Acaricidal Activity and Chemical Characterization of *Helichrysum bracteatum* and *Salvia officinalis* Leaf Extracts Against *Tetranychus urticae* and Its Predator, *Stethorus gilvifrons* (Coccinellidae)

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ABSTRACT

This study presents an attempt to evaluate the acaricidal activity of the two medicinal plants, *Helichrysum bracteatum* L. (Asteraceae) and *Salvia officinalis* L. (Lamiaceae) against *Tetranychus urticae* Koch and its predator, *Stethorus gilvifrons* Muls. under laboratory conditions. The ethanolic leaf extracts of *H. bracteatum* [$HbLE_{EtOH70\%}$] and *S .officinalis* [$SoLE_{EtOH70\%}$] were assessed for their direct toxicity against *T. urticae* and *S. gilvifrons* adult females. Both extracts were assessed for their effects on the egg-laying capacity, repellent and oviposition deterrent effects of the *T. urticae* adult females, as well as their direct effects on *T. urticae* eggs. The chemical characterization of both extracts was conducted to investigate their bioactive components by gas chromatography (GC-MS). Based on acute toxicity, results showed that $HbLE_{EtOH70\%}$ displayed the highest acaricidal activity (LC_{50}=1.27%) followed by $SoLE_{EtOH70\%}$ (3.39%) against *T. urticae* adult females. Whereas, against *S. gilvifrons* adult females, $SoLE_{EtOH70\%}$ was less toxic (LC_{50}=37.06%) than $HbLE_{EtOH70\%}$ (LC_{50}=0.76%). Our results revealed that $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ had a significant effect on the egg-laying capacity of *T. urticae* adult females. According to the repellent index (RI), the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ were classified as repellent. Both extracts revealed a potent ovicidal effect, where the egg hatching percentage was 48.75±15.38 and 45.34±11.73% for $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$, as compared to their control groups 98.81±0.7%, respectively. GC-MS analysis showed that phytol was the major component of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$. The current study confirmed the potent acaricidal effect of *H. bracteatum* and *S. officinalis* leaf extract against *T. urticae.* Thus, the *S. officinalis* leaf extract was the safe acaricidal product suitable for use in integrated pest management strategies as it was safe for *S. gilvifrons*.

Key words: *Tetranychus urticae*; *Stethorus gilvifrons*; *Helichrysum bracteatum; Salvia officinalis*; leaf extract; acute toxicity.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is the chief agricultural pest of approximately 1,100 plant species invading more than 140 different families (Grbic *et al*., 2011). Given that *T. urticae* breeds healthily through adapting to various plant species, it could be explained as it deactivated various secondary metabolites such as toxins, repellants and nutritional inhibitors, which are the key units of defense mechanisms (Smith and Clement, 2012). Hence, to get over this mite resistance, we conducted many research works to establish an alternative method in use of novel acaricides from natural plant products whether in form of extract or essential oil taking in consideration to be exceedingly specific to *T. urticae* compatible to its predator at the prescribed dosage (El Ouali *et al.*, 2016).

The spider mites biological control has focused on two bunches of biocontrol agents, the predatory mites within the family Phytoseiidae and the different Stethorini species (Coleoptera, Coccinellidae) (Roy *et al*., 2005). The ladybird beetle, *Stethorus gilvifrons* (Muls.) is the gluttonous predator in the Middle East that can effectually control spider mite populations as it feeds on both juvenile and adult spider mites (Biddinger *et al*., 2009).

Since the plant extract compounds are found in nature so they do not release toxic substances into the environment and did not cause water pollution by decomposing quickly (Salman *et al*., 2014). Therefore, several plant extracts has been investigated, as potential sources of commercial mite control agents because of their nature multi components that are less susceptible to the development of resistance (Kumral *et al.*, 2010).

Accordingly, *Helichrysum bracteatum* (Asteraceae), the golden everlasting as commonly known, contains a distinctive reservoir of various secondary metabolites that imparts them great medicinal properties. In the Mediterranean region, *Helichrysum* is widely used in traditional medicine as skin repair, likewise, the genus is described to possess anti-inflammatory, antimicrobial and antioxidant properties (Sala *et al*., 2002).

Similarly, *Salvia officinalis* L. (Lamiaceae), called meramiya in the neighborhood, is native also to the Mediterranean region. It is a potent medicinal herb used in folk medicine, as it is very rich in biologically active compounds increasing its medicinal importance (Beheshti-Rouy *et al*., 2015).

Different *Helichrysum* and *Salvia* species extracts were used as potential insecticides (Pascual-

Villalobos and Robledo, 1999; Pavela, 2004). Few studies dealt with their acaricidal activities (Erdogan *et al*., 2012; Salman *et al*., 2014; Waked, 2016). However, no studies have demonstrated the impact of these plant extracts on natural enemies, thus increasing the need to study their effects on beneficial organisms.

Therefore, this study aims to screen the bioactivity of the leaf extracts of two medicinal plant species against the adult stage of *T. urticae* and *S. gilvifrons* for the first time. Furthermore, we investigate their phytochemical active components through a GC-MS analysis.

MATERIALS AND METHODS

1. Plants collection and extract preparations:

H. bracteatum and *S. officinalis*, the nominated plants for the study were collected in April 2017 from El Orman Garden, Cairo. Identification and extraction of the collected plants were executed at the Botany and Chemistry Dept., Faculty of Science, Suez Canal University, Ismailia, Egypt, respectively.

According to Breuer and Devkota (1990), the fresh leaf samples from the collected plants with known weight were air dried in the shade, grounded into a fine powder, and then immersed in a sufficient quantity of ethanol (EtOH) for 24 h with continuous shaking. Extracted solute was separated from the insoluble plant materials, collected and the later re-extracted again with EtOH for another 24 h to ensure good extraction of ethanol soluble constituents, then the solvent was separated. The extract was then filtrated and ethanol was evaporated at reduced pressure at 40°C, to dryness in a rotation 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 avoid contamination.

2. *T. urticae* and *S. gilvifrons* stock cultures:

The susceptible strain of *T. urticae* and *S. gilvifrons* used in this study was originated from infested leaves of castor bean plants at Ismailia Agriculture Research Station, Ismailia, Egypt. The susceptible mite population was reared on sweet potato leaves in a climate-controlled room 27±2°C, under 60±5% R. H. and 16/8 h of Light/Dark photoperiod). Adults of *S. gilvifrons* were reared on potato leaves heavily infested with *T. urticae* as described by Rott and Ponsonby (2000).

3. Bioassays:

3.1. Adulticidal Bioassays:

3.1.1. Acute toxicity for *T. urticae* and its predators *S. gilvifrons* adult females:

The acaricidal activity bioassay was carried as described by Erdogan *et al*. (2012). Leaf spray method using a Sigma glass spray (unit No. S 3135) and leaf disc method were used (Helle and Overmeer, 1985). Before the proper test, the toxicity of the ethanolic leaf extracts of *H. bracteatum* [$HbLE_{EtOH70\%}$] and *S. officinalis* [$SoLE_{EtOH70\%}$] was assessed by some preliminary tests to establish the appropriate concentration ranges needed. Bioassays were carried out by spraying five serially diluted concentrations (0.375, 0.75, 1.5, 3, and 6%) for $HbLE_{EtOH70\%}$ and (0.5, 1, 2, 4, and 8%) for $SoLE_{EtOH70\%}$ in triplicates. Thirty adult female mites were gently transferred onto the sweet potato leaf-disc (3 cm diameter) in Petri dishes (9 cm diameter). According to Kumral *et al*. (2010), two ml of the two ethanolic leaf extracts were applied topically and allowed to dry for 30 min at 27±2°C. After drying, the Petri dishes were kept at 27±2°C, under 60±5% R. H. and 16/8 h (L/D), in a climatically controlled chamber. In all the experiments, an EtOH (70%) solution was used as a control treatment. 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.

The Petri-leaf disc method technique was adopted for the bioassay of the *S. gilvifrons* adult females with some amendments according to James (2003) with the same procedure and concentrations of *T. urticae* bioassay. However, bigger Petri-dishes (12 cm) and detached raspberry leaf discs (6 cm diameter) were used and put on its lower surface, then twenty adult females of *S. gilvifrons* were confined (20 females/replicate). For each treatment, two ml of the leaf extract were applied topically. Four replicates were used. All the treated leaf discs were allowed to dry completely at room temperature and kept in a climate chamber at 27±2°C and 60±5% R. H. Thirty adult females of *T. urticae* were provided as prey to *S. gilvifrons* on each Petri-dish for each treatment. Adult female mortality was assessed 24 h post-treatment.

3.1.2. Egg-laying capacity of *T. urticae* adult females:

The egg-laying capacity was evaluated for *T. urticae* adult females according to Erdogan *et al*. (2012) using one sublethal concentration (0.5%) of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$. The application of the extract and the spider mites transferring to Petri dishes were the same as in the toxicity bioassays. Six replicates were used (15 ♀/leaf disc) and each treatment was repeated ten times. The EtOH (70%) was used as a control. The Petri dishes were putted in a climate chamber at 27±2°C, under 60±5% R. H. and 16/8 h (L/D). Daily monitoring and the eggs deposited on the discs during 72 h were counted. 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.

3.1.3. Repellent and oviposition-deterrent effects on *T. urticae* adult females:

The repellency test was performed using the leaf disc method where the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ were applied with one sublethal concentration (0.5%). Repellent and oviposition-deterrent effects were assessed in a two-choice bioassay carried out in six replicates. Sweet potato leaf disks of 4.5 cm in diameter were used. Half of the disc was immersed for five seconds in the extract solution 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. 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% R. H. and 16/8 h (L:D). At 24, 48, and 72 h, the number of mites and the eggs they laid present on treated and untreated leaf disc was counted. Repellency index (RI) and oviposition deterrence index (ODI) was calculated according to Kogan and Goeden (1970) and Dimetry et al. (1993), respectively.

3.2. Ovicidal Bioassay:

To assess the ovicidal activity of the $HbLE_{EtOH70\%}$ and the $SoLE_{EtOH70\%}$, a wide range of concentrations was tested to define the effective concentrations needed. Each disc of sweet potato leaves (3 cm diameter) carried 100 eggs (24 h old) was sprayed by two ml of crude extract (8%). A control group with EtOH (70%) was considered. Three replicates were used. All treated leaf discs were allowed to dry completely at room temperature and kept at 27 ±2˚C and 60±5% R. H. in the laboratory. All discs were examined daily to record the number of hatched larvae (Chiasson *et al*., 2004). The eggs viability was checked for a period of seven days after oviposition. The eggs that did not hatch during this period were counted as non-viable.

4. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the leaf extracts:

The identification of the *H. bracteatum* and *S. officinalis* ethanolic leaf extract compounds was performed on a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS instrument using a TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). Operating conditions were as follows: Helium as the carrier gas with a flow rate of 1mL/min; column temperature 50˚C for 2 min then increasing to 150˚C for 2 min at an increasing rate of 7˚C/min, then to 270˚C for 2 min at an increasing rate of 5˚C/min, then to 310 for 10 min as a final temperature at an increasing rate of 3.5˚C/min; injector temperature, 280˚C; volume injected, 1 µL (Thangavel *et al*., 2014). The GC/MS was recorded in electron ionization mode with energy of 70 eV. The total running time was completed within 58 min. The relative percentage of each extract constituents was expressed as a percentage with peak area normalization. The identification of the bioactive phytochemical components was achieved through retention time and mass spectrometry by comparing the mass spectra of unknown peaks with those stored in WILEY and NIST libraries in the data system.

Statistical Analysis:

Mite and predator mean mortality rates (± S.E.) 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 was estimated using the POLO Plus software (LeOra Software, Berkeley, CA, USA). Mortality, fecundity and repellency data were analyzed by one-way analysis of variance and the mean values were compared by Tukey's test ($p \leq 0.05$). For studying differences between groups, data were analyzed by one-way analysis of variance (ANOVA) after data normalization. All the statistical tests were performed using the software package SPSS 15.0.0.

RESULTS AND DISCUSSION

Acute toxicity of the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ against *T. urticae* and its predators *S. gilvifrons* adult females:

In the current investigation, we assessed the acute toxicity of $Hb_{EtOH70\%}$ and $So_{EtOH70\%}$ ethanolic leaf extracts against *T. urticae* and *S. gilvifrons* adult females (Figure 1). The five serially diluted concentrations used were 0.375, 0.75, 1.5, 3 and 6% for $HbLE_{EtOH70\%}$ and 0.5, 1, 2, 4 and 8% for $SoLE_{EtOH70\%}$, respectively. The $HbLE_{EtOH70\%}$ showed strong acaricidal activity against *T. urticae* and *S. gilvifrons* adult females. After 24 h, the $HbLE_{EtOH70\%}$ at 6% showed the highest mean mortality rate (±SE) 96.7±3.3 and 100% against *T. urticae* and *S. gilvifrons* adult females, respectively, as compared to no mortality in their corresponding control groups ($P < 0.000$). Whereas, the lowest mean mortality rate (±SE) was 17.8±2.2 and 30±2.9% at 0.375%, respectively, as compared to no mortality in their corresponding control groups ($P < 0.000$). Results showed that the LC_{50} value of $HbLE_{EtOH70\%}$ predicted by Probit analysis for adult females at 24 h was 1.27 and 0.76% for *T. urticae* and *S. gilvifrons* adult females, respectively. On the other hand, the LC_{50} of $SoLE_{EtOH70\%}$ was 3.39 and 37.06% for *T. urticae* and *S. gilvifrons* adult females, respectively.

Our results showed that *H. bracteatum* ethanolic leaf extract was more effective than that of the crude extract of *H. arenarium* tested by Erdogan *et al.*

(2012) who recorded its highest mean mortality rate 82.38±1.92% at 12% while the lowest mortality rate was 39.76±5.18 at 1%. On the other hand, the highest mean mortality rate (±SE) of $SoLE_{EtOH70\%}$ at 8% 24 h post-treatment was 85.3±3.5 and 20±5% against *T. urticae* and *S. gilvifrons* adult females, respectively as compared to no mortality in their corresponding control groups ($P < 0.000$). Whereas, the lowest mean mortality rate (±SE) was 5.3±1.3% for *T. urticae* with no mortality in *S. gilvifrons* at 0.5% as compared to their corresponding control groups ($P < 0.000$).

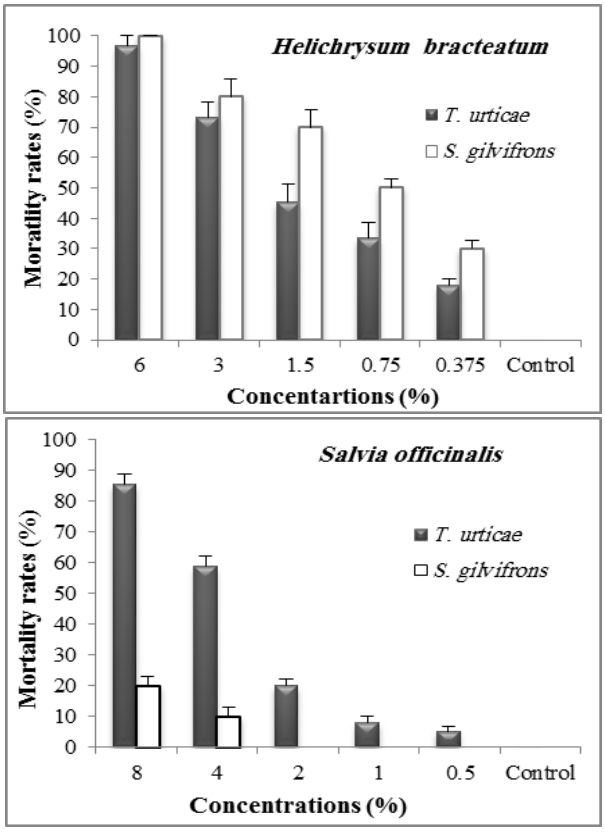

Fig. (1): Mean mortality rates (±SE) of *T. urticae* and *S. gilvifrons* adult females treated with $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ 24 h post-treatments.

According to our result, it was found that the ethanolic leaf extract of *S. officinalis* (8%) get a higher toxicity and mortality rates 24 h post-treatment than the methanolic leaf extract (12%) exhibiting 62.2±0.03% mortality rates after 6 days as recorded by Salman *et al.* (2014). Similarly, Waked (2016) recorded the mortality rates of *T. urticae* adult females 44.28 and 31.42% for the methanol and aqueous leaf extracts of *S. officinalis*, respectively at the highest concentration used (20%) after 24h.

No previous studies have investigated the impact of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ on *S. gilvifrons*. However, the findings of other plant extracts belonging to the same plant families are similar to those of our study where Sarmah *et al.* (2009) found no mortality for the aqueous plant extract of *Xanthium strumarium* (Asteraceae) and *Clerodendron infortunatum* (Lamiaceae). In addition to the different plant families as *Acorus calamus* (Acoraceae) and *Polygonum hydropiper* (Polygonaceae) even at higher concentration (10%) for 14 days did not show any mortality against *S. gilvifrons*.

Another plant extracts belonging to different families were reported having acaricidal activity with similar mortality rates against *S. gilvifrons* including the ethanol leaf extract of *Datura stramonium* (Solanaceae) as recorded by Kumral *et al.* (2013) exhibited an LC_{50} of 0.18% after 24 h. Similarly, Mazhawidza *et al.* (2018) found that the aqueous plant extract of *Solanum delagoense* (Solanaceae) was less toxic with an LC_{50} of 49%, followed by *D. stramonium* 34% to *Hippodamia variegata* (Coleoptera: Coccinellidae). These results showed that *D. stramonium* and *S. delagoense* extracts were relatively safer to *H. variegate.* The present results indicated the ethanolic leaf extracts of *H. bracteatum* exhibited more significant effect than *S. officinalis* in the treatment of *T. urticae* but the latter was safer against its predator and can be included in integrated pest management programs.

Assessment of the egg-laying capacity of *T. urticae* adult females:

The effect of a sublethal concentration 0.5% of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ on the egg-laying capacity of *T. urticae* adult females after 24, 48 and 72 h is shown in Table (1). Our results revealed that $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ had a significant effect on the egg-laying capacity of the treated adult females.

They decreased the egg numbers to 10.43±0.3 and 16.70±0.47 eggs per female, respectively as compared to their control group (24.82±0.73) ($P < 0.000$) summed after 72 h post-treatment. In contrast to our findings, Erdogan *et al*. (2012) found that *H. arenarium* crude extract even at (12%) did not affect *T. urticae* fecundity. Regarding $SoLE_{EtOH70\%}$, our results was in agreements with Mohamed *et al*. (2015) who found that the ethyel acetate of *S. officinalis* extract reduced the total number of *T. urticae* eggs. Similarly, Tomczyk and Suszko (2011) found that the ethanolic leaf extract of *S. officinalis* diminished *T. urticae* female's fecundity.

Assessment of the repellent and oviposition-deterrent effects on *T. urticae* adult females:

The repellent index (RI) and the oviposition deterrence index (ODI) of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ at 0.5% on *T. urticae* adult females 24, 48 and 72 h post-treatment is shown in Tables (2 & 3). The behaviour of *T. urticae* adult

Table (1): The egg laying capacity of *T. urticae* adult females treated with a sublethal concentration of the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ (0.5%) after 24, 48, and 72 h.

Treatments	24 hr.		48 hr.		72 hr.	
	Mean Egg No.	Mean Egg/Female	Mean Egg No.	Mean Egg/Female	Mean Egg No.	Mean Egg/Female
$HbLE_{EtOH70\%}$	56.7±2.02[c]	3.78±0.13[c]	102.8±3.04[c]	6.85±0.20[c]	156.5±4.59[c]	10.43±0.30[c]
$SoLE_{EtOH70\%}$	72.8±1.40[b]	4.86±0.09[b]	139.3±1.45[b]	9.29±0.09[b]	250.5±7.11[b]	16.70±0.47[b]
Control (EtOH 70%)	93.8±1.47[a]	6.26±0.1[a]	172.3±1.28[a]	11.49±0.08a	372.3±10.98[a]	24.82±0.73[a]

Table (2): Repellence index (RI) of the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ summed after 72 h on *T. urticae* adult females using one sublethal concentration (0.5%)**.**

Plant extracts	RI [1]			Classification
	24 h	48 h	72 h	
$HbLE_{EtOH70\%}$	0.00±0.00	0.00±0.00	0.16±0.10	Repellent
$SoLE_{EtOH70\%}$	0.33±0.33	0.27±0.11	0.40±0.17	Repellent

[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 disk; <1 repellent; 1 neutral; 1 > attractant.

Table (3): Oviposition deterrent index (ODI) of the $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ summed after 72 h on *T. urticae* adult females using one sublethal concentration (0.5%)**.**

Plant extracts	ODI[1]			Classification
	24 h	48 h	72 h	
$HbLE_{EtOH70\%}$	100	100	96.93	Oviposition deterrent
$SoLE_{EtOH70\%}$	83.33	76.91	72.24	Oviposition deterrent

[1]**Oviposition Deterrent Index;** [(C-T)/(C+T)]*100 was calculated, where C and T represent eggs laid on control and treated disk.

females was considerably affected by $HbLE_{EtOH70\%}$ than $SoLE_{EtOH70\%}$, where repellency was strongest after 24 and 48 h post-treatment. Both extracts were classified as repellent as their RI value was lower than 1-SD. Our results were in agreement with Pascual-Villalobos and Robledo (1999) who showed that *Helichrysum decumbens* leaf extract was repellent to the stored grain pest, *Tribolium castaneum*. As well, other members of the Asteraceae family had shown repellency effect against *T. urticae* (Mozaffari *et al*., 2012). *S. officinalis* leaf extract was found to be extremely repellent to *T. urticae* adult females as stated by Mohamed *et al*. (2015) and Waked (2016).

$HbLE_{EtOH70\%}$ prevents the adult females to lay eggs where the ODI ranging from 96.93 -100.0% while 72.24 - 83.33% of ODI was recorded for $SoLE_{EtOH70\%}$ after 24, 48 and 72 h, respectively. This finding was in accordance with that of Dimetry *et al*. (1993) who showed 100% ODI of neem azal-S (Meliaceae) at 0.1% against *T. urticae*. Strong oviposition deterrent (ODI=100) was noted for ethanol crude plant extracts (up to 10%) belonging to different families against *Tetranychus truncates viz.* neem and chinaberry (Meliaceae), cube root (Fabaceae) and sweet oleander (Apocynaceae) (Sakunwarin *et al*. 2004).

Direct toxicity to *T. urticae* zero-time eggs

Daily egg hatching percentage (±SE) of zero-time eggs of *T. urticae* adult females treated with 8% of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ at 27±2°C is shown in Table (4). Results showed that both extracts significantly affected egg mortality rates at 8%. The egg hatching starts four days after treatment (DAT) for both extracts as compared to their control groups. Interestingly, all larvae turned into nymphs with no larval mortality in the control groups while treatment caused the death of all hatched larvae before reaching the nymphal stages.

Our results are in accordance with other studies related to the Asteraceae family where *X. strumarium* aqueous plant extracts exhibited 87.09% of *T. urticae* egg mortality (Sarmah *et al*., 2009). In contrast to our findings, Erdogan *et al*. (2012) determined that *H. arenarium* crude extract (12%) did not show any ovicidal effects against *T. urticae* eggs. Regarding $SoLE_{EtOH70\%}$ on the other hand, our results showed that $SoLE_{EtOH70\%}$ at 8% caused 45.34±11.73% egg mortality which was higher than the methanolic leaf extract of *S. officinalis* as recorded by Salman *et al*. (2014) that caused 30.2% eggs mortality at 12%. This also was in accordance with Waked (2016) who recorded 35% of egg mortality when treated with both the methanolic and

aqueous leaf extracts of *S. officinalis* (20%). In contradiction, Tomczyk and Suszko (2011) recorded the low ovicidal effect of the ethanolic leaf extract of *S. officinalis* against *T. urticae* eggs.

GC-MS analysis of the $Hb_{EtOH70\%}$ and $So_{EtOH70\%}$ leaf extracts:

The extracts were injected into the GC-MS analyzer to identify the major bioactive components. The $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$ chromatograms is shown in (Figure 2). In total, five bioactive compounds from various classes of phytochemicals were identified in $HbLE_{EtOH70\%}$ as shown in Table (5). The main compounds were Phytol (58.79%), 2-Bromolauric acid (8.18%), Squalene (3.09%), Phenols (2.69%) and Benzene (0.45%). The identified bioactive compounds of $SoLE_{EtOH70\%}$ is shown in Table (6). The main compounds were Phytol (82.08%), Squalene (2.05%), Neophytadiene (1.61%), 6,9,12,15-Docosatetraenoic acid methyl ester (0.69%) and 2,2,3,3,4,4-Hexadeutero Octadecanal (0.12%).

To the best of our knowledge, the phytochemistry of *H. bracteatum* leaf extract remained scarce, as it has not been investigated previously. According to our results, the acyclic diterpene alcohol, phytol was the major constituent of both the *H. bracteatum* and S. *officinalis* ethanolic leaf extracts. This was significantly different from the one previously reported by Veličković *et al.* (2003) who found that manool was the major component (9%) in the ethanolic leaf extract of *S. officinalis.* While, Waked (2016) found that (1S,4R,5R) 4-Methyl-1-(propan-2-yl)bicycle[3.1.0]hexan-3-one (29%) was the major compound in the methanolic leaf extract of *S. officinalis*. This difference observed in phytochemical screening could be ascribed to the ecological circumstances, the plant age, the extraction techniques, the solvent used, the concentration of its active components, the physicochemical characteristics, and the chemical composition of the extracts all these factors must influence the performance of the plant extracts (Ouahida *et al.*, 2016).

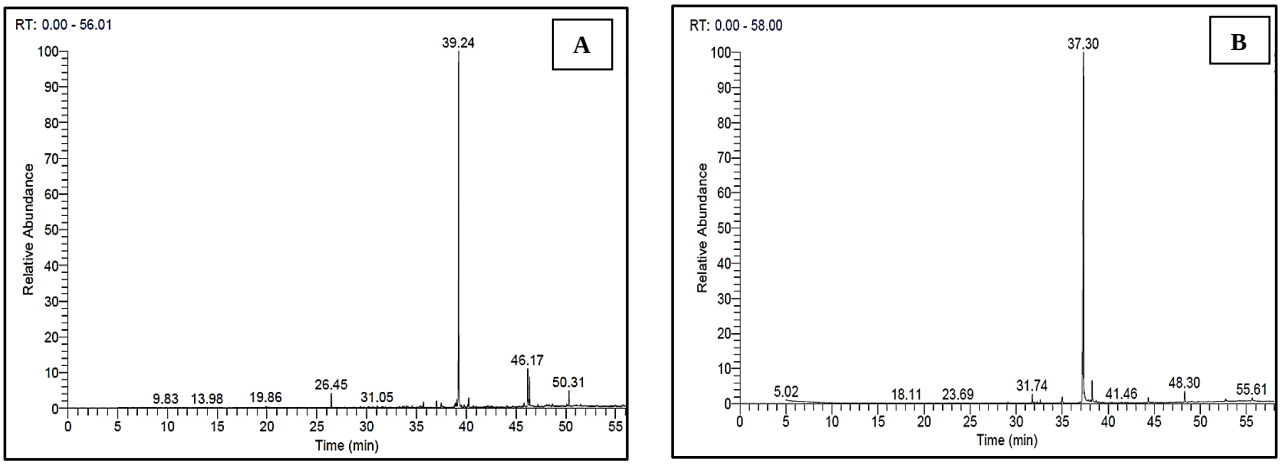

Fig. (2): GC-MS chromatogram of $HbLE_{EtOH70\%}$ (A) and $SoLE_{EtOH70\%}$ bioactive components (B).

Table (4): Egg hatching percentage (± SE) of *T. urticae* zero-time eggs treated with 8% of $HbLE_{EtOH70\%}$ and $SoLE_{EtOH70\%}$

	% Egg hatching				% Larval mortality			
	4 DAT	5 DAT	6 DAT	7 DAT	4 DAT	5 DAT	6 DAT	7 DAT
$HbLE_{EtOH70\%}$	20.05±4.25^{b}	41.46±12.68^{b}	48.75±15.38^{b}	48.75±15.38^{b}	60.56±11.07^{a}	70.96±6.45^{a}	100±0.0^{a}	100±0.0^{a}
$SoLE_{EtOH70\%}$	11.31±5.40^{b}	40.01±15.30^{b}	45.34±11.73^{b}	45.34±11.73^{b}	46.43±8.20^{a}	64.31±11.04^{a}	100±0.0^{a}	100±0.0^{a}
$Control_{EtOH70\%}$	73.67±7.72^{a}	98.81±0.7^{a}	98.81±0.7^{a}	98.81±0.7^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.0±0.00^{b}

*DAT: Day After Treatment

Table (5): Chemical composition of the different bioactive components of the $HbLE_{EtOH70\%}$ using GC-MS analysis

Peak	Retention Time (min)	Chemical Name	Molecular Formula	M wt.	Area %
1.	26.45	Phenol	$C_{15}H_{24}O$	220	2.69
2.	31.05	Benzene	$C_{17}H_{28}$	232	0.45
4.	39.24	Phytol	$C_{20}H_{40}O$	296	58.79
3.	46.17	2-Bromolauric acid	$C_{12}H_{23}BrO_2$	278	8.18
5.	50.31	Squalene	$C_{30}H_{50}$	410	3.09

Table (6): Chemical composition of the different bioactive components of the $SoLE_{EtOH70\%}$ using GC-MS analysis

Peak	Retention Time (min)	Chemical Name	Molecular Formula	M wt.	Area %
1.	31.74	Neophytadiene	$C_{20}H_{38}$	278	1.61
2.	37.30	Phytol	$C_{20}H_{40}O$	296	82.08
3.	41.46	2,2,3,3,4,4-Hexadeutero Octadecanal	$C_{18}H_{30}D_6O$	268	0.12
4.	48.30	Squalene	$C_{30}H_{50}$	410	2.05
5.	55.61	6,9,12,15-Docosatetraenoic acid methyl ester	$C_{23}H_{38}O_2$	346	0.69

Phytol showed many biological activities viz. antibacterial, anti-inflammatory, and insecticidal potentials (Kumar *et al*., 2010). It was observed by Odalo *et al*. (2005) that phytol had a high repellent activity against *Anopheles gambiae*. Squalene is the most common triterpene found in high concentration in different Helichrysum *species* (Lourens *et al*., 2008). These species are generally enriched source of phenolics, α-pyrone and acetophenones derivatives responsible for its biological activities (Kladar *et al*., 2015). It was established that phenols and its derivatives are toxic to humans and animals due to the formation of phenoxyl radicals (Andersen, 2006). Salvia *species* are also characterized by different secondary metabolite constituents responsible for their bioactivities (Simmonds and Blaney, 1992). In addition to phytol, squalene, a tripenoid hydrocarbon, the neophytadiene was also detected possessing strong bactericidal**,** antifungal and antimicrobial activities (Venkata *et al*., 2012). Hence, the extracts toxicity and repellency were possibly associated with the presence of diterpene, triterpene, and phenolic compounds (Tomczyk and Suszko, 2011). The observed acaricidal characteristics were mostly attributed to the synergetic effect of multiple major and minor compounds present at various concentrations.

In conclusion, the current study confirmed that *H. bracteatum* leaf extract had both lethal and repellent effects stronger than *S. officinalis* against *T. urticae* under laboratory conditions. *H. bracteatum* leaf extract can be used effectively as an acaricidal product for the management of *T. urticae*, but it is not suitable for use in integrated pest management (IPM) strategies due to its toxic effects against *S. gilvifrons*. Meanwhile, *S. officinalis* leaf extract can be used effectively as a harmless phyto-pesticidal product suitable for use in IPM strategies as it was safe for *S. gilvifrons*. Thus, the aromatic medicinal plant extracts tested in our study demonstrated potent acaricidal properties that have the potential to be established as natural insecticides. The permanent plant investigations have opened up a new perspective in finding alternatives to chemical acaricides.

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