Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Acaricidal, ovicidal, and repellent effects of *Tagetes patula* leaf extract against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Vol. 59, No. 2: 151–159, 2019

DOI: 10.24425/jppr.2019.129285

Received: October 6, 2018
Accepted: May 17, 2019

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Abstract

This study investigates the acaricidal, ovicidal, and repellent effects of the *Tagetes patula* Linn. (Asteraceae) leaf extract against both the adult female and egg stages of *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) under laboratory conditions. The *Tagetes patula* ethanolic leaf extract [$Tp_{EtOH70\%}$] was screened for adulticide and ovicide bioassays in order to consider its acute toxicity. One sublethal concentration was used to assess egg-laying capacity (fecundity), repellent, and oviposition deterrent activities. The chemical characterization was conducted by gas chromatography-mass spectrometry (GC-MS) analysis to identify the $Tp_{EtOH70\%}$ bioactive components. Results showed that the LC_{50} value of $Tp_{EtOH70\%}$ leaf extract predicted by Probit analysis against *T. urticae* adult females at 24 h was 0.99%. The $Tp_{EtOH70\%}$ leaf extract showed a significant toxic effect as the highest mean mortality rates (± SE) of the treated adult females was 88.9 ± 3.7%. However, the $Tp_{EtOH70\%}$ leaf extract was insignificant in affecting the egg-laying capacity of the adult females treated with a sublethal dose of 0.5% even after 72 h. The $Tp_{EtOH70\%}$ leaf extract was classified repellent since the repellent index (*RI*) value was lower than 1 – SD. In addition, it had a high oviposition deterring effect based on a 100% reduction of the total number of eggs. The $Tp_{EtOH70\%}$ leaf extract had a significant ovicidal effect on *T. urticae* eggs, with 56.04% reduction in hatching. Five bioactive compounds from various classes of phytochemicals were identified in the $Tp_{EtOH70\%}$ leaf extract and the major compound was phytol (62.72%). This pioneering investigation reveals the adulticidal, ovicidal, and repellent activities of the $Tp_{EtOH70\%}$ leaf extract against *T. urticae*. A combination of multiple modes of action of different plant components may act alone or in synergism to delay the development of mite resistance.

Keywords: acaricide, GC-MS analysis, leaf extract, repellent, *Tetranychus urticae*, *Tagetes patula*

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is an extremely polyphagous agricultural pest that can colonize more than 1,100 plant species (Migeon *et al.* 2010). *Tetranychus urticae* is now designated as the "pesticide resistance champion" since it is resistant to many selective acaricides accepted for integrated pest management programs, such as organophosphate, abamectin, clofentezine, hexythizox, bifenthrin, and chlorfenapyr (Van Leeuwen *et al.* 2014).

Plant extracts may demonstrate the potential to control mites due to their secondary metabolites, such as terpenoids, alkaloids, flavonoids, and polyacetylenes, which have a variety of biological activities including repellence, oviposition deterrence, toxicity, and growth regulatory activity (Singh and Saratchandra 2005; Dąbrowski and Seredyńska 2007). When selecting potential botanical pesticides, several properties must be present, including effectiveness to target pests at low concentrations, non-toxic to other animals, and it must be easily obtainable, handled, and applied. Botanical pesticides should have little or no residual on plants, in order to be used safely in the environment in sustainable agriculture (Isman 2006).

Moreover, plant-based pesticides often contain a mixture of active substances, which can delay or prevent resistance development (Rattan 2010). In this context, the toxic effects of some medicinal plant extracts on phytophagous mites are promising substitutes for synthetic pesticides, since they are rich sources of bioactive substances (Dubey 2011).

When searching for natural pesticides, *Tagetes patula* Linn. (French marigold; Asteraceae) is considered an excellent plant species, since it produces thiophenes and many polyacetylenic compounds that possess strong biocidal activity (Marotti *et al.* 2010). It is a strong-scented bushy annual, widespread throughout the world, easily cultured and propagated with high germination rates (Bano *et al.* 2002). Despite its famous usages in gardens as ornamental plants, *Tagetes* sp. is one of the most important medicinal plants of the Asteraceae family. It is renowned for its therapeutic values to treat colic, diarrhea, vomit, cancer, inflammation, skin diseases, and hepatic disorders (Priyanka *et al.* 2013). *Tagetes* sp. has been used as an alternative for the control of many pests and diseases, prominently in nematode management programs (Hooks *et al.* 2010; Salinas-Sánchez *et al.* 2012). Furthermore, many studies have shown that *T. patula* exhibits antibacterial, antifungal, and insecticidal activities (Faizi *et al.* 2008; Vidal *et al.* 2009).

Politi *et al.* (2012) penned the first report on the acaricidal activity of the ethanolic extract of *T. patula* against dog ticks, *Rhipicephalus sanguineus* Latreille. So far, no work on the acaricidal activity of the species *T. patula* against spider mites, *T. urticae* has been published, and experimental data about its acaricidal properties are scarce.

This study aims to investigate for the first time the acaricidal, ovicidal, repellent, and oviposition-deterrent effects of the ethanolic leaf extract of *T. patula* against both the adult female and egg stages of *T. urticae* under laboratory conditions. The bioactive components that chemically characterize the $Tp_{EtOH70\%}$ leaf extract were identified using gas chromatography (GC-MS) analysis.

Materials and Methods

Collection of plant material and extract preparation

Tagetes patula, the selected plant for the study, was collected in June 2017 from El Orman Garden, Cairo, Egypt and identified at the Botany Department Faculty of Science, Suez Canal University, Ismailia, Cairo, Egypt.

Fresh leaf samples from the collected plants with a known weight (75.12 g) were air dried in the shade, ground into a fine powder, then immersed for 24 h in a quantity of ethanol (EtOH) that was enough to cover the plant material. This procedure was repeated for 3 days to ensure thorough extraction of ethanol soluble constituents. The extract was then filtrated and ethanol was evaporated at reduced pressure at 40°C in a rotary evaporator to obtain the crude extract. After complete evaporation of the solvent, the extract was lyophilized and stored in a refrigerator at 4°C to be protected from humidity and contamination (Breuer and Devkota 1990).

Maintenance of *Tetranychus urticae* colony

The susceptible strain of *T. urticae* originated from infested leaves of castor bean plants on the borders of the Suez Canal region. The susceptible population was reared on sweet potato leaves in a climate-controlled room (27 ± 2°C, under 60 ± 5% relative humidity (RH) and 16 : 8 h of light (L) : dark (D) photoperiods, starting in January 2017.

Bioassays

The acaricidal activity of the plant extract on *T. urticae* was assessed for acute toxicity (mortality), egg-laying capacity (fecundity), repellency, and ovicidal activities. Ethanol was used as a solvent (Erdogan *et al.* 2012). A leaf spray method using a Sigma glass spray (unit No. S 3135) and a leaf disc method were used (Helle and Overmeer 1985).

Adulticidal bioassays

Assessment of acute toxicity (LC_{50})

The ethanolic extract toxicity was assessed in pre-tests using different diluted concentrations to establish the five concentrations needed. Bioassays were carried out by spraying five serially diluted concentrations (0.25, 0.5, 1.0, 2.0, and 5.0%) in triplicates dissolved in EtOH (70%). Thirty adult female mites (2–3 days old) were gently transferred directly from stock culture to the sweet potato leaf-disc (3 cm diameter) and placed on moistened cotton wads in Petri dishes (9 cm diameter). Two ml of $Tp_{EtOH70\%}$ leaf extract were sprayed with a Sigma glass sprayer and allowed to dry for 30 min at 27 ± 2°C (Kumral *et al.* 2010). In all the experiments, an EtOH (70%) solution was used as a control treatment. After drying, the Petri dishes were kept in a climate chamber at 27 ± 2°C, under 60 ± 5% RH and 16 : 8 h (L : D). The experiment was repeated five times. Mortality of the adult females was observed and the number of living and dead mites was recorded after 24 h using a stereo microscope. The assessment of mite mortality corresponded to the mites' failure to respond positively by leg movements to a fine brush.

Assessment of egg-laying capacity (fecundity)

Egg-laying capacity was evaluated for the *T. urticae* adult females according to Erdogan *et al.* (2012), using one sublethal concentration (0.5%) of $Tp_{EtOH70\%}$ leaf extract. On different sweet potato leaves, inactive deutonymphs were separated before the fecundity test. Then one newly matured female (1-day-old) and two spider mite males were used for each application. The methodology for extract application and transferring the spider mites to Petri dishes was the same as in the toxicity bioassay. Six replicates were used (15 ♀/leaf disc). The Petri dishes were placed in a climate chamber at 27 ± 2°C, under 60 ± 5% RH and 16 : 8 h (L : D). Eggs deposited on the discs during 72 h were counted. EtOH (70%) was used as a control. Each treatment was repeated ten times. Fecundity of the treated females, i.e. the number of eggs laid per female at a 24-h interval summed over 72 h was calculated.

Assessment of repellent and oviposition-deterrent effects

For the repellency test, the leaf disc method was used. The $Tp_{EtOH70\%}$ leaf extract was applied with one sublethal concentration (0.5%). Repellent and oviposition-deterrent effects were evaluated in a two-choice bioassay. Sweet potato leaf discs of 4.5 cm in diameter were used. Half of the disc was immersed for five seconds in a solution of the extract at a concentration of 0.5% and after drying at room temperature, the other half was immersed in EtOH (70%) serving as a control. Each half circle was immersed in a way that permitted a free area of 0.3 cm between the two halves where the mites were initially released. The leaf discs were placed on filter paper, on polyethylene foam, and the entire arrangement was placed on a plastic tray containing water. Each disc was infested with ten adult females of *T. urticae* and each treatment was replicated six times. All Petri dishes were kept in a climate chamber at 27 ± 2°C, under 60 ± 5% RH and 16 : 8 h (L : D). At 24, 48, and 72 h post-treatment, the number of mites and eggs present on treated and untreated leaf disc was counted. The repellent index (*RI*) and oviposition deterrent index (*ODI*) was calculated according to Kogan and Goeden (1970) and Dimetry *et al.* (1993), respectively.

Ovicidal bioassay (direct toxicity to zero-time eggs)

To determine the ovicidal activity of the $Tp_{EtOH70\%}$ leaf extract, a wide range of concentrations was tested to define the effective concentration needed. Each disc of sweet potato leaves (3 cm diameter) with 100 eggs (24 h old) was sprayed with 2 ml of crude extract (8%) in triplicates. A control group with EtOH (70%) was used. After drying, the Petri dishes were kept in a climate chamber at 27 ± 2°C, under 60 ± 5% RH and 16 : 8 h (L : D). All discs were examined daily to record the number of hatched larvae (Chiasson *et al.* 2004). The viability of the eggs was checked for a period of 7 days after oviposition. The eggs that did not hatch during this period were counted as non-viable.

Gas chromatography-mass spectrometry (GC-MS) analysis of $Tp_{EtOH70\%}$ leaf extract

The identification of the $Tp_{EtOH70\%}$ leaf extract compounds was performed using a trace gas chromatograph interfaced to a Polaris Q mass spectrometer (Thermo Finnigan, Hertfordshire, UK). According to Thangavel *et al.* (2014), DB5-MS capillary standard non-polar column (30 m × 0.25 mm × 0.25 µm) and helium as a carrier gas were used at a constant flow rate of 1 ml · min^{-1}. The temperature of the oven was kept at 70°C and adjusted to reach 260°C at an increasing rate of 6°C/min for 2 min. Mass range was 50 to 650 (m/z). The total running time was 51 min. The relative percentage of the identified constituents was expressed as a percentage with peak area normalization. The phytochemical components were identified by comparing the retention time (*RT*) and mass spectra with those of WILEY and NIST libraries.

Statistical analysis

Mite mean mortality rates (± SE) were calculated as a percentage of dead females. Concentration and mortality data were subjected to Probit analysis and LC_{50} value with a 95% confidence limit and slopes ± SE of the regression were estimated using the POLO Plus software (LeOra Software, Berkeley, CA, USA). Mortality, repellency, and fecundity data were analyzed through one-way analysis of variance and treatment means were compared by Tukey's test ($p \leq 0.05$). For studying differences between groups, data were analyzed by analysis of variance (ANOVA) after data normalization. All the statistical tests were performed using the software package SPSS 15.0.0.

Results

Assessment of the acute toxicity

For the evaluation of the efficacy of the $Tp_{EtOH70\%}$ leaf extract as an acaricide against *T. urticae* adult females, five serially diluted concentrations (0.25, 0.5, 1.0, 2.0 and 5.0%) were used. The mean mortality rates (± SE) of the treated *T. urticae* adult females 24 h post-treatment at 27 ± 2°C are shown (Fig. 1). The mean mortality rates (± SE) were 88.9 ± 3.7, 72.2 ± 1.4, 48.6 ± 3.7, 29.2 ± 4.8 and 15.3 ± 1.4%, respectively as compared

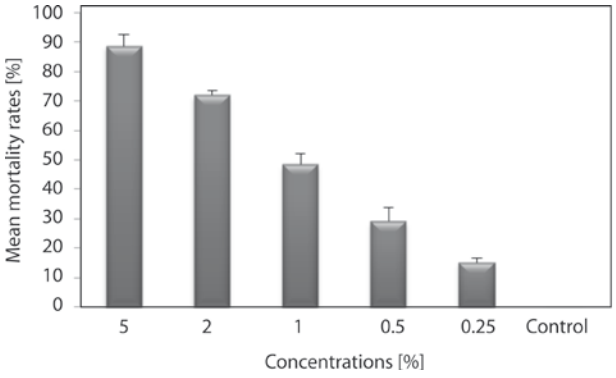

Fig. 1. Mean mortality rates (±SE) of *Tetranychus urticae* adult females treated with 5.0, 2.0, 1.0, 0.5 and 0.25% of $Tp_{EtOH70\%}$ leaf extract 24 h post-treatment

to no mortality in their corresponding control groups ($p < 0.000$). Results showed that the LC_{50} value of $Tp_{EtOH70\%}$ leaf extract predicted by Probit analysis for the treated adult females 24 h post-treatment was 0.99% with 95% CI (confidence interval) ranging from (0.82 to 1.20%).

Assessment of the egg-laying capacity (fecundity)

The egg-laying capacity of *T. urticae* adult females treated with a sublethal concentration of $Tp_{EtOH70\%}$ leaf extract (0.5%) after 24, 48, and 72 h is shown (Table 1). Our results revealed that the egg-laying capacity of the treated adult females was not affected by the $Tp_{EtOH70\%}$ leaf extract even after 72 h.

Assessment of the repellent and oviposition-deterrent effects

The *RI* and *ODI* of the $Tp_{EtOH70\%}$ leaf extract on *T. urticae* adult females 24, 48, and 72 h post-treatment using one sublethal concentration (0.5%) is shown (Table 2). The behavior of *T. urticae* females was considerably affected, and repellency was strongest 24 and 48 h post-treatment. However, after 72 h, a smaller number of females was found on the treated halves than on the untreated ones. The *RI* of the $Tp_{EtOH70\%}$ leaf extract was classified as a repellent since the *RI* value was lower than 1 – SD.

As for the oviposition behavior, the $Tp_{EtOH70\%}$ leaf extract prevented the adult females from laying eggs where the *ODI* reached 100% 24 h post-treatment. Then in the following 24 h, *T. urticae* females laid significantly fewer eggs on the treated halves and *ODI* decreased to 83.3. The repellent and oviposition deterrent effect of $Tp_{EtOH70\%}$ leaf extract on adult females of *T. urticae* 24, 48, and 72 h post-treatment using one sublethal dose (0.5%) is given (Fig. 2 A–B).

Table 1. The egg laying capacity of *Tetranychus urticae* adult females treated with a sublethal concentration of $Tp_{EtOH70\%}$ leaf extract (0.5%) after 24, 48, and 72 h

Plant extract	Mean no. of eggs	Mean eggs/female
	after 24 h	
$Tp_{EtOH70\%}$ leaf extract	78.7 ± 9.2 a	5.24 ± 0.6 a
Control (ethanol 70%)	88.7 ± 2.7 a	5.91 ± 0.2 a
	after 48 h	
$Tp_{EtOH70\%}$ leaf extract	146.7 ± 14.9 a	9.78 ± 0.9 a
Control (ethanol 70%)	152.3 ± 1.6 a	10.15 ± 0.1 a
	after 72 h	
$Tp_{EtOH70\%}$ leaf extract	269 ± 17.4 a	17.93 ± 1.2 a
Control (ethanol 70%)	304.3 ± 23.7 a	20.29 ± 1.6 a

Table 2. Repellent index (*RI*) and oviposition deterrent index (*ODI*) of $Tp_{EtOH70\%}$ leaf extract summed after 72 h on *Tetranychus urticae* adult females using one sublethal concentration (0.5%)

Parameters	$Tp_{EtOH70\%}$ leaf extract			Classification
	24 h	48 h	72 h	
RI[1]	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.22	repellent
ODI[2]	100	83.3	81	oviposition deterrent

[1]repellent index; $2G/(G + P)$, where G = the number of mites in the treated disk and P = the number of mites in the control disc; <1 – SD repellent; 1 ± SD neutral; 1+ SD > attractant

[2]oviposition deterrent index; $[(C - T)/(C + T)]*100$ was calculated, where C and T represent eggs laid on control and treated disc

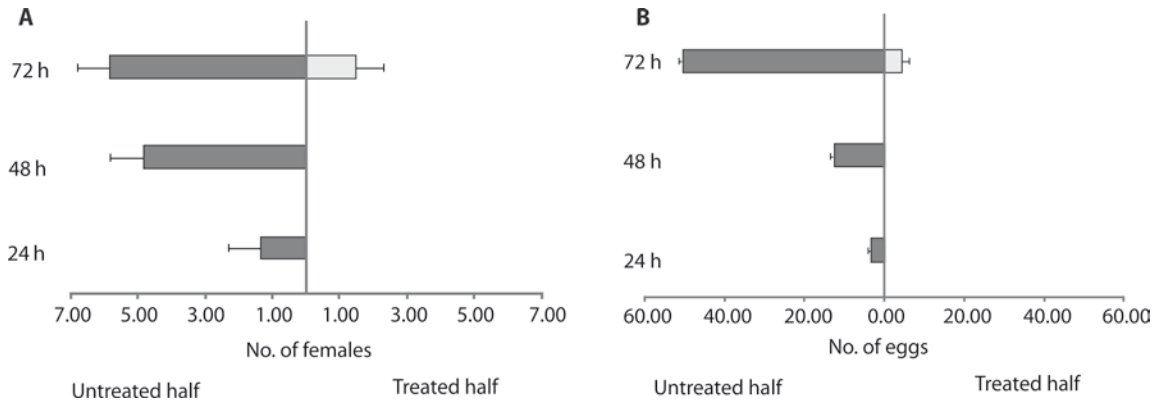

Fig. 2. Repellent (A) and oviposition deterrent (B) effects of the $Tp_{EtOH70\%}$ leaf extract on both *Tetranychus urticae* adult females and its oviposition summed after 72 h using one sublethal concentration (0.5%)

Assessment of the ovicidal effect

Egg hatching percentage (± SE) of zero-time eggs of *T. urticae* adult females treated with 8% of $Tp_{EtOH70\%}$ leaf extract at 27 ± 2°C is shown (Table 3). The percentage of egg hatching on the 4th day after treatment (DAT) was 4.17 ± 0.65% as compared to its corresponding control group 69.18 ± 7.35% ($p < 0.001$). Egg hatching percentage at 5 and 6 DAT was 52.97 ± 7.84, 56.04 ± 7.35%, respectively as compared to its corresponding control group 97.79 ± 0.20% ($p < 0.005$). Moreover, 44.44 ± 5.56, 65.92 ± 7.75 and 100 ± 0.00% of larvae were dead after hatching on the 4th, 5th and 6th days, respectively. Remarkably, all hatched eggs turned into nymphs in the control group,while treatment reduced the number of hatched eggs, juvenile development was extended by approximately 1–2 days and all hatched larvae died before reaching nymphal stages.

Yield and GC-MS analysis of $Tp_{EtOH70\%}$ leaf extract

The extract was injected into a GC-MS analyzer to identify the major bio-active compounds. The extract yield from 75.12 g of *T. patula* leaves was 17.92 g. The $Tp_{EtOH70\%}$ leaf extract chromatogram is shown (Fig. 3). Five bio-active compounds representing different classes of phytochemicals were identified (Table 4). The main compounds were phytol (62.72%), hexadecanoic acid or palmitic acid ethyl ester (9.81%), ethyl linoleate or linoleic acid ethyl ester (4.29%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.83%), and 1-docosene (1.32%).

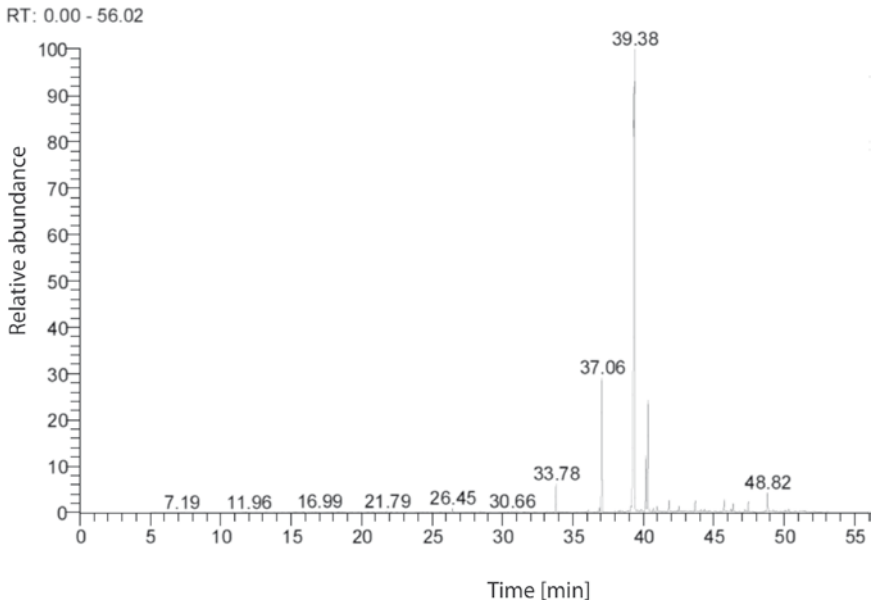

Fig. 3. GC-MS chromatograms of the $Tp_{EtOH70\%}$ leaf extract bioactive components

Table 3. Egg hatching percentage (± SE) of *Tetranychus urticae* zero-time eggs treated with 8% of $Tp_{EtOH70\%}$ leaf extract

Plant extract	% Egg hatching			
	4 DAT	5 DAT	6 DAT	7 DAT
$Tp_{EtOH70\%}$ leaf extract	4.17 ± 0.65 b	52.97 ± 7.84 b	56.04 ± 7.35 b	56.04 ± 7.35 b
Control (ethanol 70%)	69.18 ± 7.35 a	97.79 ± 0.20 a	97.79 ± 0.20 a	97.79 ± 0.20 a
	% Larval mortality			
$Tp_{EtOH70\%}$ leaf extract	44.44 ± 5.56 a	65.92 ± 7.75 a	100 ± 0.00 a	100 ± 0.00 a
Control (ethanol 70%)	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b

DAT – Day After Treatment

Table 4. Chemical composition of the different bioactive components of $Tp_{EtOH70\%}$ leaf extract by using GC-MS analysis

Peak	Retention time [min]	Chemical name	Molecular formula	M. wt.*	Area [%]
1	33.78	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.83
2	37.06	Hexadecanoic acid, or palmitic acid ethyl ester	$C_{18}H_{36}O_2$	284	9.81
3	39.38	Phytol	$C_{20}H_{40}O$	296	62.72
4	40.19	Ethyl linoleate, or linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	4.29
5	48.82	1-Docosene	$C_{22}H_{44}$	308	1.32

*molecular weight

Discussion

The Asteraceae family is one of the most distinguished botanical families, and is a source of acaricides and repellents against ticks, as reported by Benelli *et al.* (2016). Screening bioassays for the $Tp_{EtOH70\%}$ leaf extract against *T. urticae* adult females for the first time revealed significant toxic effects. The LC_{50} value of $Tp_{EtOH70\%}$ leaf extract predicted by Probit analysis for the treated adult females 24 h post-treatment was 0.99%. Our findings are in agreement with Santos *et al.* (2016) who found that the LC_{50} of *T. patula* and *T. erecta* L. ethanolic leaf extracts was 1.76 and 1.21%, respectively, against *Sitophilus zeamais* Mots. after 24 h. This was in contradiction to that of Khan *et al.* (2017) who found that *T. minuta* methanolic extract had no toxic effects against aphids, and the LC_{50} couldn't be calculated at 2% crude extract. Regardless of the concentrations tested, Politi *et al.* (2012) found that *T. patula* leaf extract had no lethal effect on adult dog ticks, *Rh. sanguineus* L.

The highest mean mortality rate (± SE) of the treated *T. urticae* adult females after 24 h at 27 ± 2°C was 88.9 ± 3.7% and the lowest mean mortality rate (± SE) was 15.3 ± 1.4%. Our results showed that the $Tp_{EtOH70\%}$ leaf extract was more effective than that of Mmbone *et al.* (2014) who reported 35 and 69% mortality rates of *T. urticae* adult females, caused by long soaking in *T. minuta* crude extract with high concentrations for 24 and 48 h, respectively. Another study on the Asteraceae family showed parallel results to our findings as reported by Yanar *et al.* (2011b), who found that methanol leaf extracts of *X. strumarium* L. and *Anthemis vulgaris* L. significantly affect the adult female mite mortality (79.85 ± 0.83 and 76.63 ± 2.08%, respectively). They also showed significantly higher mortality rates than synthetic pesticides tested at 5% 24 h post-treatment where azadirachtin, bromopropylate, dicofol, and spirodiclofen showed mean mortality of 16.28 ± 3.07, 19.07 ± 0.54, 23.21 ± 0.32 and 22.41 ± 2.38%, respectively. In addition, Vidal *et al.* (2009) noted that the ethanolic leaf extract of *T. patula* L. caused significant mortality rates of 92 and 77%, respectively against larvae and pupae of *Aedes aegypti* L. 48 h post-treatment.

Our results also showed no significant effect on the egg-laying capacity of *T. urticae* adult females treated with a sublethal concentration of $Tp_{EtOH70\%}$ leaf extract (0.5%) over 72 h. This result contradicts that of Politi *et al.* (2012) who found that Tp_{EtOH} at 5% reduced egg laying (21.5%) of dog ticks, *Rh. sanguineus* L. In addition, El-Hawary and Sammour (2006) detected that hexane or ethanol extracts of *A. monosperma* (Asteraceae) were able to reduce the fecundity of aphids.

The repellent index value of the $Tp_{EtOH70\%}$ leaf extract against *T. urticae* adult females was lower than 1 – SD, thus it was classified as a repellent. This is in agreement with Mmbone *et al.* (2014) who observed 55% repellence of *T. urticae* treated with *T. minuta*. It also demonstrated a good potential repellent activity against adults of the red flour beetle, *Tribolium castaneum*

Herbst in the laboratory as recorded by Padín *et al.* (2013). The crude extract of *T. patula* was used formerly as a repellent insecticide (Chadha 1976). Similarly, Morallo-Rejesus *et al.* (1982) found that the leaf extract of *T. patula* was more repulsive than *T. erecta* L. against black bean aphid. Likewise, many Asteraceae species, as reported by El-Sharabasy (2010), have a tendency to repel *T. urticae* adult females. With regard to the *ODI* of the $Tp_{EtOH70\%}$ leaf extract, results showed that *ODI* was 100 after 24 and 48 h. The females significantly preferred the untreated halves of leaves than the treated ones. In addition, their oviposition was significantly greater on the untreated halves in the first 24 h and over 72 h. This was in accordance with Vasanthakumar *et al.* (2012) who found that the leaf extract of *Wedelia chinensis* (Asteraceae) at 7.5% exerted ovipositional deterrent effects against the red spider mite, *Oligonychus coffeae* Nietner. Conversely, Elango *et al.* (2010) recorded that the oviposition active index was –0.72 and –0.64 for hexane and chloroform extracts of *T. erecta* L. against *Culex tritaeniorhynchus* Giles.

Regarding the ovicidal effect of the $Tp_{EtOH70\%}$ leaf extract, it significantly affected *T. urticae* eggs, recording 56.04% reduction in its hatching percentage. Remarkably, all hatched eggs turned into nymphs in the control groups while treatment reduced the number of hatched eggs. In addition, larvae development was extended for approximately 1–2 days, then all hatched larvae died before reaching nymphal stages. Our findings are in accordance with many studies on the Asteraceae family, Yanar *et al.* (2011a) recorded 57.45 ± 0.67 and 25.38 ± 1.13% mortality rates of *T. urticae* eggs treated with 10% of methanolic leaf extracts of *X. strumarium* L. and *A. vulgaris* L., respectively. Furthermore, Elango *et al.* (2010) observed 100% mortality of mosquito eggs treated with 0.1% of *T. erecta* hexane extract. Similarly, Bharadwaj and Sharma (2007) found that *T. patula* leaf extract inhibited the hatching of root-knot nematode eggs due to the presence of terthienyls, thiophenes, and terpenoids in these plant parts. Our results revealed 44.44 ± 5.56, 65.92 ± 7.75 and 100 ± 0.00% of larval death after hatching on the 4th, 5th and 6th days, respectively. This concurs with Politi *et al.* (2012) who observed that all hatched larvae of dog ticks, *Rh. sanguineus* L., treated with Tp_{EtOH} at 5%, died on the 4th, 5th and 6th days after hatching. Hence, the mortality can be attributed to direct contact of the newly emerged larvae with the extract or to the adverse effects on egg development. This confirms that the $Tp_{EtOH70\%}$ leaf extract (8%) had a marked effect on zero-time eggs with 100% larval mortality.

Studies on the biological activity of the *T. patula* leaf extract against mites remain scarce. The *T. patula* flower, root and oil extracts are well-described in literature. However, few studies explore the phytochemical components of its leaf extract. In the present study, the identification of the bioactive compounds of the $Tp_{EtOH70\%}$ leaf extract showed a total of five compounds from various classes of phytochemicals: phytol (62.72%), palmitic acid ethyl ester (9.81%), linoleic acid ethyl ester (4.29%), 3,7,11,15-tetramethyl-2-hexadecen--1-ol (1.83%), and 1-docosene (1.32%). Our results are similar to the findings of Devika and Koilpillai (2014) who registered more bioactive components in the methanolic leaf extract of *T. erecta* L., namely, phytol, hepta, hexa, tetradecanoic acid, celidoniol, citronellyl isobutyrate, phosphorothoic acid 0-0diethyl 0-(3,5,6--trichloro-2pridinyl) ester, alpha-tacopherol-beta-D--mannoside, stigmasterol and butanoic acid 3,7-dimethyl6-octenyl ester. Conversely to our results, Politi *et al.* (2012) registered 12 o-glycosylated flavonoids from *T. patula* leaf extract. Likewise, the Asteraceae species may possess some toxic chemicals *viz.* aromatic ester, *n*-hexyl salicylate, and a long-chain ketone detected in the aqueous leaf extract of *Xanthium strumarium* L. which may be responsible for its toxic and repellency effects against *Callosobruchus chinensis* L. (Roy *et al.* 2014). The biological activities of *Tagetes* sp. were attributed to several compounds in their tissue, as recorded by Vasudevan *et al.* (1997). *Tagetes patula* contains numerous thiophenes, steroids, and terpenoids in its roots, leaves, and flowers as pinpointed by Bano *et al.* (2002) and Ramya *et al.* (2012). In our study, phytol (62.72%), representing the major constituent of $Tp_{EtOH70\%}$ leaf extract, exhibited a high repellent activity, as similarly observed by Odalo *et al.* (2005). In addition, linoleic acid (4.29%) exerted some toxic effects, as stated by Rahuman *et al.* (2008) who showed high mortality rates of mosquito larvae exposed to the oleic and linoleic acid extracted from *Citrullus colocynthis* Linn Schrad (Cucurbitaceae). Likewise, Politi *et al.* (2016) found that the (70%) ethanolic extract of aerial parts of *T. patula* showed a cytotoxicity index with 58% of cell lysis. This was in accordance with Adel *et al.* (2010) who observed that the epithelium membrane of *Spodoptera littoralis* midgut was exfoliated and completely destroyed after treatment with *Artemisia monosperma* (Asteraceae). Palmitic acid ethyl ester (9.81%), found also in *T. erecta* L., showed nematicidal activity as reported by Debprasad *et al.* (2000). Therefore, the active chemical constituents of plant extracts may differ in their composition, depending on the plant species and the parts used, the method of extraction, and undoubtedly the solvent used (Tiwari *et al.* 2011). A combination of multiple modes of action of different plant components may act alone or in synergism to delay the development of mite resistance (Rattan 2010).

Botanical phytochemicals with acaricidal potential are now recognized as potent alternative insecticides to replace synthetic ones in mite management. Taken together, the findings reported above emphasize the promising role of medicinal plant extracts in the battle

against mites, alongside the data already reported in scientific literature. It is therefore clear that the species of the Asteraceae contain several chemical classes of bioactive compounds. The present study reveals the adulticidal, ovicidal, repellent and oviposition-deterrent activities of the ethanol leaf extract of *T. patula*, even though it has no effect on fecundity. Thus, the ability of this extract to kill and repel mites, while also reducing its egg hatching, makes this botanical plant a potential for the development of a new precautionary tool for the control of the *T. urticae*. It is easily available, accessible, and affordable for even small farmers. Nevertheless, these results were obtained under laboratory conditions, therefore it is necessary to investigate the efficacy of the $Tp_{EtOH70\%}$ leaf extract under field and greenhouse conditions to confirm its efficacy as a safe controlling agent for management programs of *T. urticae*.

In conclusion, this pioneering investigation revealed that this extract had toxicant, ovicidal, repellent and oviposition-deterrent activities. A combination of multiple modes of action of the different plant components, acting either alone or in synergism, is very promising because it may delay the development of mite resistance.

Acknowledgements

We are thankful to Prof. Dr. Mona M.A. Ghallab, Vegetable & Ornamental Plant Mites Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), for her kind help and contribution to this work. We would also like to extend our thanks to the Orman Garden staff.

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