

## **Acaricidal and ovicidal efficacies of *Leucaena glauca* Benth. seed crude extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae)**

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### **ABSTRACT**

The preliminary screening under laboratory conditions is a great value for evaluating the acaricidal and ovicidal activities of hexane, dichloromethane and methanol crude extracts of *Leucaena glauca* Benth. (Leguminosae) seeds against adults and eggs of *Tetranychus urticae* Koch (Acariformes). The leaf disc no choice and leaf disc choice tests were used to determine the acaricidal effects while direct spray method was used to investigate the ovicidal effects. Hexane and dichloromethane extracts of dried seeds demonstrated higher acaricidal effect than other extracts under leaf disc no choice test. The hexane and dichloromethane extracts of *L. glauca* dried seeds at 10000 ppm (v/v) induced the highest mortality rates of 80.6 and 98.9% of *T. urticae* female adults, respectively. These extracts also demonstrated oviposition deterring effects based on a 100% reduction of the total number of eggs on leaf disc treated with the extracts. This investigation indicated that hexane and dichloromethane extracts of dried seeds at all treated concentrations (100, 500, 1000, 5000 and 10000 ppm) also had a good potential for repelling this mite and decreasing the egg laying on the treated surfaces under leaf disc choice test since < 50% of alive mites and eggs were found on the treated sides. In the side of ovicidal activity, the hexane and dichloromethane extracts of *L. glauca* fresh and dried seeds caused higher percentage of egg mortality compared to the control. Egg mortalities of 59.8-98.8% and 82.3-97.6% were detected in hexane and dichloromethane extracts of *L. glauca* fresh seeds at concentrations ranging from 10000 to 50000 ppm, respectively. Egg mortalities of 95.8-99.6% and 64.9-97.5% were recorded when treated with hexane and dichloromethane extracts of dried seeds at the same concentrations of the fresh seeds, respectively. Hence, the hexane and dichloromethane extracts of *L. glauca* have the efficacy to be developed as botanical acaricides for *T. urticae* management.

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### **INTRODUCTION**

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Acariformes: Tetranychidae) is a well known and important agricultural pest damaging several plants such as tomato, eggplant, pepper, cucumber, bean and potato in both outdoors and greenhouses with a global distribution (Jeppson *et al.*, 1975; Helle and Sabelis 1985; Hincapie *et al.*, 2008; Yanar *et*

*al.*, 2011). *Tetranychus urticae* reduces both quality and quantity of yields since it feeds by puncturing cells, resulting in yellow spotting of the leaf surface due to chlorophyll depletion, necrosis of young leaves and stems defoliation or even the death of the plant (Badawy *et al.*, 2010).

Several synthetic acaricides had been employed to control this mite (Choi *et al.*, 2004; Liburd *et al.*, 2007; Ochiai *et al.*, 2007).

Few selective acaricides such as organophosphate, carbamates, dicofol, abamectin, clofentezine, hexythizox, organotins, bifenthrin, chlorfenapyr are also accepted for integrated pest management (Van Pottelberge *et al.*, 2009). However, some populations of mite have developed resistance to synthetic acaricides (Van Pottelberge *et al.*, 2009). Consequently, the producers have to increase both quantity of chemical acaricide and application time in order to control these mites that develop resistance. In addition, synthetic acaricides are known for their negative effect for human health and environment (Cavalcanti *et al.*, 2010; Kumral *et al.*, 2010). Consequently, problems dealing with use of synthetic chemicals lead researchers to find effective techniques to control phytophagous mites, friendly to other animal planets and their surroundings and not expensive to produce.

The natural pesticides which derived from plants and microorganisms have gradually increased interest for application to mite control. Natural chemicals are assumed to be safer than synthetic chemicals, effective to target pests, specific to natural enemies, degradable, resulting in less residual on plants as well as in the environment, and also appropriate for use in sustainable agriculture, nevertheless in fact, they are not as popularly used as they should be (Kelany, 2001; Park *et al.*, 2002; Mansour *et al.*, 2004; Isman, 2006; Wei *et al.*, 2011). Plant may show the potentiality to control mites due to their secondary metabolites such as terpenoids, alkaloids, flavonoids, tannin and polyacetylenes which have effect to reduce feeding injury by phytophagous mites, inhibit growth and behavior of mites, or show toxicity on mites (Copping and Duke, 2007; Chandler *et al.*, 2011; Lim *et al.*, 2011). Some plant extracts have been demonstrated as acaricide effect on the mites for example *Azadirachta indica* A. Juss. (Ascher, 1993); *Allium sativum* L. (Boyd and Alverson, 2002; Hincapie *et al.*, 2008); *Datura stramonium* (Mateeva *et al.*, 2003); *Capparis aegyptia* L. (Hussein *et al.*, 2006); *Ailanthus altissima* L.

and *Convolvulus krauseanus* Regel and Schmalh (Chermenskaya *et al.*, 2010).

*Leucaena*, belonging to the family Leguminosae, is a genus which has 24 species of leguminous trees and shrubs (Mabberley, 1997). *Leucaena glauca* (L.) Benth. can be used for livestock folder green manure, fire wood crops. Its seed extracts have anthelmintic medicinal quantities and the bark is used for internal pain (Duke, 1981). In Senegal, the seeds of *L. leucocephala* are useful in repelling *Ascaris* spp. worms (Adebowale, 1993). The plant in genus *leucaena* contains hydrocyanic acid, leucaenine, quercitrin, tannin acid. By the way, terpenes, flavonoids, coumarins and sterols were also found in *L. glauca* growing in Egypt (Hassan and Radwan, 2010). *Leucaena* is reported to reduce resistance to the pests and diseases prevalent in Hawaii (Hassan and Radwan, 2010). *Leucaena leucocephala* extracts showed insecticidal against *Bemisia tabaci* biotype B (Vasconcelos *et al.*, 2006).

The purpose of this study is to investigate the efficacy of *L. glauca* seed crude extracts in terms of mortality, repellency and ovicidal activity on *T. urticae* female. The received information gained from this investigation not only provides the new knowledge concerning the acaricidal and ovicidal activities of *L. glauca* seed extracts on *T. urticae* but also guides the producer to select seeds of *L. glauca* for extraction and use it to control polyphytophagous mites.

## MATERIALS AND METHODS

### Preparation of Extracts

Three kilograms of fresh and dried *L. glauca* seeds were collected from Phetchaburi province located in western Thailand in November 2012. Each type of seeds was ground into small pieces by motor grinder before putting in the glass bottle and extracting with hexane for 2 days at room temperature. The hexane solution was filtered with a Whatman #1 filter paper and concentrated by a rotatory evaporator under reduced pressure to give the crude hexane extract. The residual was re-extracted with

dichloromethane and methanol in the same method as hexane extraction to obtain crude dichloromethane and methanol extracts, respectively. All dried crude extracts were weighed and kept in the refrigerator at 10-12 °C for further bioassays. The extraction rate was calculated (Wang *et al.*, 2002; Shi *et al.*, 2004; Shi *et al.*, 2006) as follows: Extraction rate = (extract weight (g) × 100%) / 3000 (g).

#### **Tested Mites**

The stock colonies of *T. urticae* used in this study originated from Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. They were cultured on the lower surface of mulberry leaf (*Morus alba* L.) placed on moistened cotton pads resting on sponges in the plastic boxes. The colonies were maintained at 28 ± 2°C and 70 ± 5% R.H. under laboratory conditions. The mulberry leaves were examined every few days and replaced with fresh ones when over-crowding of mites and yellow leaves were observed. All bioassays were conducted and carried out under the same environmental conditions as the culture.

#### **Acaricidal effect**

##### **Leaf disc no choice test**

Toxicity, repellency and egg-laying activities were determined for studying the effect of residual of *L. glauca* seed crude extracts on leaf discs against *T. urticae* females (3- days old). Mulberry leaf discs (2 cm diam) were punched with a cork borer before painting with 50 µL of different concentrations (100, 500, 1000, 5000, 10000 and 90000 ppm (9%)) of crude extracts. Mulberry leaf discs painted with 95% ethanol were used as control. Treated leaf discs were allowed to completely dry at room temperature and then placed on moistened cotton pads in glass Petri dishes (9 cm diam) (3 leaf discs/ replication, 3 replications/ concentration). Twenty *T. urticae* females from the culture were introduced into the center of each leaf disc using a fine artist's brush. The experiment was kept at 28 ± 2°C and 70 ± 5% R.H. in the laboratory. The number of alive mites, dead mites on the leaf discs, mites off the leaf discs and eggs oviposited on the leaf discs was recorded at 24 hrs after experiment.

##### **Leaf disc choice test**

The crude extracts of *L. glauca* seeds were also used to evaluate the repellency activity and oviposition deterrent index against *T. urticae* females (3-days old) by using leaf disc choice test. Each mulberry leaf disc (2 cm diam) was cut with a cork borer and divided into two equal parts by midrib of the leaf disc. One-half of leaf disc was painted with 25 µL of tested material at different concentrations (100, 500, 1000, 5000, 10000 ppm) while the other half was treated with its solvent (95% ethanol). Treated leaf discs were allowed to completely dry at room temperature and placed on the moistened cotton pad in 9 cm diam of glass Petri dishes (3 leaf discs/ replication, 3 replications/ concentration). Twenty females were transferred from stock colony and placed on the center of each leaf disc. All petri dishes were maintained at 28 ± 2°C and 70 ± 5% R.H. in the laboratory. Number of alive *T. urticae* females and eggs laid on treated side of leaf discs was recorded at 24 hrs after exposure. Oviposition deterrent index (ODI) (Dimetry *et al.*, 1993) was calculated as follows:

$$\text{ODI} = \{(C-T)/(C+T)\} \times 100$$

where C was the number of eggs laid on the control side and

T was the number of eggs laid on the treated side.

##### **Ovicidal effect**

To determine an ovicidal activity of the crude extracts from *L. glauca*, 10-adult female mites were introduced to each of the 2 cm diam mulberry leaf disc cut by cork borer for oviposition overnight. Leaf discs were placed on moistened cotton pads in glass Petri dishes (9 cm diam) (3 leaf discs/ replication, 3 replications/ concentration). The introduced mites were removed from the leaf disc to another food source after 24 hrs. Eggs laid on the 3 leaf discs were sprayed with 500 µL at different concentrations of crude extracts by using hand sprayer. The control eggs were prepared in the same method as mentioned above and treated with 95% ethanol. All treated leaf discs were allowed to completely dry completely at room temperature and kept

at  $28 \pm 2^\circ\text{C}$  and  $70 \pm 5\%$  R.H. in the laboratory. The viability of eggs was checked for a period of 7 days after oviposition. The eggs that did not hatch at this period were counted as non-viable.

**Statistical Analysis**

All data were analyzed using Analysis of Variance (ANOVA) by the SPSS program 19.0. Treatment means were compared by Duncan’s Multiple Range Tests (DMRT) at  $p = 0.05$ .

**RESULTS AND DISCUSSIONS**

**Acaricidal effect**

**Leaf disc no choice test**

Our results showed that 3 organic solvents, hexane, dichloromethane and methanol, used to extract fresh and dried seeds of *L. glauca* had effects on extract weights, extract rates and extract feature of these seeds (Table 1). The highest extract weights and extract rates were detected in methanol extract of dried seeds while the lowest extract weights and extract rates were detected in hexane extract of fresh seeds.

**Table 1.** Extract weight (g), extraction rate (%) and extract feature of crude extracts from *Leucaena glauca* seeds.

Solvents	Seeds	Extract weight (g)	Extraction rate (%)	Extract feature
Hexane	Fresh	8.23	0.27	green liquid
	Dry	25.47	0.85	yellow liquid
Dichloromethane	Fresh	18.73	0.62	dark green liquid
	Dry	27.74	0.92	dark green liquid
Methanol	Fresh	30.24	1.01	dark blown liquid
	Dry	44.40	1.48	dark blown solid

The acaricidal effects of 9% *L. glauca* fresh and dried seed extracts against *T. urticae* female adults are shown in Table 2. When *T. urticae* contacted the residual of the seed extracts, the maximum death rate was 100.0%, followed by 92.2% in the dichloromethane and hexane extracts of dried seeds, respectively.

On the other hand, mites contacted with hexane and dichloromethane

extracts of fresh seeds resulted in a death rate ranging between 33.3-52.8%. Consequently, these extracts presented significant difference in mortality when compared to the control and methanol crude extracts ( $F_{6,14}=37.906, P<0.05$ ). According to this experiment, the mortality rates caused by hexane and dichloromethane extracts of the dried seeds were 1.75 and 3.0 times of those obtained from fresh seeds. Therefore, it can be inferred that the hexane and dichloromethane extracts of dried seeds were effective than those of fresh seeds in controlling *T. urticae*. However, the treatment with hexane and dichloromethane extracts of fresh seeds showed the repellency rate (37.2-41.1%) which is differing significantly from the control ( $F_{6,14}=14.856, P<0.05$ ). At the end, all hexane and dichloromethane extracts caused significantly lower in survival rates when compared to the methanol and control ( $F_{6,14}=43.015, P<0.05$ ).

The effect of egg laying activity of mites contacting the residual of 9% *L. glauca* seeds extracts is shown in Table 2. The results revealed that *T. urticae* contacted with hexane and dichloromethane extracts of fresh seeds laid only 0.1 and 1.5 eggs per female, respectively. In addition, the total numbers of eggs oviposited by female contacted with both treated with active extracts were extremely lower than those oviposited by female contacted with the control and methanol extracts. As a result, hexane and dichloromethane extracts caused egg reduction ranging between 87.2-100.0%. In the next step, we examined the effectiveness of hexane and dichloromethane extracts from dried seeds of *L. glauca* at lower concentrations (Table 3). Mortalities due to treatment with 5000 and 10000 ppm of hexane and dichloromethane extracts differ significantly from their controls ( $F_{5,12}=91.018, P<0.05$  and  $F_{5,12}=201.075, P<0.05$  for hexane and dichloromethane extracts, respectively). However, the highest mortalities of 80.6 and 98.9% were detected at maximum concentration (10000 ppm) of hexane and dichloromethane respectively.

**Table 2.** Mortality and repellency rates of *T. urticae* females and their eggs at 24 hrs after staying on mulberry leaf discs treated with 9% of *L. glauca* seeds crude extracts, using leaf disc no choice test.

Seeds	Solvent used	% of Mite (Mean ± S.E.) <sup>1/2</sup>			Number of egg laid (Mean ± S.E.) <sup>1/2</sup> Egg/female	Total egg production	% Egg reduction
		Alive	Dead	Repelled			
Fresh	Hexane	4.5±2.3bc	52.8 ± 10.2 b	37.2 ± 8.2 a	0.1 ± 0.1c	5	99.5
	Dichloromethane	25.6 ±19.0b	33.3 ± 15.4 c	41.1 ± 8.0 a	1.5 ± 1.5c	136	87.2
	Methanol	98.9 ±1.1a	0.0 ± 0.0 d	1.1 ± 1.1b	11.3 ± 0.1ab	1006	5.6
Dry	Hexane	0.0 ±0.0 c	92.2 ± 2.9 a	7.8 ± 2.9b	0.0 ± 0.0 c	0	100.0
	Dichloromethane	0.0 ±0.0 c	100.0 ± 0.0 a	0.0 ± 0.0b	0.0 ± 0.0 c	0	100.0
	Methanol	91.0 ± 4.0 a	1.1 ± 1.1 d	7.9 ± 2.9b	9.5 ± 0.4 b	844	20.83
Control		100.0 ± 0.0 a	0.0 ± 0.0 d	0.0 ± 0.0b	11.8 ± 0.4 a	1066	

<sup>1/2</sup> Means ± SE followed by the same letters are not significantly different as determined by Duncan's Multiple Range Tests (DMRT) ( $\alpha = 0.05$ ).

**Table 3.** Mortality and repellency rates of *T. urticae* females and their eggs at 24 hrs after staying on mulberry leaf discs treated with different concentrations of crude extracts of *L. glauca* dried seeds, using leaf disc no choice test.

Solvent used	Concentration (ppm, v/v)	% of Mite (mean ± S.E.) <sup>1/2</sup>			Egg laying (Mean ± S.E.) <sup>1/2</sup> Egg/female	Total egg production	%Egg reduction
		Live	Dead	Repelled			
Hexane	100	93.3 ± 1.0 a	1.1 ± 0.6 c	5.6 ± 1.5 c	5.7 ± 0.3 a	1017	13.5
	500	70.7 ± 0.7 b	1.1 ± 1.1 c	28.2 ± 1.0 b	3.4 ± 0.3 b	613	47.9
	1000	47.8 ± 12.0 c	2.2 ± 1.5 c	50.0 ± 13.5 a	2.0 ± 0.3 c	364	69.0
	5000	8.3 ± 5.0 d	38.3 ± 2.5 b	53.3 ± 4.8 a	0.1 ± 0.1 d	23	98.0
	10000	2.2 ± 1.5 d	80.6 ± 7.8 a	17.2 ± 6.6 bc	0.0 ± 0.0 d	0	100.0
	Control	100.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c	6.4 ± 0.7 a	1176	
Dichloromethane	100	86.1 ± 4.0 b	0.6 ± 0.6 c	13.3 ± 4.2 c	3.8 ± 0.4 a	680	12.4
	500	44.8 ± 8.5 c	1.7 ± 1.0 c	53.5 ± 9.4 b	1.0 ± 0.1 b	180	76.8
	1000	13.3 ± 2.5 d	6.1 ± 2.4 c	80.6 ± 3.6 a	0.0 ± 0.0 c	5	99.4
	5000	11.6 ± 2.5 de	28.7 ± 6.1 b	59.7 ± 7.0 b	0.0 ± 0.0 c	1	99.9
	10000	0.0 ± 0.0 e	98.9 ± 1.1 a	1.1 ± 1.1 c	0.0 ± 0.0 c	0	100.0
	Control	99.4 ± 0.6 a	0.0 ± 0.0 c	0.6 ± 0.6 c	4.3 ± 0.0 a	776	

<sup>1/2</sup> Means ± SE followed by the same letters are not significantly different as determined by Duncan's Multiple Range Tests (DMRT) ( $\alpha = 0.05$ ).

Higher repellency rates were recorded at the concentrations lower than 10000 ppm where 50-53% ( $F_{5,12}=11.964$ ,  $P<0.05$ ) was found at 1000-5000 ppm hexane extract. In contrast, 53.5% ( $F_{5,12}=41.276$ ,  $P<0.05$ ) repellency rate was recorded where the mites contacted with 500 ppm dichloromethane extract. They were found statistically different from the control groups. As for egg laying activity, both extracts at 500, 1000, 5000 and 10000 ppm showed a significantly deterrent effect on egg laying per female when compared to its

control ( $F_{5,12}=54.036$ ,  $P<0.05$  and  $F_{5,12}=121.607$ ,  $P<0.05$  for hexane and dichloromethane extracts respectively) (Table 3). Mites contacted the highest concentration (10000 ppm) of hexane extract did not lay any egg and as a result their eggs had been greatly reduced (100%). Moreover, more than 50% egg reduction (69-98%) was also recorded by 1000 and 5000 ppm of hexane crude extract while 500, 1000 and 5000 ppm of dichloromethane extract caused egg reduction ranging between 77-99%.

**Leaf disc choice test**

The repellency effect as indicated by the leaf disc choice test showed that both hexane and dichloromethane extracts from *L. glauca* dried

seeds had a significant effect on the activity of *T. urticae* (Table 4).

**Table 4.** Percentage of *T. urticae* females alive and percentage of eggs laid on the treated side of leaf discs at 24 hrs after painting with 25 µL of *L. glauca* dried seeds crude extracts, using leaf disc choice test.

Solvent used	Concentration of the extract (ppm, v/v)	Mean % of live mite <sup>1</sup>	Mean % of egg laid <sup>1</sup>	Oviposition deterrent index <sup>1</sup>
Hexane	100	33.7 ± 3.6 a (20.0/59.7)	37.9 ± 3.9 a (56.0/147.7)	23.4
	500	20.8 ± 3.8 b (12.3/59.3)	31.7 ± 10.6 a (22.0/69.3)	33.5
	1000	22.7 ± 4.3 b (13.7/60.3)	24.8 ± 4.8 ab (61.0/245.7)	50.4
	5000	12.2 ± 3.6 b (7.3/60.0)	17.5 ± 13.1 a (10.0/57.0)	45.1
	10000	0.0 ± 0.0 c (0.0/60.0)	0.0 ± 0.0 b (0.0/166.0)	100.0
Dichloromethane	100	0.6 ± 0.5 a (0.3/60.0)	35.5 ± 8.4 a (51.7/145.7)	33.7
	500	5.0 ± 4.2 a (3.0/59.7)	2.4 ± 1.2 a (5.0/209.7)	95.2
	1000	6.7 ± 5.9 a (4.0/59.7)	24.6 ± 16.3 a (19.3/78.7)	43.8
	5000	1.7 ± 1.0 a (1.0/60.3)	2.4 ± 10.1 a (2.0/83.7)	73.1
	10000	1.6 ± 0.9 a (1.0/60.1)	14.3 ± 5.6 a (0.3/2.3)	55.6

<sup>1</sup> For each extract, Means ± SE followed by the same letters are not significantly different as determined by Duncan's Multiple Range Tests (DMRT) (α = 0.05) (mites or eggs on treated side/total mites or eggs).

The alive mites and eggs laid on the treated side were less than 50% at all tested concentrations of both crude extracts. The results revealed that mites avoided to stay at the treated side and chosen non-treated side to live and lay their eggs. The repellency effect of hexane crude extract was dose-dependent whereas this effect was not found on dichloromethane crude extract. Alive mites and eggs were absent on the treated side with highest concentration of hexane crude extract while 33.7% (F<sub>4,10</sub>=13.225, P<0.05) and 37.9% (F<sub>4,10</sub>=3.385, P<0.05) of those were recorded at the lowest concentration. In contrast, the dichloromethane extract, maximum alive mites (6.7%) (F<sub>4,10</sub>=0.627, P<0.05) and eggs (35.5%) (F<sub>4,10</sub>=1.970, P<0.05) were noted on the side treated with 1000 and 100 ppm. However, the maximum oviposition deterrent (95.2%) was recorded at

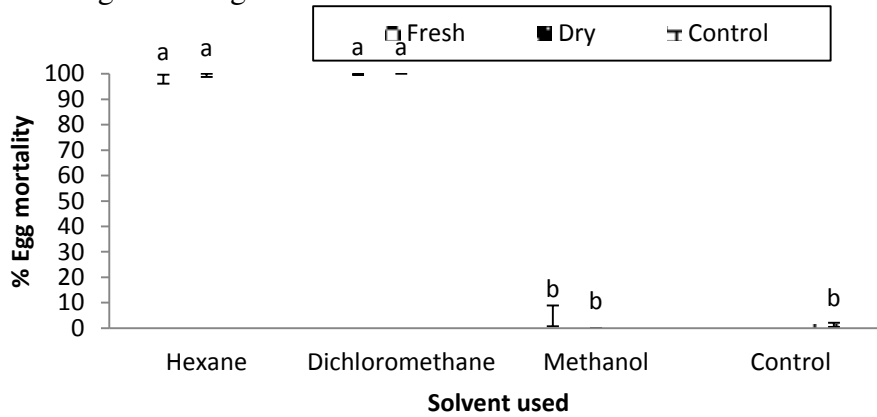
500 ppm dichloromethane extract. From 5 concentrations of mentioned extracts, 3 concentrations of hexane extract and dichloromethane extract deterred mites to lay eggs on the treated side with oviposition deterrent index (ODI) over 50%.

**Ovicidal effect**

The ovicidal effect of *L. glauca* fresh and dried seed crude extracts against *T. urticae* adult female was shown in Fig 1. The egg mortality which was caused by hexane and dichloromethane crude extracts of *L. glauca* at 9% (v/v) concentration was significantly higher than that of the control (P<0.05). The highest egg mortality was found in the dichloromethane crude extract of *L. glauca* dried seeds, followed by dichloromethane crude extract of *L. glauca* fresh seeds, hexane crude extract of *L. glauca* dried seeds and hexane crude extract of *L. glauca* fresh seeds.

However, these extracts were not significantly different from each others. The lowest egg mortalities ranging from 0.0-4.8% was observed in methanol crude extracts from both *L. glauca* seeds where 1.3% was found in control. Our results gave strong evidence that

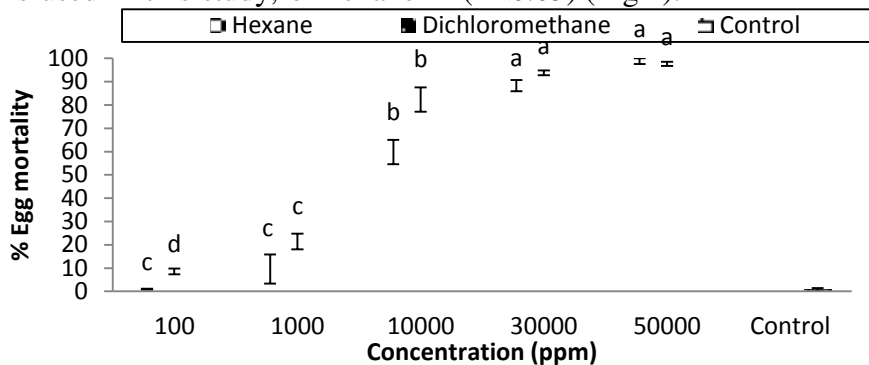
hexane and dichloromethane crude extracts of both fresh and dried *L. glauca* seeds have the ability to control eggs of *T. urticae*.



**Fig. 1.** Percentage of egg mortality of *T. urticae* after treated with *L. glauca* seed crude extracts. Means followed by the same letter are not significantly different as determined by DMRT ( $\alpha = 0.05$ ).

All extracts with egg mortality over 50% were selected for further study to investigate the ovicidal activity against *T. urticae* eggs. From 5 concentrations used in this study, of hexane

and dichloromethane extracts from fresh seeds induced egg mortalities (> 50%) which were significantly different from the control ( $P < 0.05$ ) (Fig 2).



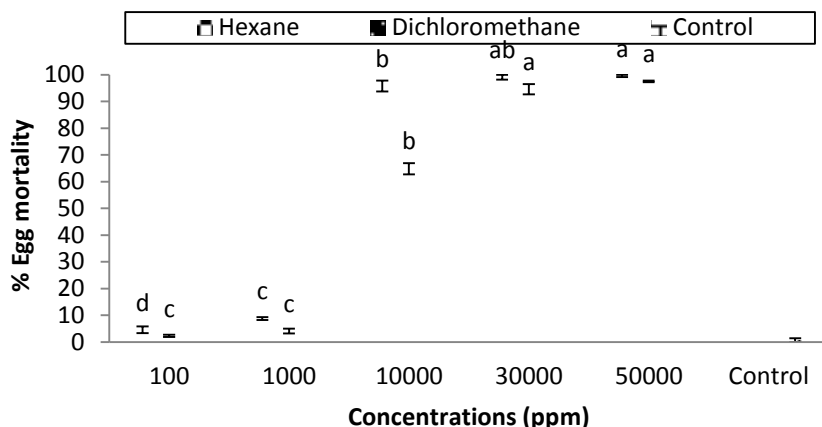
**Fig. 2.** Percentage of egg mortality induced by various concentrations of fresh *L. glauca* seed crude extracts on *T. urticae* eggs. For each solvent used, means followed by the same letter are not significantly different as determined by DMRT ( $\alpha = 0.05$ ).

The best concentration of hexane and dichloromethane extracts were 50000 ppm, where 98.8 and 97.6% egg mortalities were obtained which were higher significantly than 59.8 and 82.3% those of 10000 ppm concentration. The results demonstrated that egg mortalities were based on the concentrations of the extracts from *L. glauca* fresh seeds. The percentage of egg mortality caused by *L. glauca* dried seed extracts is shown in Fig 3. Among the tested concentrations, the 10000, 30000 and 50000

ppm of hexane and dichloromethane extracts also illustrated over 90 egg mortalities, respectively except for the 10000 ppm of dichloromethane extract where only 65% egg mortality was observed ( $P < 0.05$ ). The highest effect of 99.6% was determined at maximum concentration (50000 ppm) of the hexane extract and 97.5% was recorded for dichloromethane extracts at the same concentration. The results indicated that the increased concentration led to increased egg mortality.

In this study, it was noted that different *L. glauca* seeds and solvents used for extracting gave different extract weights and features. Dried seed extracted with methanol gave highest extract weight and rate. This result is similar to the result of Wang *et al.* (2010) and

Shi *et al.* (2006) who reported that methanol was the most effective solvent for extracting



**Fig. 3.** Percentage of egg mortality induced by various concentrations of dried *Leucaena glauca* seed crude extracts on *Tetranychus urticae* eggs. For each solvent used, means followed by the same letter are not significantly different as determined by DMRT ( $\alpha = 0.05$ ).

*Wikstroemia chamaedaphne* Meissn. (Thymelaeaceae) and *Kochia scoparia* (L.) Schrad (Macrophomina), respectively with higher extraction rate than those of petroleum ether and chloroform solvents.

All extracts, except methanol, showed different acaricidal and ovicidal effects on *T. urticae*. Both fresh and dried seeds extracted with low polar solvents, hexane and dichloromethane, demonstrated higher acaricidal and ovicidal effects than those extracted with high polar solvent, methanol. However, hexane and dichloromethane extracts of *L. glauca* dried seeds were found to have more acaricidal effects on *T. urticae* adults than extracts of *L. glauca* fresh seeds. The hexane and dichloromethane extracts of *L. glauca* seeds are liquid which is similar to oil. From this feature, these extracts are more advantageous than methanol extracts (solid material) to control the mites. The fact that mites do not prefer to stay and lay their eggs on the leaves that had smell or surface different from the natural. When contacted with hexane and dichloromethane extracts, sticky, on the treated leaf disc, the mites were difficult to live and lay their eggs on the treated leaf disc resulting in higher contact toxicity and oviposition deterrence of the mites.

The 9% hexane and dichloromethane *L. glauca* dried seeds extracts affected on the mortality (over 90%) of *T. urticae* and reduced its eggs (100%) after 24 hours. By the way, treated with low concentrations, 5000 and 10000 ppm concentrations, these extracts also had contact toxicity (38.3-80.6%) and 98.0-100% egg reduction. In some prior studies, the effects of extract of *Leucaena* genus on pests were reported such as aqueous extract of *L. leucocephala* dried seeds had anthelmintic activity ( $LC_{50} = 0.586$  mg/mL) on *Haemonchus contortus*-infective larvae (Ademola and Idowu, 2006). Okonkwo *et al.* (1995) reported that chicken at 1 to 5 weeks old fed on meal containing 15% *L. leucocephala* seed powder for 12 days exhibited a reduction in feeding and body weight gain. In addition, *L. leucocephala* leaves extracts had improved the egg and worm count reductions in lamb feces by oral administration (Hernandez *et al.*, 2014). Aqueous extracts of *L. leucocephala* leaves and roots reduced nematode population, galling, nematode reproduction rate and enhanced fruit weight of okra plant (Adekunle and Akinlua, 2007).

Unfortunately, there is a lack of information on the acaricidal effect of *L. glauca* in the scientific literature. Besides, there are some



plants belonging to the family Leguminosae showed acaricidal effects on *T. urticae*, for example, aqueous extract of *Astragalus ocephalus* Boiss leaves showed acaricidal activities on nymphs and adults of *T. urticae* (Al-Alawi, 2014). Moreover, methanol extracts of *Cassia sp.* and *Acacia saligna* (Labill.) H.L.Wendl. leaves demonstrated acaricidal effect ( $LC_{50} = 140$  and  $250$  mg/L, respectively) against *T. urticae* (Moussa *et al.*, 2010), methanol extracts of *Albizzia coreana* Nakai twigs and leaves and *Sophora flavescens* Aiton roots had acaricidal and egg laying effect on *T. urticae* (Kim *et al.*, 2005). It can be noted that their methanol extracts showed acaricidal effects which were not detected by our methanol extracts. Furthermore, other plant extracts belonging in different families were reported having acaricidal activity against *T. urticae* including the ethanolic extract of *Syzigium cumini* (L.) (Myrtaceae) (Abd El-Moneim *et al.*, 2011), ethanolic extract of *Cnidioscolus aconitifolius* leaves (Euphorbiaceae) (Numa *et al.*, 2015), ethanolic extract of *Metha pulegium* L. leaves (Lamiaceae) (Mozaffari *et al.*, 2012), ethanol extracts of both leaf and seed in the Thorn apple (*Datura stramonium* L.) (Solanaceae) (Kumral *et al.*, 2010), methanol extract obtained from *Ammi visnaga* (L.) Lam (Apiaceae) seeds (Pavela, 2015), methanol extracts of *Salvia officinalis* L. and *Rosmarinus officinalis* L. (Yorulmaz Salman *et al.*, 2014) and aqueous extracts of *Ruta chalepensis* L. leaves and stems (Rutaceae) (Al-Alawi, 2014) and ethanol extracts of *Helichrysum arenarium* L. (Asteraceae), *Allium sativum* L. (Amaryllidaceae), *Veratrum album* L. (Liliaceae), *Tanacetum parthenium* L. (Asteraceae) and *Rhododendron luteum* S. (Ericaceae) (Erdogan *et al.*, 2012). All results differed from our results which included methanol extract that did not have any acaricidal effects on *T. urticae* adults. On the other hand, results of Tewary *et al.* (2005) seem to be similar to our result since aqueous methanol of *Hedera nepalensis* L. leaves (Araliaceae) showed no acaricidal activity on *T. urticae* while petroleum ether extract, non

polar extract, of this plant showed moderate acaricidal effect on the same mite.

In the leaf disc choice test, we found that *T. urticae* female preferred to stay on the non-treated side than the treated side in all treated concentrations of hexane and dichloromethane extracts of *L. glauca* dried seeds. These extracts also deterred the egg-laying effect of *T. urticae* on the treated side. Our results are similar to those of El-Sharabasy (2010) who reported that petroleum ether extract, non polar extract, of *Artemisia judaica* L. leaves (Asteraceae) had repellency effect on *T. urticae* and those of Auamcharoen and Chandrapatya (2015) who indicated that chloroform extract of *Diospyros cauliflora* Blume (Ebenaceae) showed repellency activity against *T. urticae*. In addition, various plant extracts belonging to many families also had repellency effect on *T. urticae* such as the extract of wild tomato (*Solanum lycopersicum* L.) (Solanaceae) (Antonious *et al.*, 2006), the ethanol extract of *M. pulegium* leaves (Mozaffari *et al.*, 2012), ethanol extracts of both leaf and seed of *D. stramonium* (Kumral *et al.*, 2010), methanol extracts of *A. coreana* Nakai twigs and leaves and *S. flavescens* Aiton roots (Leguminosae) (Kim *et al.*, 2005). Besides, the ethanol extract of *Ailanthus altissima* L. leaves (Simarubaceae), *Plantago major* L. aerial part (Plantaginaceae), *Anabasis aphylla* L. seeds (Chenopodiaceae), *Stachys tschatkalensis* Knorr. aerial part (Lamiaceae), *Vinca erecta* Regel. and Schmalh. aerial part (Apocynaceae), *Convolvulus krauseanus* Regel. and Schmalh. roots (Convolvulaceae), *Prangos lipskyi* Korov. aerial part and root (Umbelliferae), *Allium obliquum* L. whole plant (Liliaceae), *Hedysarum darantkurganicum* Sultanova top, *Senecio saposhnikovii* Krasch et. Schipcz. aerial part (Fabaceae) and *Mediasia macrophlla* (Regel. and Schmalh.) M. Pimen. aerial part (Umbelliferae) significantly deterred *T. urticae* female on treated leaves and their eggs were also absent on the treated leaves (Chermenskaya *et al.*, 2010) which were agreed with our study.

The methanolic extract of both *L. glauca* seeds did not have contact toxicity, repellency to *T. urticae* under leaf disc choice test. These extracts also did not show ovicidal effect on *T. urticae*. The ovicidal activities of these extract continued to behave as they were in the control. Most of the eggs treated with methanol extracts were able to hatch. In contrast, the hexane and dichloromethane extracts of both seeds significantly (10000-50000 ppm) decreased the hatch ability of *T. urticae* (over 60%). The result revealed that dichloromethane is a suitable organic solvent to extract fresh seeds of *L. glauca* whereas hexane is appropriate for extracting its dried seeds in order to control *T. urticae* eggs.

No previous studied have reported the ovicidal effect of *L. glauca* extract on *T. urticae*. However, there are methanolic extract of *Trigonella elliptica* Boiss. leaves belonging to family Leguminosae were tested for ovicidal effect on *T. urticae* and caused 40.11% egg mortality (Ghaderi *et al.*, 2013) which contrasted to our study. Moreover, methanol extracts of *S. officinalis* and *R. officinalis* (Yorulmaz Salman *et al.*, 2014), methanol extract of *Eucalyptus camaldulensis* Dehn leaves (Myrtaceae), *Xanthium strumarium* L. fruits and leaves (Asteraceae), *Solanum nigrum* L. fruits (Solanaceae) (Yanar *et al.*, 2011), methanol extract of *Ammi visnaga* seeds (Pavela, 2015) also had high ovicidal effect on *T. urticae* eggs. *Tetranychus urticae* has a very high reproduction ability its female laying numerous eggs. If the producer finds the chemical materials that can reduce visible eggs on the plants, the population of mite will certainly be below the economic injury threshold (Yanar *et al.*, 2011). Consequently, no synthetic chemicals were applied in the fields and the quality of life producer and consumer is improved.

Our study demonstrated that mortality, repellency and ovicidal effects of *L. glauca* seed extracts was detected in hexane and dichloromethane extracts. The biological effect of both extracts was assumed by some chemical components involved in the extracts. Some studies showed that genus *Leucaena* contains hydrocyanic acid, leucaenine,

epicatechin-3-O-gallate, quercetin-3-O-arabinofuranoside, quercetin-3-O-rhamnoside, apigenin (Aderogba *et al.*, 2010). All plant parts of *Leucaena* contain mimosine. The young leaves and mature seeds contain the greatest 2.66 and 2.38% of dry weight respectively (Xuan *et al.*, 2006). Leaves of *L. leucocephala* showed the presence of phenolic compound, aromatic amide and carboxylic acid while roots contain phenolic compound and carboxylic acid (Adekunle and Akinlua, 2007). Moreover,  $\alpha$ -pinene, limonene,  $\alpha$ -phelandrene, sabinene and  $\beta$ -pinene were the main constituents in the volatile oil of *L. glauca* dried seeds which was extracted by petroleum ether (Hassan and Radwan, 2010). However, this presumption is not encouraged by our experiments. Future investigations on the acaricidal activity of *L. glauca* seeds using the elucidated chemical constituents to clarify the relations between the acaricidal and ovicidal effects and chemical constituents are highly recommended.

In conclusion, it is for the first time reported that hexane and dichloromethane *L. glauca* seed extracts had acaricidal and ovicidal on *T. urticae* adults and eggs. The repellency activity on *T. urticae* was also detected in these extracts. This combination of multiple modes of action found in plants is good because it may delay the development of resistance in insects or mites (Feng and Isman, 1995). Nevertheless, these results were obtained under laboratory conditions. Then, it is very necessary to investigate the efficacy of *L. glauca* seed hexane and dichloromethane extracts under greenhouse and the field conditions to confirm that these extracts can be effective as a safe controlling agent for the management *T. urticae* and also friendly to its natural enemies and non target organisms.

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**REFERENCES**

- Abd El-Moneim, M. R. A., Hossam, S. El. B., Sayed, A. F. and Emad, Ash. 2011. Acaricidal activity of different extracts from *Syzygium cumini* L. Skeels (Pomposia) against *Tetranychus urticae* Koch. *Asian Pacific Journal of Tropical Biomedicine*, **11**: 359-364.
- Adebowale, E. A. 1993. Some ethno-veterinary and traditional management practices in liveatock production. In: *Proceedings of a workshop on indigenous knowledge in agriculture and rural development*, Ibadan Nigeria, 51-59 PP.
- Adekunle, O. K. and Akinlua, A. 2007. Nematicidal effects of *Leucaena leucocephala* and *Gliricidia epium* extracts on *Meloidogyne incognita* infecting okra. *Journal of Agricultural Sciences*, **52**: 53-63.
- Ademola, I. O. and Idowu, S. O. 2006. Anthelmintic activity of *Leucaena leucocephala* seed extract on *Haemonchus contortus*-infective larvae. *Veterinary Record*, **158**: 485- 486.
- Aderogba, M. A., Mc Gaw, L. J., Bezabih, B. T. and Abegaz, B. M. 2010. Antioxidant activity and cytotoxicity study of *Leucaena leucocephala* (Lam.) de wit leaf extract constituents. *Nigerian Journal of Natural Products and Medicine*, **13**: 65-68.
- Al-Alawi, M. S. 2014. Acaricidal activity of medicinal plants against the development stages of the two spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *International Journal of Agricultural Research*, **9**: 38-64.
- Antonious, G. F. Meyer, J. E. and Snyder, J. C. 2006. Toxicity and repellency of hot pepper extracts to spider mite, *Tetranychus urticae* Koch. *Journal of Environmental Science and health, Part B*, **41**: 1383-1391.
- Ascher, K. P. S. 1993. Nonconventional insecticidal effects on pesticides available from the neem tree, *Azadirachta indica*. *Archives of Insect Biochemistry and Physiology*, **22**: 433-449.
- Auamcharoen, W. and Chandrapatya, A. 2015. Potential control of two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) by crude extracts of *Duabanga grandiflora* (Lythraceae) and *Diospyros cauliflora* (Ebenaceae). *Pakistan Journal of Zoology*, **47**: 953-964.
- Badawy, M. E. I., El-Arami, S. A. A. and Abdelgaleil, S. A. M. 2010. Acaricidal and quantitative structure activity relationship of monoterpenes against the two-spotted spider mite, *Tetranychus urticae*. *Experimental and Applied Acarology*, **52**: 261-274.
- Boyd, D. W. and Alverson, D. R. 2000. Repellency effects of garlic extracts on two spotted spider mite, *Tetranychus urticae* Koch. *Journal of Entomological Science*, **35**: 86-90.
- Cavalcanti, S. C. H., Dos Niculau, E., Blank, A. F., Camara, C. A. G., Araujo, I. N. and Alves, P. B. 2010. Composition and acaricidal of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresource Technology*, **101**: 829-832.
- Chandler, D., Bailey, A. S., Tatchell, G. M., Davidson, G., Greaves, J. and Grant, W. P. 2011. The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B*, **366**: 1987-1998.
- Chermenskaya, T. D., Stepanycheva, E. A., Shchenikova, A.V. and Chakaeva, A. Sh. 2010. Insecto-acaricidal and deterrent activities of extracts of Kyrgyzstan plants against three agricultural pests. *Industrial Crops and Products*, **32**: 157-163.
- Choi, W., Lee, S., Park, H. and Ahn, Y. 2004. Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Economic Entomology*, **97**: 553-558.
- Copping, L. G. and Duke, S. O. 2007. Natural products that have been used

- commercially as crop protection agents-a review. *Pest Management Science*, **63**: 524-554.
- Dimetry, N. Z., Amer, S. A. A. and Reda, A. S. 1993. Biological activity of two neem seed kernel extracts against the two-spotted spider mite *Tetranychus urticae* Koch. *Journal of Applied Entomology*, **116**: 308-312.
- Duke, J. A. 1981. Hand book of legumes of world economic importance. Plenum Press, New York.
- El-Sharabasy, H. M. 2010. Acaricidal activities of *Artemisia judaica* L. extracts against *Tetranychus urticae* Koch and its predator *Psytoseiulus persimilis* Athias Henriot (Tetranychus : Phytoseiidae). *Journal of Biopesticides*, **3**: 514-519.
- Erdogan, P., Yildirim, A. and Sever, B. 2012. Investigations on the effects of five different plant extracts on the two-spotted mite *Tetranychus urticae* Koch (Arachnid: Tetranychidae). *Psyche*, **PP**: 1-5.
- Feng, R. and Isman, M. B. 1995. Selective for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experientia*, **51**: 831-833.
- Ghaderi, S., Minaei, K., Rowshan, V and Ghamadyari, M. 2013. Toxicity and ovicidal activity of different plant extracts on two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetrachidae). *Archives of Phytopathology and Plant Protection*, **46**: 120-126.
- Hassan, R. A. and Radwan, H. M. 2010. The lipids and volatile oil constituents of *Leucaena glauca* (L.) Benth. Growing in Egypt and their biological activity. *Journal of Applied Sciences Research*, **6**: 478-482.
- Helle, W. and Sabelis, M. W. 1985. Spider mite: their biology, natural enemies and control, vol 1B, Elsevier, Amsterdam.
- Hernandez, P. M., Salem, A. Z. M., Elghandour, M. M. M. Y., Cipriano-Salazar, M., Cruz-Lagunas, B. and Camacho, L.M. 2014. Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs. *Tropical Animal Health and Production*, **46**: 173-178.
- Hincapie, C. A. LI., Lopez, G. E. P. and Torres R. Ch. 2008. Comparison and characterization of garlic (*Allium sativum* L.) bulbs extracts and their effect on mortality and repellency of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Chilean Journal of Agricultural Research*, **68**: 317-327.
- Hussein, H., Abou-Elelia, M., Amer, S. A. A. and Momen, F. M. 2006. Repellency and toxicity of extracts from *Capparis aegyptia* L. to *Tetranychus urticae* Koch. (Acari: Tetranychidae). *Acta Phytopathologica et Entomologica Hungarica*, **41**: 331-340.
- Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and as increasingly regulated world. *Annual Review of Entomology*, **51**: 45-66.
- Jeppson, L. R., Keifer, H. H. and Baker, E. W. 1975. Mite injurious to economic plants, University of California Press, Berkeley, 383 **PP**.
- Kelany, I. M. 2001. Plant extracts and utilization of their products for safe agricultural production and for reducing environmental pollution. [http://www.trifolium.de/info\\_service/workshops/Abstracts\\_2001/abstracts\\_2001.html#0-top](http://www.trifolium.de/info_service/workshops/Abstracts_2001/abstracts_2001.html#0-top).
- Kim, D. I., Park, J. D., Kim, S. G., Kuk, H., Jang, M. S. and Kim, S. S. 2005. Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. *Journal of Asia-Pacific Entomology*, **8**: 93-100.
- Kumral, N. A., Cobanoglu, S. and Yalcin, C. 2010. Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L. against adult *Tetranychus urticae* (Koch). *Journal of Pest Science*, **83**: 173-180.

- Liburd, O. E., White, J. C., Rhodes, E. M. and Browdy, A. A. 2007. The residual and direct effects of reduce-risk and conventional miticides on two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae) and predatory mites (Acari: Phytoseiidae). *Florida Entomologist*, **90**: 249-257.
- Lim, E. G., Roh, H. S., Coudron, T. A. and Park, C. G. 2011. Temperature-dependent fumigant activity of essential oils against two-spotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology*, **104**: 414-419.
- Mabberley, D. J. 1997. The plant book, 2nd Ed., Cambridge University Press, United Kingdom.
- Mansour, F., Azaizeh, H., Saad B. Tadmor Y. and Abo-Moch, F. 2004. The potential of middle eastern flora as a source of new safe bioacaricides to control *Tetranychus cinnabarinus*, the carmine spider mite. *Phytoparasitica*, **32**: 66-72.
- Mateeva, A. A., Christov, C., Stratieva, S. and Palagatscheva, N. 2003. Alternative plant protection means against *Tetranychus urticae* Koch. In: *International symposium on plant health in urban horticulture*, Berlin, Germany.
- Moussa, A. M., Emam, A. M., Mohamed, M. A. and Diab, Y. M. 2010. *In vitro* evaluation of some Egyptian plants against the rot bacteria and spider mite and isolation the active constituent(s) from *Myrtus communis* leaves. *International Food Research Journal*, **17**: 287-294.
- Mozaffari, F., Abbasipour, H., Garjan, A. S., Saboori, A. R. and Mahmoudvand, M. 2012. Various effects of ethanolic extract of *Mentha pulegium* on the two-spotted spider mite, *Tetranychus urticae* (Tetranychidae) *Archives of Phytopathology and Plant Protection*, **45**: 1347-1355.
- Numa, S., Rodriguez, L., Rodriguez, D. and Coy-Barrera, E. 2015. Susceptibility of *Tetranychus urticae* Koch to an ethanol extract of *Cnidocolus aconitifolius* leaves under laboratory conditions. *SpringerPlus*, **4**: 1-10.
- Ochiai, N., Mizuno, M., Mimori, N., Miyake, T., Dekeyser M., Canlas, L. J. and Takeda, M. 2007. Toxicity of bifentazate and its principal active metabolite, diazene, to *Tetranychus urticae* and *Panonychus citri* and their relative toxicity to the predaceous mites, *phytoseiulus persimilis* and *Neoseiulus californicus*. *Experimental and Applied Acarology*, **43**: 181-197.
- Okonkwo, A. C., Wamagi, I. T., Okon, B. I. and Umon, B. I. 1995. Effect of *Leucaena leucocephala* seed meal on the performance and carcass characteristics of broiler chicken from five to twelve weeks of age. *Nigeria Journal of Animal Production*, **22**: 44-48.
- Park, B. S., Lee, S. E., Choi, W. S., Jeong, C. Y., Song, C. and Cho, K. Y. 2002. Insecticidal and acaricidal activity of piperonaline and piperocetadecalidine derived from dried fruits of *Piper longum* L. *Crop Protection*, **21**: 249-251.
- Pavela, R. 2015. Acaricidal properties of extracts and major furanochromens from the seeds of *Ammi visnaga* Linn. against *Tetranychidae urticae* Koch. *Industrial Crops and Products*, **67**: 108-113.
- Shi, G. L., Liu, S. Q., Cao, H., Zhao, L. L., Li, J. and Li, S. Y. 2004. Acaricidal activities of extracts of *Stellera chamaejasme* against *Tetranychus viennensis* (Acari: Tetranychidae). *Journal of Economic Entomology*, **97**: 1912-1916.
- Shi, G. L., Zhao, L. L., Liu, S. Q., Cao, H., Clarke, S. R. and Sun, J. H. 2006. Acaricidal activities of extracts of *Kochia scoparia* against *Tetranychus urticae*, *Tetranychus cinnabarinus*, and *Tetranychus viennensis* (Acari: Tetranychidae). *Horticultural Entomology*, **99**: 858-863.
- SPSS Inc. 2015. SPSS base 19.0 for Windows user's guide. Chicago (IL): SPSS Inc.

- Tewary, D. K., Bhardwaj, A. and Shanker, A. 2005. Pesticidal activities in five medicinal plants collected from mid hills of western Himalayas. *Industrial Crops and Products*, **22**: 241-247.
- Van Pottelberge, S., Van Leeuwen T., Nauen R. and Tirry L. 2009. Resistance mechanisms to mitochondrial electron transport inhibitors in a field-collected strain of *Tetranychus urticae* Koch. (Acari: Tetranychidae). *Bulletin of Entomological Research*, **99**: 23-31.
- Vasconcelos, G. J. N., Junior, M. G. C. G. and Barros, R. 2006. Aqueous extracts of *Leucaena leucocephala* and *Sterculia foetida* to the control of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae). *Ciencia Rural*, **36**: 1353-1359.
- Wang, N. J., Liu, J. J., Ling, Z. Z. and Lai, Y. F. 2002. A preliminary study on *Wisktoemia chamaedaphne* and its toxicity in caijiachuan river basin, Shaanxi province. *Shaanxi Forest Science and Technology*, **3**: 1-5.
- Wang, Y. N., Bu, C. Y., Jin, J. S., Ren, J. J., Guo, H. I., Zhao, L., Du, J., Liu, Y.B., Zhao, L.L., Cheng, J. and Shi, G.L. 2010. Acaricidal activities of *Wikstroemia chamaedaphne* extracts against *Tetranychus urticae* and *Tetranychus cinnabarinus* (Acari: Tetranychidae) In: *4<sup>th</sup> International Conference on Bioinformatics and Biomedical Engineering*, Chengdu, China, 1-5 **PP**.
- Wei, J., Ding, W., Zhao, Y. G. and Vanichpakorn, P. 2011. Acaricidal activity of *Aloe vera* L. leaf extracts against *Tetranychus cinnabarinus* (Boisduval) (Acarina: Tetranychidae). *Journal of Asia-Pacific Entomology*, **14**: 335-356.
- Xuan, T. D., Elzaawely, A. A., Deba, F., Fukuta, M. and Tawata, S. 2006. Mimosine in *Leucaena* as a potent bio-herbicide. *Agronomy for Sustainable Development*, **26**: 89-97.
- Yanar, D., Kadioglu, I. and Gokce, A. 2011. Ovicidal activity of different plant extracts on two spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae). *Scientific Research and Essays*, **6**: 3041-3044.
- Yorulmaz Salman, S., Saritas, S., Kara, N. and Ay, R. 2014. Acaricidal and ovicidal effects of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Agricultural Sciences*, **20**: 358-367.

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