schuster, D. J. 1993. Insecticidal Effects of Selected Soaps, Oils and Detergents on the Sweet Potato Whitefly (Homoptera: Aleyrodidae). *Florida Entomol.*, **76:** 161-167.
5. Chapman, P. J. 1967. Petroleum Oils for the Control of Orchard Pests. *Agri. Exp. Stn. Bull*., N Y. **814**:1-22.
6. Chaurasiya, R. S. and Hebbar, H. U. 2017. Reverse Micelles for Anoparticle Synthesis and Biomolecule Separation. *Nanosci. Food Agric*., **4**:181-211.
7. Chiasson, H., Bostanian, N. and Vincent, C. J. J. O. E. E. 2004. Acaricidal Properties of a Chenopodium-Based Botanical. *J. Econ. Entomol*., **97**: 1373-1377.
8. Cui, J., Yu, B., Zhao, Y., Zhu, W., Li, H., Lou, H. and Zhai, G. J. I. J. O. P. 2009. Enhancement of Oral Absorption of Curcumin by Elf-Microemulsifying Drug Delivery Systems. *Int. J. Pharm.,* **371**: 148-155.
9. Currier, H. J. H. 1951. Herbicidal Properties of Benzene and Certain Methyl Derivatives. *Hilgardia*, **20**: 383-406.
10. Dai, J., Kim, S. M., Shin, I. -S., Kim, J. D., Lee, H. Y., Shin, W. C. and Kim, J. -C. 2014. Preparation and Stability of Fucoxanthin-Loaded Microemulsions. *J. Ind. Eng. Chem.*, **20**: 2103-2110.
11. Davidson, N. A. 1991. *Managing Insects and Mites with Spray Oils.* Div. Agric Natur. Resour., Univ. Calif., Oakland, California.
12. Elshazly, M. 2015. Effect of Temperature and Relative Humidity on Certain Acaricides Toxicity. *Proc. 4th Int. Conf. Inf., Environ. Energy. Appl.*, PP. 8-14.
13. Esmaeelian, M. 2016. Formulation and Fuzzy Modelling of Emulsion Stability of Neroli Essential Oil, Gum Arabic and Maltodextrin. *Biosci. Biotechnol. Res. Commun.,* **9**: 189-194.
14. Hu, X. l., Sun, Z. w., Wang, J. J., An, M. and Duan, S. 2014. Sublethal Toxic Effects of Nonylphenol Ethoxylate and Nonylphenol to *Moina macrocopa*. *Bull. Environ. Contam. Toxicol.,* **93**: 204-208.
15. INSO. 2011. *Pesticides-Volk Oil-Specifications*. Iran National Standard No. 14588, PP. 1-17.
16. Ismail, M. S. M., Ghallab, M., Soliman, M. F. M. and AboGhalia, A. H. 2011. Acaricidal Activities of Some Essential and Fixed Oils on the Two-Spotted Spider Mite, *Tetranychus urticae*. *Egyptian Acad. J. Biol. Sci., B. Zool.*, **3**: 41-48.
17. Ivanov, D. S., Lević, J. D., Sredanović, S. A. and Research, F. 2010. Fatty Acid Composition of Various Soybean Products. *Food Feed Res.*, **37**: 65-70.
18. Khodayari, S., Fathipour, Y. and Kamali, K. 2008. Biology, Life Table, and Predation of *Zetzellia mali* (Acari: Stigmaeidae) on *Tetranychus urticae* (Acari: Tetranychidae). *Acarina*, **16**: 191-196.
19. Knight, H., Chamberlin, J. C. and Samuels, C. D. 1929. On Some Limiting Factors in the Use of Saturated Petroleum Oils as Insecticides. *Plant Physiol.,* **4**: 299.
20. Koc, A. B. 2009. Ultrasonic Monitoring of Glycerol Settling during Transesterification of Soybean Oil. *Bioresour. Technol.***, 100**: 19-24.
21. Lancaster, A. L., Deyton, D. E., Sams, C. E., Cummins, J. C., Pless, C. D. and Fare, D. C., 2002. Soybean Oil Controls Two-Spotted Spider Mites on Burning Bush. *J. Environ. Hort.*, **20(2):** 86-92.
22. Lee, Y. S., Song, M. H., Ahn, K. S., Lee, K. Y., Kim, J. W. and Kim, G. H. 2003. Monitoring of Acaricide Resistance in Two-Spotted Spider Mite (*Tetranychus urticae*) Populations from Rose Greenhouses in Korea. *J. Asia-Pac. Entomol.*, **6**: 91-96.
23. Lewis, M. A. 1991. Chronic and Sublethal Toxicities of Surfactants to Aquatic Animals: A Review and Risk Assessment. *Water Res.*, **25**: 101-113.
24. Li, Y., Zhong, M., Xie, F., Sun, Y., Zhang, S. and Qi, B. 2020. The Effect of pH on the Stabilization and Digestive Characteristics of Soybean Lipophilic Protein Oil-in-Water Emulsions with Hypromellose. *Food Chem*., **309**: 125579.
25. Liburd, O. E., White, J. C., Rhodes, E. M. and Browdy, A. A. 2007. The Residual and Direct Effects of Reduced-Risk and Conventional Miticides on Two-Spotted Spider Mites, *Tetranychus urticae* (Acari: Tetranychidae)

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

and Predatory Mites (Acari: Phytoseiidae). *Florida Entomol.*, **90**: 249-258.
26. Manthey, F. A., Matysiak, R. and Nalewaja, J. D. 1992. Petroleum Oil and Emulsifier Affect the Phytotoxicity of Imazethapyr. *Weed Technol.*, **6**: 81-84.
27. McClements, D. J. 2015. Food Emulsions: Principles, Practices, and Techniques. CRC Press, Boca Raton.
28. Miles-Richardson, S., Pierens, S., Nichols, K., Kramer, V., Snyder, E., Snyder, S. A., Render, J., Fitzgerald, S. and Giesy, J. 1999. Effects of Waterborne Exposure to 4-Nonylphenol and Nonylphenol Ethoxylate on Secondary Sex Characteristics and Gonads of Fathead Minnows (*Pimephales promelas*). *Environ. Res.*, **80**: 122-137.
29. Mohammadi, S., Ziaee, M. and Seraj, A. A. 2016. Sublethal Effects of Biomite® on the Population Growth and Life Table Parameters of *Tetranychus turkestani* Ugarov and Nikolskii on Three Cucumber Cultivars. *Syst. Appl. Acarol.*, **21**: 218-226.
30. Motazedian, N., Ravan, S. and Bandani, A. R. 2012. Toxicity and Repellency Effects of Three Essential Oils against *Tetranychus urticae* Koch (Acari: Tetranychidae*). J. Agric. Sci. Tech.*, **14:** 275-284.
31. Musser, F. R. and Shelton, A. M. 2005. The Influence of Post-Exposure Temperature on the Toxicity of Insecticides to *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Pest Manage. Sci.*, **61**: 508-510.
32. Naylor, C. G. 1995. Environmental Fate and Safety of Nonylphenol Ethoxylates. *Text. Chem. Color.*, **27**: 29-33.
33. Nejadmansouri, M., Hosseini, S. M. H., Niakosari, M., Yousefi, G. H. and Golmakani, M. T. 2016. Physicochemical Properties and Oxidative Stability of Fish Oil Nanoemulsions as Affected by Hydrophilic Lipophilic Balance, Surfactant to Oil Ratio and Storage Temperature. *Colloids Surf. A.*, **506**: 821-832.
34. Ogunniyi, D. S. 2006. Castor Oil: A Vital Industrial Raw Material. *Bioresour. Technol.*, **97**: 1086-1091.
35. Özdemir, C. and Güner, A. 2007. Solubility Profiles of Poly (Ethylene Glycol) Solvent Systems, i: Qualitative Comparison of Solubility Parameter Approaches. *Eur. Polym. J.*, **43**: 3068-3093.
36. Park, J. H., Ryu, K. Y., Lee, B. M. and Jee, H. J. 2008. Effect of Coy (Cooking Oil and Yolk Mixture) on Control of *Tetranychus urticae*. *J. Appl. Entomol*., **47:** 249-254.
37. Petrović, Z. S. and Fajnik, D. J. 1984. Preparation and Properties of Castor Oil-Based Polyurethanes. *J. Appl. Polym. Sci.*, **29**: 1031-1040.
38. Puritch, G. S. 1981. Pesticidal Soaps and Adjuvants: What Are They and How Do They Work. *Proc. 23rd Annu. Lower Mainland Hort. Improv. Assoc*., Canada, PP. 53-37.
39. Rahate, A. R. and Nagarkar, J. M. 2007. Emulsification of Vegetable Oils Using a Blend of Nonionic Surfactants for Cosmetic Applications. *J. Dispersion Sci. Technol.*, **28**: 1077-1080.
40. Santos, L. A., Pozza, E. A. and Souza, P. E. D. 2017. Phytotoxicity of Mineral, Vegetable Oils and Foliar Fertilizers in Coffee Seedlings. *Coffee Sci.*, **12(1):** 108-113.
41. Sarı, A. J. E. and Buildings. 2014. Composites of polyethylene glycol (PEG600) with Gypsum and Natural Clay as New Kinds of Building PCMS for Low Temperature-Thermal Energy Storage. *Energ. Build.*, **69**: 184-192.
42. Sciarini, L., Maldonado, F., Ribotta, P., Perez, G. and Leon, A. 2009. Chemical Composition and Functional Properties of *Gleditsia triacanthos* Gum. *Food Hydrocol.*, **23**: 306-313.
43. Sedaratian, A., Fathipour, Y. and Moharramipour, S. 2009. Evaluation of Resistance in 14 Soybean Genotypes to *Tetranychus urticae* (Acari: Tetranychidae). *J. Pest Sci.*, **82**: 163-170.
44. Seifi, R., Moharramipour, S. and Ayyari, M. 2018. Acaricidal Activity of Different Fractions of *Moringa peregrina* on Two Spotted Spider Mite *Tetranychus urticae* (Acari: Tetranychidae). *Ind. Crops Prod.*, **125**: 616-621.
45. Souza, R. F., Alencar, M. A., Nascimento, C. M., da Silva, M. G., Meneghetti, M. R. and Hickmann, J. M. 2006. Thermal Optical Nonlinearity Enhanced by Gold Nanoparticles. Plasmonics: Metallic Nanostructures and Their Optical Properties IV. *Int. Soc. Opt. Photonics*, 63231T PP. doi: 10.1117/12.680072
46. SPSS. 2011. IBM SPSS Software Statistics for Windows: Version 20.0. IBM Corp, New York, NY.
47. Tsolakis, H. and Ragusa, S. 2008. Effects of a Mixture of Vegetable and Essential Oils and

[DOR: 20.1001.1.16807073.2021.23.6.13.6]

Fatty Acid Potassium Salts on *Tetranychus urticae* and *Phytoseiulus persimilis*. *Ecotoxicol. Environ. Safe.*, **70(2):** 276-282.

48. Yang, H. J., Cho, W. G. and Park, S. N. 2009. Stability of Oil-in-Water Nano-Emulsions Prepared Using the Phase Inversion Composition Method. *J. Ind. Eng. Chem.*, **15**: 331-335.
49. Yu, J. and Chen, B. 2009. Acaricidal Efficacy of Three Vegetable Oils on *Tetranychus urticae* Koch (Acari: Tetranychidae). *J. Taiwan Agric. Res.*, **58**: 136-145.

تولید فرمولاسیون کنه کش گیاهی بی خطر بر پایه روغن های گیاهی علیه کنه تارتن دولکه ای

م. بشیری، س. محرمی پور و م. نگهبان

چکیده

کنه تارتن دو لکه‌ای، *Tetranychus urticae* Koch، یکی از آفات چند خواری است که انتشار جهانی دارد. به دلیل مشکلات مرتبط با سموم شیمیایی از جمله مقاومت در برابر آفت کش‌ها و آلودگی‌های زیست محیطی، روغن‌های گیاهی برای استفاده جهت کنترل کنه‌ها مورد توجه هستند. اما، حلالیت کم آنها در آب و ایجاد گیاه‌سوزی از عمده‌ترین مشکلاتی است که کاربرد آنها را محدود می‌کند. در این پژوهش، فرآیند غربالگری روی برخی از امولسیون کننده‌های تجاری و تثبیت کننده‌ها برای به دست آوردن فرمولاسیون یک کنه‌کش ایمن از روغن‌های گیاهی مناسب از جمله روغن کرچک و روغن سویا انجام شد. از بین هشت امولسیفایر، نونیل فنل اتوکسیلات 6 مول (NPE6) قدرت امولسیون‌کنندگی بالا با اثر گیاه‌سوزی کمتر و تا حدودی اثر سمی روی کنه را دارا بود. در میان دو روغن گیاهی مورد آزمایش، روغن کرچک اثر کنه کشی بالاتری نسبت به روغن سویا داشت. همچنین، پلی اتیلن گلیکول 400 (PEG 400) از پایداری دمایی بیشتری در فرمولاسیون برخوردار بود. در آخرین مرحله غربالگری، بهترین نسبت‌های NPE6، روغن کرچک و PEG 400 از نظر کشندگی، پایداری و اثر گیاه‌سوزی مورد بررسی قرار گرفت. بر اساس نتایج بدست آمده، نسبت‌های 1: 8: 1 و 2: 6: 2 (NPE6:روغن کرچک:PEG 400) به عنوان بهترین محصول نهایی بودند که می‌تواند در گلخانه گل رز مورد استفاده قرار گیرد. همچنین، کارایی نسبت‌های مختلف امولسیفایر-روغن-تثبیت کننده با دو روش مختلف روی کنه تارتن دو لکه‌ای بررسی شد. نتایج نشان داد که روش آزمایش پتری دیش در مقایسه با روش آزمون برگ ایستاده باعث برآورد بیش از حد در میزان مرگ و میر کنه می‌شود. استفاده از روش‌های جدید مانند فرمولاسیون‌های پلیمری می‌تواند افق جدیدی در کنترل آفات بدون مصرف سموم شیمیایی خطرناک را ترسیم نماید.

[DOR: 20.1001.1.16807073.2021.23.6.13.6]