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Acaricide effect of some extracts and fractions on *Tetranychus urticae* Koch (Acari: Tetranychidae)

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The acaricidal potential of *Melia azedarach*, *Peganum harmala*, *Nigella sativa* and *Trigonella foenum-graecum* seeds was investigated on adult *Tetranychus urticae* under laboratory conditions. After the treatment of foliar discs using dichloromethane and ethanol extracts of these seeds, the statistical analysis of the mortality percentage revealed that *N. sativa* was the most potent one. In fact, using the dichloromethane extracts, the LC_{50}s were 398.64, 410.48, 73.95 and 520.74 ppm for the four species respectively. Whereas, for ethanol extracts, the LC_{50}s were 994.93, 617.26, 92.96 and 1074.81 ppm respectively. *n*-Hexane, trichloromethane and ethyl acetate fractions of *N. sativa* dichloromethane extract used in the same manner showed that *n*-Hexane fraction was the most toxic with LC_{50}=74.34 ppm followed by trichloromethane fraction with 96.74 ppm then ethyl acetate with 104.27 ppm. The phytochemical analysis of these fractions revealed the presence of some metabolites which could explain their toxicity.

Key words: Acaricidal potentials, extract, fractions, ethanol, dichloromethane, *Tetranychus urticae*, phytochemical analysis.

INTRODUCTION

The large-scale use of pesticides began after the World War II with the organochlorine and organophosphorus compounds. Other chemical groups have been subsequently developed. Nowadays, pesticides are widely used in agriculture in view of their ease of application and rapidity of action (Dhaliwal and Arora, 2001; Hamilton and Crossley, 2004). However, since the publication of Rachel Carson's book "Silent Spring" in the 1960s (Carson, 1965), the public concern about the impact of pesticides on the environment has increased. Much of this concern was associated with the organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and dieldrin (Hamilton and Crossley, 2004). In fact, the continuous and indiscriminate use of these substances has caused adverse effects, not only on mammals health, but also on other benefit members of the ecosystem and the environment in which they are immersed (Theiling and Croft, 1988; Çelik et al., 2005; Chauhan and Gupta, 2005). In addition to these hazardous effects, the insect

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pest problems were aggravated in many crops, due to the continuous application of pesticides. Indeed, many hitherto unknown species of pests have assumed serious status and many pest species have developed resistance to one or more groups of pesticides (Dhaliwal and Arora, 2001). Therefore, the consumption of pesticides has increased resulting in the phenomenon of pesticide treadmill (Altieri, 1995). To overcome increasing problems encountered with the use of pesticides, efforts were focused on reducing reliance on chemical compounds (Dhaliwal and Arora, 2001). One of the available alternatives is the use of plant chemicals, the "botanical pesticides" used by farmers since ancient times to prevent their colonization by insects (Arora and Dhaliwal, 1994).

Two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a serious pest on more than 180 host plants, including more than 100 cultivated species (Hale and Williams, 2003). It appears on the first row of the top 20 resistant arthropods whose first case of resistance was detected in 1943 (Whalon et al., 2008). Actually, this specie showed a resistance to more than 80 pesticides (Whalon et al., 2008). Thus, the present study was carried out, aiming to investigate the biological activity of the crude extract of some seeds and the fractions of the best one against *T. urticae*.

MATERIALS AND METHODS

Plant material

Ripe fruits from Chinaberry *Melia azedarach* L. (Meliaceae) were used; they were collected from trees of the garden of the National School of Agriculture of Meknès and de-pulped manually, then their kernels (endocarps) were thoroughly washed with distilled water. Seeds of Syrian rue *Peganum harmala* L. (Zygophyllaceae), black cumin *Nigella sativa* L. (Ranunculaceae) and fenugreek *Trigonella foenum-graecum* L. (Leguminosae) were obtained from herbal stores. The four species were ground to a powder with a mortar and pestle then stored at room temperature in hermetically sealed plastic bags prior to extraction.

Preparation of the crude extract

The plants powder were extracted with dichloromethane (Dichloromethane, stabilized with ~ 20 ppm of amylene (PAR) PAI; Panreac Química S.A.U.) and ethanol (Ethanol absolut puriss. p.a., ACS-ISO; Sigma-Aldrich®) in Soxhlet extraction apparatus for 3 h and the solvent was removed by vacuum evaporation in a rotary evaporator (BŰCHI RE 111 Rotavapor). The residues were diluted in distilled water containing one drop of emulsifier Tween® 20 (Tween® 20; Sigma-Aldrich®), to ensure complete solubility of the material in water, and then to obtain concentrations of 100, 500, 1000 and 5000 ppm.

Fractionation of the best crude extract

The best crude extract, which showed the highest mortality rate at low concentration and the lowest lethal concentration 50 (LC_{50}) was redissolved in 95% methanol (Mathanol PA-ACS-ISO; Panreac Química S.A.U.) and washed with the same volume of n-Hexane (n-Hexane PA; Polysciences, INC). The separated methanol extract was dried then partitioned between water and Trichloromethane ($CHCl_3$) (Trichloromethane R.G. stabilized with approx. 1% Ethanol; Riedel-de Haën®) (1:1). The water layer was washed once again with the same volume of fresh ethyl acetate (EtOAc) (Ethyl Acetate (PAR) PAI; Panreac Química S.A.U.). The n-Hexane, trichloromethane and ethyl acetate soluble fractions were concentrated under vacuum in rotary evaporator and kept at room temperature. Serial dilutions of about 100, 200 and 300 ppm were prepared in the same manner as the crude extracts.

Two-spotted spider mites rearing

The two-spotted spider mites, *T. urticae*, used in this experiment was obtained from a colony collected initially in the field then had been reared in a greenhouse on tomato seedlings *Lycopersicon esculentum* Mill. (Solanaceae).

Bioassay

The tests were accomplished following Campos et al. (1995) methodologies. The method consists of placing *T. urticae* adults on a lower face of leaf discs confectioned using a 2 cm diameter cork-borer. Once transferred, the discs are placed in Petri dishes containing hydrophilic cotton sufficiently soaked in sterile distilled water in order to prevent the escape of the tested mites and to provide moisture needed to prevent desiccation. The Petri dishes were kept in a growth room at 25 ± 1°C with a (16 h:8 h) (L:D) photoperiod provided by cool white fluorescent lamps. 24 h later, the foliar discs were recovered and dipped for 10 s in the tested solutions: 0 ppm (control: distilled water + one drop of Tween® 20); 100; 500; 1000 and 5000 ppm in the case of crude extracts and 100; 200 and 300 ppm in the case of the best extract fractions. The discs were kept at room temperature for 2 h to allow the evaporation of water then retransferred to Petri dishes which were placed in the growth room. Mortality was controlled after 24 h using fine brush. The tested adults were considered dead when they did not show any movement, even after being touched with the brush. On each foliar disc, 20 individuals were placed, three Petri dishes per concentration were used and each concentration was repeated tree times. To eliminate mortality due to natural causes, this rate was corrected using the Abbott formula (Abbott, 1925).

Phytochemical study of the best extract's fractions

These tests were based on the addition of specific reagents to aliquots that contained the tested fractions. Changes in coloration or precipitate formation were observed. Tests were performed as follows:

(i) Lieberman–Burchard test, a chloroform ($CHCl_3$) solution of the extract (2 ml) was mixed with acetic anhydride (Acetic anhydride PA-ACS-ISO; Panreac Química S.A.U.) (1 ml) and three drops of concentrated sulfuric acid (Sulfuric acid, ACS reagent, 95.0 to 98.0%, Sigma-Aldrich®). The development of a blue to green color indicates the presence of steroids while the development of a red to brown color indicates the presence of triterpenoids (Maciel et al., 2006).
(ii) Salkowski reaction, 200 mg of the sample was dissolved in 2 ml $CHCl_3$, then 3 ml concentrated sulfuric acid was added, forming two phases. The development of a red color indicates the presence of terpénoïdes (Egwaikhide and Gimba, 2007).
(iii) Iron-III-chloride reagent, 1 g of $FeCl_3$ (Iron(III) Chloride) anhydrous, 97% PS; Panreac Química S.A.U.) was dissolved in 5

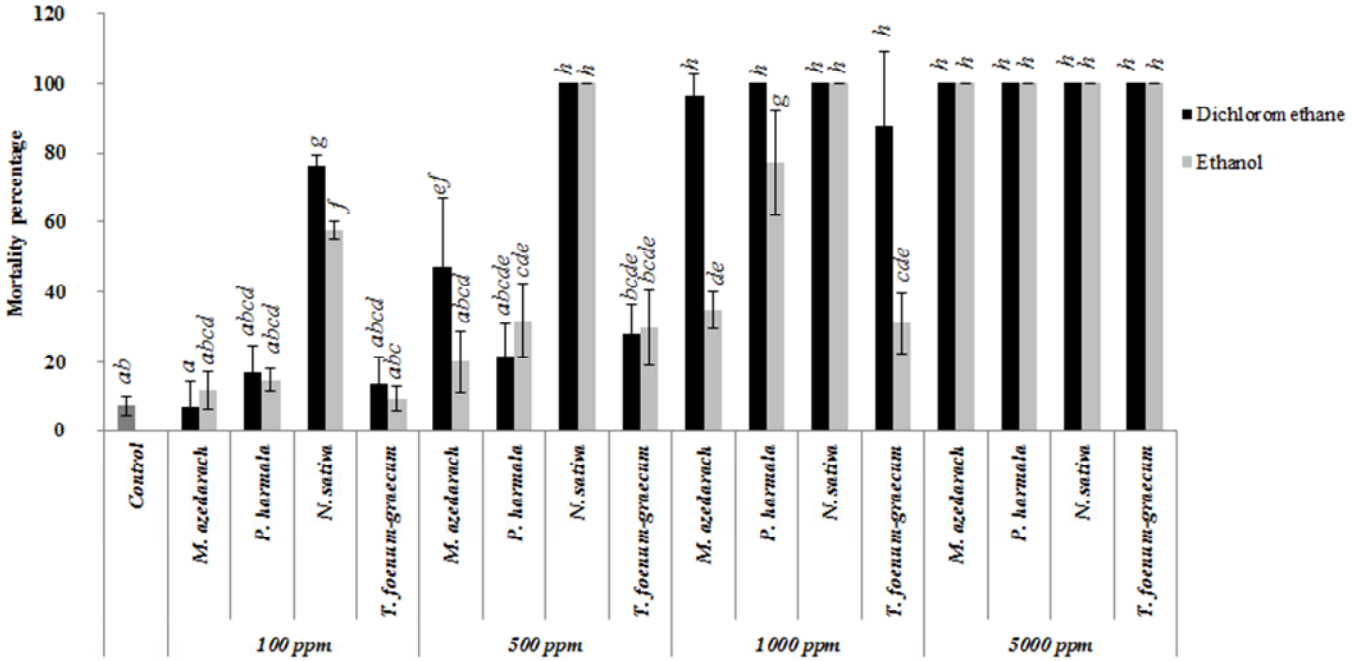

Figure 1. Effect of *M. azedarach*, *P. harmala*, *N. sativa* and *T. foenum-graecum* dichloromethane and ethanol extracts on *T. urticae* adults. Histograms represent the mortality percentage ± Standard deviation of three replicates. Histograms with the same letters are not statistically different (SNK test: p≤0.05).

ml water then diluted to 100 ml with ethanol. In the presence of this reagent, alcoholic extracts (3 ml) shows a dark blue precipitate in the presence of hydrolysable tannins while it shows a green precipitate in the presence of condensed tannins or catechins (Maciel et al., 2006).

(iii) Sulfuric acid, flavones and flavonols dissolve into concentrated H_2SO_4, producing a deep yellow colored solution. Chalcones and aurones produce red or red-bluish solutions. Flavanones show orange to red colors (Jones and Kinghorn, 2006).

(iv) Wagner reagent, 1.27 g I_2 (sublimed) (IODE BISUBLIME; PROLABO) and 2 g KI (Potassium iodide puriss.; Riedel-de Haën®) were dissolved in 20 ml water then diluted to 100 ml with water. A brown precipitate in acidic solutions indicates the presence of alkaloids (Jones and Kinghorn, 2006).

(v) Mayer reagent, Solution I: 1.36 g $HgCl_2$ (Mercury (II) chloride PA-ACS; Panreac Química S.A.U.) was dissolved in 60 ml water. Solution II: 5 g KI was dissolved in 10 ml water. Procedure: the two solutions were combined and diluted with water to 100 ml. The development of a white to yellowish precipitate after the addition of a few drops to an acidified extract solution (diluted Hydrochloric Acid (HCl) 37% PA-ACS-ISO; Panreac Química S.A.U.) suggests the presence of alkaloids (Jones and Kinghorn, 2006).

Statistical analysis

The statistical package SPSS V16.0.1 was used for the analysis of the collected data and the experiments were laid out as completely randomized designs. The mortality percentages were subjected to analysis of variance (ANOVA) after an angular transformation [arcsine (mortality percentage)$^{1/2}$] to stabilize the variances (Gomez and Gomez, 1984). If significant F-values were observed, differences between the treatments were determined by Student-Newman-Keuls (SNK) multiple range test (P ≤0.05). The LC_{50} was calculated by the method of Probits (Finney, 1971).

RESULTS

Effect of crude extracts on two-spotted spider mite adults

Statistical analysis of the biocidal effect of crude extracts of *M. azedarach*, *P. harmala*, *N. sativa* and *T. foenum-graecum* seeds against *T. urticae* adults showed that all treatments have toxic effect on this pest. In comparison with control, all treatments showed an increase in mortality rate depending on the concentration. Indeed, the mortality rate was depending on the plant species from which we extract the active ingredient and the solvent used for extraction. Crude extracts of *N. sativa* were the most toxic ones, at 500 ppm, with 100% mortality and 73.95 ppm LC_{50} for dichloromethane extracts and 92.95 ppm LC_{50} for ethanol extracts. Our results also showed that dichloromethane is the most suitable solvent for extraction, since, nearly all plant species evaluated, showed that the extracts obtained using dichloromethane were more toxic than those obtained using ethanol (Figure 1 and Table 1). Thus, in terms of toxicity, *N. sativa* was followed by *M. azedarach* which dichloromethane extracts gave 398.64 ppm LC_{50}, then by *P. harmala* dichloromethane extracts (LC_{50} = 410.48 ppm) and ethanol extracts (LC_{50} = 617.26 ppm), followed by *T. foenum-graecum* dichloromethane extracts (LC_{50} = 520.74 ppm) and finally, by extracts of *M. azedarach* and *T. foenum-graecum*, both obtained using ethanol as extraction solvent (LC_{50} = 994.93 ppm and

Table 1. LC_{50} of *M. azedarach*, *P. harmala*, *N. sativa* and *T. foenum-graecum* dichloromethane and ethanol extracts for *T. urticae* adults after 24 h.

Species	Extract	Lethal concentration 50 (ppm)
Melia azedarach	Dichloromethane	398.64
	Ethanol	994.93
Peganum harmala	Dichloromethane	410.48
	Ethanol	617.26
Nigella sativa	Dichloromethane	73.95
	Ethanol	92.96
Trigonella foenum-graecum	Dichloromethane	520.74
	Ethanol	1074.81

ppm: Parts per million.

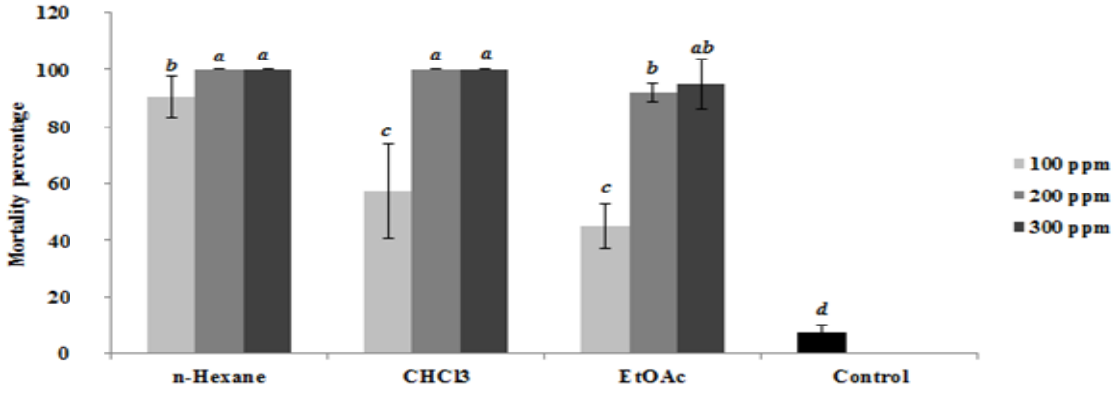

Figure 2. Effect of the fractions of *N. sativa* dichloromethane extract on *T. urticae* adults. Histograms represent the mortality percentage ± Standard deviation of three replicates. Histograms with the same letters are not statistically different (SNK test: p≤0.05).

LC50 = 1074.81 ppm, respectively) (Table 1). During toxicological tests it was observed that the dose of 5000 ppm causes phytotoxicity and destruction of foliar tissue in all treatments.

Effect of *N. sativa's* fractions on two-spotted spider mite adults

Based on the results of toxicity of crude extracts towards the adults of *T. urticae*, which showed that *N. sativa* dichloromethane extract was the most toxic one, by giving 76.22% mortality at only 100 ppm, a separation of this extract's fractions was carried out to assess their toxicity towards the same pest. Thus, the statistical analysis of the effect of "Fractions" and "Concentration" on the mortality rate has exhibited a significant difference between treatments ($F_{Fractions}$ = 21.474, $P_{Fractions}$ ≤0.05 and $F_{Concentration}$ = 84.117, $P_{Concentration}$ ≤0.05). All fractions proved to be effective, since it was observed that nearly all tested groups were died, at a concentration of 200 ppm, especially for *n*-Hexane and trichloromethane fractions (Figure 2). The tests also showed that *n*-Hexane fraction is the most potent giving a LC_{50} of around 74.34 ppm, followed by trichloromethane with a LC_{50} of 96.74 ppm and finally by ethyl acetate fraction with a LC_{50} of 104.27 ppm.

Phytochemistry of *N. sativa* fractions

The analysis was performed with *n*-Hexane, trichloromethane and ethyl acetate fractions of *N. sativa* dichloromethane extracts. These tests showed the presence of different types of organic compounds, which revealed their structural characteristics via specific reagents. The reaction of these fractions with a $FeCl_3$ solution did not show any coloration and that is indicative of the absence of tannins. In the Lieberman–Burchard test, *n*-Hexane fraction showed a green olive color

Table 2. Coloration or precipitation obtained after the addition of specific reagents to the fractions of *N. sativa* dichloromethane extract.

Reagents	*N. sativa* fractions		
	***n*-Hexane**	**$CHCl_3$**	**EtOAc**
Lieberman–Burchard	Green olive	Red	Brown
Salkowski	Red	Red	Red
Iron-III-chloride	-	-	-
Sulfuric acid	Orange to red	Orange to red	Orange to red
Wagner	Brown precipitate	-	-
Mayer	White precipitate	-	-

(-) No reaction.

indicative of steroids, trichloromethane fraction showed a red color while ethyl acetate fraction showed a brown color, indicating the presence of triterpenoids. All the fractions showed a red color in Salkowski test and orange to red color in Sulfuric acid test indicating the presence of terpenoids and flavanones respectively. Alkaloids were detected only in *n*-Hexane fraction, which was confirmed by Wagner reagent and Mayer reagent that gave a brown precipitate and a white precipitate, respectively (Table 2).

DISCUSSION

It was clear from the results that *M. azedarach*, *P. harmala*, *N. sativa* and *T. foenum-graecum* seed extracts were all toxic to adults of *T. urticae*, especially the dichloromethane extracts of *N. sativa* witch showed a LC_{50}<100 ppm. In fact, McLaughlin and Rogers (1998) mentioned that pure substances with LC_{50} values ≤1 µg/ml are worthy of commercial development. Similar observations have been reported on the same plants extract effects on several insects. Chiu et al. (1984) showed that a reduction of 85.6% was observed in the population of the citrus red mite *Panonychus citri* (Mc Gregor) (Acari: Tetranychidae), 1 day post treatment with an emulsion of seed oil of *M. azedarach* at 0.25%. The effectiveness was comparable to the treatment with Amitraz. A second application on the same trees produced 98.8% reduction either with the vegetal extract or with Amitraz (Chiu et al., 1984). Yanar et al. (2011) demonstrated that methanol extracts of *M. azedarach* fruits were effective against adult *T. urticae* and showed contact and residual toxicity after 24 h (76.45 and 74.57% mortality, respectively). The lack of activity of ethanolic extracts in comparison to other extracts has been reported. Borges et al. (2003) showed that the *M. azedarach* fruit extracts were toxic against the larvae of *Boophilus microplus* (Canestrini) (Acari: Ixodidae) when extracted with hexane, chloroform and ethanol and applied on filter papers, which remained in contact with the larvae for 10 min. The chloroform extract was the most potent one, exhibiting 100% mortality, followed by the hexanic extract (97%) then by ethanolic extract (50%) at a concentration of 0.125%. Hexanic and chloroformic extracts showed more acaricide effectiveness than the ethanolic extract against engorged females of the thick immersed in the solutions of extracts (Borges et al., 2003). The same tendency of activity was observed by McMillian et al. (1969) on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The insecticidal activity of plants of the Meliaceae family has been extensively studied, registering the presence of many triterpenoids possessing insecticidal activity. Carpinella et al. (2002) reported the isolation of two interchangeable isomeric limonoids (12-Hydroxyamoorastatin and Meliartenin) which were antifeedant and insecticide against *Epilachna paenulata* Germ. (Coleoptera: Coccinellidae) at 4 mg/cm^2. Two pure products were isolated from *M. azedarach* fruit (1-cinnamoilmelianolona and 1-cinnamoyl-3-11 dihydroxymeliacarpin) showing an insecticide effect against *Heliothis virescens* (Lepidoptera: Noctuidae) (Lee et al., 1987; Kraus et al., 1989; Lee et al., 1991). Italo et al. (2009) indicated that the chemical analysis of *M. azedarach* fruit polyphenols using High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) showed 14 compounds as causes of the insecticidal effect of this species, of which three would correspond to flavonoids: one catechin and two kaempherols.

With regard to *P. harmala* extract, Abbassi et al. (2003) showed that the alkaloids extracted from *P. harmala* leaves cause a significant mortality of the desert locust *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae) compared to untreated controls. Cumulative mortality rates attained 37% in adults fed with alkaloids extracted from *P. harmala*, whereas it did not exceed 12% in untreated controls during 30 days (Abbassi et al., 2003). Methanolic *P. harmala* seeds extract was the most toxic in comparison to other species with 58% mortality 10 days after treatment of last-instar larvae of *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) (Jbilou et al., 2006, 2007). The cumulative mortality of adults reached 92%, 32 days after treatment (Jbilou et al., 2006). *P. harmala* is a rich source of β-carboline alkaloids

as harmol, harmine and harmaline (Herraiz et al., 2010). These alkaloids as well as other secondary metabolites of this plant may explain the toxic effect on the studied insects (Jbilou et al., 2007).

Fenugreek (*T. foenum-graecum*) is an annual crop belonging to the legume family. Dried leaves of this crop have been reported to have insecticidal properties; in India they are used effectively in storing grains and cereals in traditional old-fashioned granaries (Petropoulos, 2002). This effectiveness was demonstrated by Khater and Shalaby (2007), who showed that commercially available *T. foenum-graecum* oil was the most toxic in comparison to other oils after treating the 4th larval instars of *Culex pipiens* (Diptera:Culicidae). The LC_{50} value was 32.42 ppm (Khater and Shalaby, 2007). Abdel Halim and Morsy (2006) studied the insecticidal efficacy of this species on the 3rd stage larvae of *Musca domestica* L. (Diptera: Muscidae) under controlled laboratory conditions, they showed that concentrations ranging from 25 to 100% killed completely the larvae while 5, 2 and 1% caused mortality percentages of 44.4, 33.3 and 22.2, respectively.

Few studies were conducted to evaluate the effect of *N. sativa* essential oils and extracts on pests. Elumalai et al. (2010) studied the ovicidal and larvicidal activity of *N. sativa* essential oils on *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Application of 50, 100, 150, 200, 250 and 300 ppm on fourth instar larvae exhibited a LC_{50} of about 35.1 ppm, while, on eggs, a percentage of hatchability of about 11.5 and 0% was recorded for 250 and 300 ppm respectively (Elumalai et al., 2010). On the other hand, Bilal et al. (2011) reported that the lowest LC_{50} was found in *N. sativa* ether extracts at a dose of 377.5 ppm after 24 h exposure of *Aedes albopictus* Skuse (Diptera: Culicidae) larvae under laboratory conditions, while the amount of extracts used was reduced to 300.8 ppm after 48 h (Bilal et al., 2011). Mahdi and Rahman (2008) tested the effect of *N. sativa* powder mixed to black gram seeds *Phaseolus bengalensis* L. on the pulse beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Data were recorded on days to 100% mortality, number of adults emerged and per cent weight loss of black gram seeds. It was found that at a dose of 30 g/kg, 100% mortality was occurred after 6 days (Control: 16 days), 10 to 20 adults were emerged (Control: 60 to 70 adults) and only 10% weight loss of black gram (Control: 35%). *N. sativa* fixed oils contain essentially glycerol esters and fatty acids as linoleic acid, oleic acid and palmitic acid (Lautenbacher, 1997). It also contains thymoquinone, dithymoquinone, thymohydroquinone and thymol (Ghosheh et al., 1999; Al-Saleh et al., 2006). The essential oil consists mainly of monoterpenes of which thymoquinone is characteristic, *p*-cimene, carvacrol, *t*-anethole, 4-terpineol and longifoline (Canonica et al., 1963; El-Dakhakhny, 1963; Burits and Bucar, 2000). The seed also contains flavonol triglycosides (Merfort et al., 1997) and alkaloids such as nigelline, nigellicine, nigellidine, nigellimine and nigellimine-N-oxide (Atta-ur-Rahman et al., 1985a, b; 1992, 1995). In the light of these results, *N. sativa* extracts were effective against *T. urticae* and can be used as insecticides. Therefore, it constitutes a real alternative to synthetic pesticides. However, a more detailed study of these extracts is needed to determine the metabolites responsible for their toxicity.

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REFERENCES

Abbassi K, Atay-Kadiri Z, Ghaout S (2003). Biological effects of alkaloïds extracted from three plants of Moroccan arid areas on the desert locust. Physiol. Entomol. 28:232–236.

Abbott WS (1925). A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

Abdel Halim AS, Morsy TA (2006). Efficacy of *Trigonella foenum-graecum* (fenugreek) on third stage larvae and adult fecundity of *Musca domestica*. J. Egypt. Soc. Parasitol. 36(1):329-334.

Al-Saleh IA, Billedo G, El-Doush H (2006). Levels of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. J. Food Comp. Anal. 19:167-175.

Altieri M (1995). Escaping the treadmill. The FAO review: *Ceres* 27:15–23.

Arora R, Dhaliwal GS (1994). Botanical pesticides in insect pest management: Ecological perspectives. In: Dhaliwal GS, Kansal BD (eds) Management of Agricultural Pollution in India. Commonwealth Publishers, India, pp. 213–245.

Atta-ur-Rahman, Malik S, Zaman K (1992). Nigellimine, a new isoquinoline alkaloid from the seeds of *Nigella sativa*. J. Nat. Prod. 55(5):676-678.

Atta-ur-Rahman, Malik S, Ahmed S, Chaudhary I, Habib-ur-Rehman (1985a). Nigellimine- N-oxide a new isoquinoline alkaloid from the seeds of *Nigella sativa*. Heterocycles 23(4):953-955.

Atta-ur-Rahman, Malik S, Cun-heng H, Clardy C (1985b). Isolation and structure determination of nigellicine, a novel alkaloid from the seeds of *Nigella sativa*. Tetrahedron Lett. 26(23):2759-2762.

Atta-ur-Rahman, Malik S, Hasan SS, Choudhary MI, Ni C-Z, Clardy J (1995). Nigellidine, a new indazole alkaloid from the seeds of *Nigella sativa*. Tetrahedron Lett. 36:1993-1996.

Borges LMF, Ferri PH, Silva WJ, Silva WC, Silva JG (2003). *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus.* Med. Vet. Entomol. 17:228–231.

Burits M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323-328.

Campos F, Dybas RA, Krupa DA (1995). Susceptibility of twospotted spider mite (Acari: Tetranychidae) populations in California to abamectin. J. Econ. Entomol. 88(2):225-231.

Canonica L, Jommi G, Scolastico C (1963). The pharmacologically active principle in *Nigella sativa*. Gazz. Chim. Ital. 93(11):1404-1407.

Carpinella MC, Ferrayoli GC, Valladares G, Defago M, Palacios S (2002). Potent limonoid insect antifeedant from *Melia azedarach*. Biosci. Biotechnol. Biochem. 66:1731–1736.

Carson R (1965). Silent Spring. Penguin Books Ltd, UK, P. 336.

Çelik A, Mazmanci B, Çamlica Y, Çömelekoğlu Ű, Aşkin A (2005). Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat

bone marrow by gavage administration. Ecotoxicol. Environ. Safety 61:128–133.
Chauhan LKS, Gupta SK (2005). Combined cytogenetic and ultrastructural effects of substituted urea herbicides and synthetic pyrethroid insecticide on the root meristem cells of *Allium cepa*. Pest. Biochem. Physiol. 82:27–35.
Chiu SF, Huang ZX, Huang DP, Huang BQ, Xu MCh, Hu MY (1984). Investigations on the extraction of toxic principles from seed kernels of Meliaceae and their effects on agricultural insects. Personal communication. pp. 29–32.
Dhaliwal GS, Arora R (2001). Role of Phytochemicals in Integrated Pest Management. In: Koul O, Dhaliwal GS (eds) Phytochemical Biopesticides. Overseas Publishers Association by license under the Harwood Academic Publishers, Netherlands, pp. 87-106.
Egwaikhide PA, Gimba CE (2007). Analysis of the Phytochemical Content and Anti-microbial Activity of *Plectranthus glandulosis* Whole Plant. Middle-East J. Sci. Res. 2(3-4):135-138.
El-Dakhakhny M (1963). Studies on the chemical constituents of Egyptian *Nigella sativa* L. seeds II: The essential oil. Planta Med11:465-470.
Elumalai K, Krishnappa K, Anandan A, Govindarajan M, Mathivanan T (2010). Larvicidal and ovicidal activity of seven essential oil against lepidopteran pest *S. litura* (lepidoptera: noctuidae). Int. J. Recent Sci. Res. 1:008-014.
Finney DJ (1971). Probit analysis. Cambridge University Press, USA, P. 272.
Ghosheh OA, Houdi AA, Crooks PA (1999). High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J. Pharm. Biomed. Anal. 19(5):757-762.
Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research. (2nd ed.), John Wiley and Sons Ltd, USA, P. 680.
Hale FA, Williams H (2003). Insects – Twospotted Spider Mites. Agricultural Extension Service. The Universiy of Tennesse.
Hamilton D, Crossley S (2004). Introduction. In: Hamilton D, Crossley S (eds) Pesticide Residues in Food and Drinking Water: Human Exposure and Risks. John Wiley and Sons Ltd, UK, pp. 1-25.
Herraiz T, González D, Ancín-Azpilicueta C, Arán VJ, Guillén H (2010). β-Carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO). Food Chem. Toxicol. 48:839–845.
Italo Chiffelle G, Amanda Huerta F, Diego Lizana R (2009). Physical and chemical characterization of *Melia azedarach* L. fruit and leaf for use as botanical insecticide. Chilean J. Agric. Res. 69(1):38-45.
Jbilou R, Amri H, Bouayad N, Ghailani N, Ennabili A, Sayah F (2007). Insecticidal effects of extracts of seven plant species on larval development, α-amylase activity and offspring production of *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae). Bioresour. Technol. 99:959–964.
Jbilou R, Ennabili A, Sayah F (2006). Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Afr. J. Biotechnol. 5(10):936-940.
Jones WP, Kinghorn AD (2006). Extraction of Plant Secondary Metabolites. In: Sarker SD, Latif Z, Gray AI (eds) Natural Products Isolation. (2nd ed.), Humana Press, USA, pp. 323-351.
Khater HF, Shalaby AA-S (2007). Potential of biologically active plant oils for controlling of *Culex pipiens* (Diptera:Culicidae). Acta Scien. Vet. 35 (2):153-160.
Kraus W, Gutzeit H, Bokel M (1989). 1,3-diacetyl-11,19-deoxa-11-oxo-meliacarpin, a possible precursor of azadirachtin from *Azadirachta indica* A. Juss (Meliaceae). Tetrahedron Lett. 30:1797–1798.
Lautenbacher LM (1997). Schwarzkümmelöl. Dtsch. Apoth. Ztg. 137:68-69.
Lee SM, Klocke JA, Balandrin MF (1987). The structure of 1-cinnamoylmelianolone, a new insecticidal tetranortriterpenoid, from *Melia azedarach* L. (Meliaceae). Tetrahedron Lett. 28:3543–3546.
Lee SM, Klocke JA, Barnby MA, Yamasaki RB, Balandrin MF (1991). Insecticidal constituents of *Azadirachta indica* and *Melia azedarach* (Meliaceae). In: Naturally Occurring Pest Bioregulators, ACS Symposium Series 449:293–304.
Maciel MV, Morais SM, Bevilaqua CML, Camurça-Vasconcelos ALF, Costa CTC, Castro CMS (2006). Ovicidal and larvicidal activity of *Melia azedarach* extracts on *Haemonchus contortus*. Vet. Parasitol. 140:98–104
Mahdi SHA, Rahman MdK (2008). Insecticidal effect of some spices on *Callosobruchus maculatus* (Fabricius) in black gram seeds. Univ. J. Zool. Rajshahi Univ. 27:47-50.
McLaughlin JL, Rogers LL (1998). The use of biological assays to evaluate botanicals. Drug. Inf. J. 32:513-524.
McMillian WW, Bowman MC, Burton RL, Starks KJ, Wiseman BR (1969). Extract of chinaberry leaf as a feeding deterrent and growth retardant for larvae of the corn earworm and fall armyworm. J. Econ. Entomol. 62:708–710.
Merfort I, Wray V, Barakat HH (1997). Flavonoid triglycerides from seeds of *Nigella sativa.* Phytochemistry 46:359-363.
Petropoulos GA (2002). Fenugreek: The genus *Trigonella*. (1st ed), Taylor and Francis, UK and USA, P. 226.
Theiling KM, Croft BA (1988). Pesticide side-effects on arthropod natural enemies: a database summary. Agric. Ecosys. Environ. 21:191–218.
Whalon ME, Mota-Sanchez D, Hollingworth RM (2008). Analysis of Global Pesticide Resistance in Arthropods. In: Whalon ME, Mota-Sanchez D, Hollingworth RM (eds) Global Pesticide Resistance In Arthropods. CAB International, Cambridge, pp. 5-31.
Yanar D, Kadıoğlu I, Gökçe A (2011). Acaricidal effects of different plant parts extracts on two-spotted spider mite (*Tetranychus urticae* Koch). Afr. J. Biotechnol. 10(55):11745-11750.