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Efficacy of synergistic activity of seed oils from *Carthamus tinctorius* (Safflower) and *Nasturtium officinale* (Watercress) on the lethality of the cattle tick *Hyalomma scupense* (Acari: Ixodidae)

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Abstract

Background: Ticks and tick-borne diseases are a severe economic and public-health problem for cattle producers. The emergence of acaricide resistance to synthetic chemical treatments has prompted interest in developing alternative tick control methods.

Aim: The main objective of the current research was to identify the chemical structure of *Carthamus tinctorius* and *Nasturtium officinale* seed oils and to assess their anti-tick properties against *Hyalomma scupense* ticks both alone and in combination (1:1).

Methods: Analytical methods were used to analyze the chemical components. For *in vitro* assays, adults of *H. scupense* were immersed in *C. tinctorius* and *N. officinale* seed oils at 100, 125, 200, and 300 mg/ml concentrations; for 5 minutes. Larvae of *H. scupense* were dipped in 25, 50, 100, 125, 200, and 300 mg/ml doses of seed oils; the mortality percentage was determined after 24 hours.

Results: The seed oil safflower was mainly composed of linoleic acid (84.48%), followed by palmitic acid (6.54%) and stearic acid (3.77%). Meanwhile, watercress seed oil was mainly composed of linolenic acid (50.78%), gondoic acid (13.57%), linoleic acid (10.58%), palmitic acid (8.02%), and erucic acid (6.62%). The Adults Immersion Test showed the sensitivity of ticks to *C. tinctorius* and *N. officinale* seed oil: *C. tinctorius* seed oil caused (95%) mortality of *H. scupense* at 300 mg/ml, while *N. officinale* seed oil induced (88.68%) mortality at the same concentration. At a 200 mg/ml concentration, *C. tinctorius* and *N. officinale* oil combined caused 100% mortality. Tested oils showed larvicidal efficacy. LC50 values for *C. tinctorius* and *N. officinale* seed oils were 84.16 and 61.78 mg/ml, respectively, in 24 hours. LC50 value of oils association (50% *C. tinctorius*: 50% *N. officinale*) was 47.96 mg/ml.

The mixture of seed oils from two plants tested against *H. scupense* larvae and adult females at a 1:1 ratio showed synergistic interaction.

Conclusion: Seed oils tested alone, and the mixture could be used as an alternative solution in the fight against ticks.

Keywords: *Carthamus tinctorius*, *H. scupense*, *Nasturtium officinale*, Acaracidal activity, synergistic.

Introduction

In North Africa, theileriosis, caused by *Theileria annulata*, represents a major tick-borne protozoan disease and poses serious limitations to the cattle sector (Bouattour *et al.*, 1996; Gharbi and Aziz Darghouth, 2014). In Tunisia, the vector tick is mainly *Hyalomma scupense* Schulze, 1919 (Gharbi *et al.*, 2013). These arthropods are the most economically important ectoparasites of cattle, causing significant costs in terms of diseases, lower productivity and fertility, and often mortality (Kumar *et al.*, 2020).

Many approaches have been used for tick management, such as biological control using pathogens or predators, pheromone-assisted control, herbal pour-on or dip preparations including green manufactured nanoparticles (Banumathi *et al.*, 2017), and vaccination (Labarta *et al.*, 1996). Acaricides and repellents

are still regarded as the easiest method for control; however, applications involve several drawbacks like cost, toxicity, waiting times, and acaricide resistance (Quadros *et al.*, 2020). Alternative anti-tick products and or strategies are therefore necessary. Natural products have been investigated for the acaricidal effect (Figueiredo *et al.*, 2018; Alimi *et al.*, 2021; Sbhatu *et al.*, 2021). More recently, oil seeds have garnered the attention of researchers and food scientists (Tanwar and Goyal, 2021). Obviously, seed oils are an excellent source of bioactive compounds used in the food, cosmetic, and pharmaceutical industries, encompassing the prevention and treatment of several diseases (Vergallo, 2020).

Carthamus tinctorius Linnaeus, or safflower, commonly called « Bok » (in Tunisia), is a thistle-like herbaceous annual plant belonging to the Asteraceae

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family (Asgarpanah and Kazemivash, 2013). It is native to Asia and the Mediterranean basin, and it can grow in dry and semi-arid areas with seasonal rainfall. Safflower is a valuable medicinal and fragrant plant utilized as a source of edible additives, natural colors, nutritious drinks, and cosmetics in many regions (Khalid et al., 2017; Jia-Xi et al., 2019).

Several pharmacological activities, such as antioxidant (Zemour et al., 2019), antidiabetic (Asgary et al., 2012), antibacterial (Ozkan et al., 2021), analgesic, and anti-inflammatory effects (Alaiye et al., 2020), are exhibited by safflower. Notably, safflower oil is excellent in nutritional value, including 70% polyunsaturated fatty acid (linoleic acid), 10% monounsaturated oleic acid, and trace levels of stearic acid (Zhou et al., 2014; Khémiri et al., 2020). Safflower oil also contains other chemicals. Phenolic molecules, which are found in the unsaponifiable phase of oil and are responsible for its stability and nutritional value, are among them (Khémiri et al., 2020).

Nasturtium officinale or watercress, also known as “jarjir” in Tunisia, is a highly uncommon aquatic or semi-aquatic plant endemic to Europe, North Africa, and the Asia Brassicaceae family (Faizy et al., 2021). This herb has well-known nutritional characteristics due to its diverse chemical components, including vitamins B, C, and E, as well as pro-vitamin A, folic acid, carotenoids, glucosinolates, and a variety of minerals such as calcium, iron, and sulfur. Furthermore, Glucosinolates, isothiocyanates, polyphenols (flavonoids, phenolic acids, proanthocyanidins), and terpenoids, including carotenoids, are the primary components discovered in the *N. officinale* plant (Afsharypuor and Salehi 2008; Jeon et al., 2017).

The European Food Safety Authority has approved *N. officinale* as a safe food plant, and it is listed in the monographs on “Leaf vegetables, herbs, and edible flowers”.

Watercress is considered an important medicinal plant largely used in traditional medicine (Teixidor-Toneu et al., 2016). Moreover, *N. officinale* oil revealed a broad spectrum of pharmacological activities, including anticancer (Hecht et al., 1995), antioxidant (Bahramikia and Yazdanparast, 2010; Zeb, 2015; Ramezani et al., 2021), antibacterial (Bahramikia and Yazdanparast, 2008), tuberculosis (Halberstein, 2005), and cardioprotective actions (Pandey et al., 2018).

According to the literature, any previous investigations were found about the oils of *C. tinctorius* and of *N. officinale*, or seed oils generally, having any anti-tick activity on *H. scupense*. Therefore, the major goal of this study was to characterize the chemical structure of *C. tinctorius* and *N. officinale* seed oils, as well as to evaluate their anti-tick activities against *H. scupense* larvae and engorged female ticks individually and in association (1:1).

Materials and Methods

Plant material

Collection of plant

Samples of *C. tinctorius* and *N. officinale* seeds were collected during the month of June 2020 from the village of Ain Draham in the gouvernate of Jendouba (Northwestern of Tunisia, alt 800 m; 36° 46' 34" N, 8° 41' 05" E). The plants' species were identified as *C. tinctorius* L and *N. officinale* by the Department of Plant Biotechnology, Higher Institute of Biotechnology of Beja, Jendouba University, Tunisia, according to the flora of Tunisia (Cuénod, 1954).

Seed oils extraction

Seeds were mechanically separated, rinsed in clean water, and air-dried at room temperature (20°C–25°C). To preserve their constituents' quality, both oils were extracted naturally by cold pressing using an oil press machine (SMIR, MUV2 65) without any chemical treatment. After filtration, seeds oils were preserved in labeled dark glass bottles at room temperature until analysis.

Fatty acid methyl esters analysis and preparation from samples

One ml of the oils were dissolved in 20 ml petroleum ether, and 2 ml of methanolic potassium hydroxide (KOH) (2M) was added for fatty acid methyl esterification. The mixture was allowed to sit for 10 minutes after being agitated for 2 minutes. The top layer, high in fatty acid methyl esters, was removed, washed with water, and examined using gas chromatography/mass spectrometry (GC/MS) (Nickavar et al., 2003). An Agilent GC-MS was used to analyze *C. tinctorius* and *N. officinale* (Agilent Technologies, Wilmington, DE, 7890) equipped with a gas chromatograph and a 5975C quadrupole mass selective detector. A fused silica capillary column from HP-5MS (30 m 0.25 mm i.d. 0.25 μm film thickness) was employed. The carrier gas was helium, with a 1 ml/min constant flow rate. A 1.0 μl sample was injected into a split 1/120 injector at 250°C. The ion source had a temperature of 230°C, whereas the quadrupole had a temperature of 150°C. The oven temperature was firstly kept at 150°C for 1 minute, then increased at a rate of 15°C/minute to 200°C for 3 minutes, then elevated to 280°C at a rate of 3°C/minute for 10 minutes, then maintained to 300°C at 15°C/minute for 10 minutes, and finally held for 10 minutes. The methyl esters of standard fatty acids were run under the same conditions (Freese et al., 1973). Their mass spectra were compared to the Wiley 275 and NIST mass spectra data bases to identify and authenticate substances. Quantitative data was obtained using Peak's area percents.

Hyalomma scupense ticks

Ticks collection

Adult-engorged females of *H. scupense* were handpicked from naturally infected cattle, without

chemical acaricidal treatment, and in the vicinity of cattle pens in a rural farm in the hamlet of Soliman (North-East of Tunisia, gouvernate of Nabeul). To allow for improved ventilation, ticks were delivered to the laboratory in perforated bottles (about 1 mm in diameter for each entire). Ticks were washed, weighted (with an average weight of 0.25 g), dried, and chosen for vitality and movement. A total of 225 adult engorged female ticks were used for the present study. Out of this, 15 ticks were separated and were held individually at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $85\% \pm 5\%$ relative humidity in a labeled glass bottle with the mouth covered by muslin cloth for oviposition. The eggs were allowed to hatch to larvae in 18–25 days under similar incubation conditions. The larvae were used for performing a “larval packet test” (LPT). The remaining 210 ticks were gathered into three groups (one for each plant and combination), each of 60 ticks and 30 ticks for two controls. Each comprising of 15 ticks as 5 ticks each in 3 replicates. Each group of ticks was used to estimate the acaricidal effects of the respective concentration of plant by adult immersion test (AIT).

AIT and evaluation of synergism

Drummond *et al.* (1973) described the immersion protocol to evaluate acaricide activity against adult ticks. Seeds oils from *C. tinctorius* and *N. officinale* were tested individually and in association at a 1:1 ratio. Ticks were dipped in increasing doses of oils for 5 minutes, while control ticks were immersed in a solution of 2% Tween-80 (negative control). This control solution was used to prepare a series of *C. tinctorius* and *N. officinale* oils solutions at different concentrations: 100, 125, 200, and 300 mg/ml. The same concentrations were used for combined seed oils. At doses of 0.0125 mg/ml, a commercial product containing amitraz was employed as a control sample. The ticks were then placed on Petri dishes over Whatman filter paper 1. All the Petri dishes with treated ticks were kept at room temperature for 24 hours. After 24 hours, ticks were transferred to glass vials covered with muslin cloth and kept in desiccators having $85\% \pm 2\%$ relative humidity and placed in an incubator at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$. These ticks were observed for oviposition and death up to 15 days. The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded compared to the control. The eggs were incubated at the same condition, and the percentage of hatched eggs was estimated visually. To evaluate reproductive and percentage inhibition of fecundity, the following equations were used (Drummond *et al.*, 1973; Ribeiro *et al.*, 2008; Matos *et al.*, 2019):

Reproductive Index (RI) = average weight of eggs laid (mg)/average weight of females before treatment (mg).
Inhibition of Oviposition (IO%) = $\frac{\text{RI (control group)} - \text{RI (treated group)}}{\text{RI (control group)}} \times 100$.
Reproduction efficiency (RE) = $\frac{\text{egg weight} \times \% \text{ hatchability}}{\text{weight of females}}$

*Constant indicating the number of eggs present in 1 g of egg-laying

$\text{RI (\%)} = \frac{\text{RE (control)} - \text{RE (treated)}}{\text{RE (control)}} \times 100$
Effectiveness of the product (PE) = $\frac{\text{RE (control group)} - \text{RE (treated group)}}{\text{RE (control group)}} \times 100$

The synergistic factor (SF) was calculated using the formula described by Kalyanasundaram and Das (1985) with a slight modification. A $\text{SF} > 1$ indicates synergistic effect, whereas the value of $\text{SF} < 1$ shows antagonism. $\text{SF value} = 1$ indicates that there is no substantial effect.

$\text{SF} = \frac{\text{LC50 value of the individual plant extract}}{\text{LC50 value of the combined plant extract}}$

Larval packet test (LPT)

The *H. scupense* larvae packet test was used for each treatment, according to Stone and Haydock (1962). Filter paper sheets (2×2 cm) were impregnated with 1 ml of *C. tinctorius*, *N. officinale*, and their combination oil at concentrations of 300, 200, 125, 100, 50, and 25 mg/ml. About one hundred larvae, 14–21 days old, were deposited on each sheet impregnated with the solution. After 24 hours of impregnation, larvae were put inside the packets and subsequently incubated (27°C and $\text{RH} > 80\%$) (Figueiredo *et al.*, 2018). After 24 hours, an assessment of percent larval mortality was performed. Three replicates were performed for each concentration, as well as for the controls groups, which were composed of: 2% Tween 80 in sterile distillate water (negative control) and amitraz (0.0125 mg/ml) (positive control). The percentage mortality of *H. scupense* was calculated with Abbott's correction.

Statistical analyses

To see any significant variations in larval and adult mortality rates between the treatments, analysis of variance (ANOVA) was used, followed by Fisher's PLSD tests. If the *p*-value was less than 0.05, it was considered to be significant. The mean and standard error of the mean was used to represent the data. Probit analysis with GraphPad Prism 9.0 software was used to compute the lethal concentration of the fixed oil for 50% (LC50) and 90% (LC90) of the tick population with a 95% confidence interval. The Statview v.5.0.1 program conducted all of these statistical studies (SAS Institute, Cary, NC).

Ethical approval

At the Department of Comparative Medicine, parasites were kept in a pathogen-free environment. All tests were carried out in compliance with IACUC procedure No. (NIH publication 86-23 modified 1985) USA (National Ethics Committee of Tunis University).

Results

Seed oils analysis

Table 1 shows the various oil compositions. Linolenic acid (84.48%) was the most abundant ingredient in *C. tinctorius* seed oil (Fig. 1), followed by palmitic acid (6.54%) and stearic acid (3.77%). Other fatty acids

such as myristic acid, oleic acid, oxiraneoctanoic acid, arachidic acid, gondoic acid, behenic acid, nervonic acid, lignoceric acid, and lignoceric acid were present in trace amounts and varied between 0.09% and 0.63%. Eleven individual compounds were characterized in *N. officinale* oil, representing 100% of the total seed oil. The main constituents of the oil were linolenic acid (50.78%) (Fig. 2), gondoic acid (13.57%), linoleic acid (10.58%), palmitic acid (8.02%), and erucic acid (6.62%).

Adult immersion test (AIT)

The results of the AIT using *C. tinctorius* and *N. officinale* and their mixture oil are shown in Table 2. The efficacy of different treatments was assessed by estimating the percent adult mortality, RI, IO, hatching

rate, and efficacy. In the high tested concentration of 300 mg/ml, seed oil from *C. tinctorius* caused 95% mortality, while at the same concentration, the seed oil of *N. officinale* showed only 88.68% mortality. The oil mixture of seed oils, at all concentrations tested, exhibited the best inhibition rate of 100% at 200 mg/ml. The results showed that seed oils tested on *H. scupense* had a dose-dependent effect in all the AIT bio-assays. Compared to the control group, there was a significant difference between all treatments. The positive control amitraz caused 82.10% mortality in engorged females of *H. scupense*.

Table 2 contains further information on tick reproductive efficiency and the efficacy of seed oils as a tick treatment. Our findings revealed that *C. tinctorius*

Table 1. Composition of seeds oils from *C. tinctorius* and *N. officinale* obtained by GC-MS.

RT(minutes)	Compounds	Molecular formula	Content (%) of total	
			<i>Carthamus tinctorius</i> L.	<i>Nasturtium officinale</i>
6.931	Myristic acid	C14H28O2	0.09	-
9.511	Palmitic acid	C16H32O2	6.54	8.02
12.876	Linoleic acid	C18H32O2	84.48	10.58
12.773	Linolenic acid	C18H30O2	-	50.78
13.265	Stearic acid	C18H36O2	3.77	3.01
16.309	Oleic acid	C18H34O2	0.33	-
16.795	Oxiraneoctanoic acid	C10H18O3	0.50	-
16.870	Eicosadienoic acid	C20H36O2	-	0.3
17.001	Gondoic acid	C20H38O2	0.21	13.57
17.677	Arachidic acid	C20H40O2	0.63	4.12
20.984	Erucic acid	C22H42O2	-	6.62
21.213	Behenic acid	C22H44O2	0.31	1.24
22.700	Nervonic acid	C24H46O2	0.17	1.05
22.912	Lignoceric acid	C24H48O2	0.16	0.71
Total			97	100

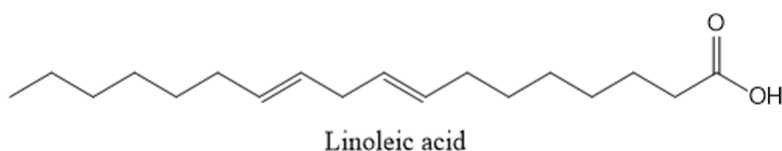


Fig. 1. The major component of seed oil of *C. tinctorius* (84.48%).

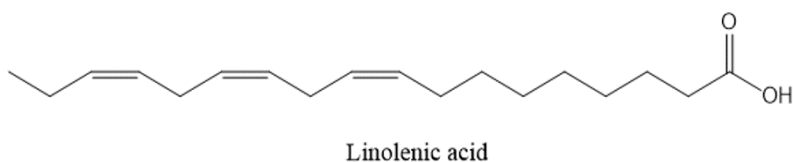


Fig. 2. The major component of seed oil of *N. officinale* (50.78%).

Table 2. Mean (\pm SE) standard deviation of biological activity of *H. scupense* engorged females after exposure to different concentrations of *C. tinctorius*, *N. officinale* and combination of seed oils.

Treatment	Concentrations (mg/ml)	FW \pm SE	MA ₁₅ \pm SE	EW \pm SE	RI \pm SE	IO (%)	Hatching (%) (visual)	REI (%)	EP (%)
(2%) Tween 80 (negative control)		0.25 \pm 0.02	0.00 \pm 0.00	0.134 \pm 0.02	0.53 \pm 0.00		100	99.14	0
<i>Carthamus tinctorius</i>	300	0.25 \pm 0.02	95.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	100	0	0	100
	200	0.26 \pm 0.02	84.77 \pm 5.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	100	0	0	100
	125	0.25 \pm 0.01	63.16 \pm 3.10a	0.0118 \pm 0.03a	0.047 \pm 0.001a	67.16	0	0	100
	100	0.25 \pm 0.01	34.11 \pm 6.10a	0.0121 \pm 0.02a	0.048 \pm 0.005a	63.88	0	0	100
<i>Nasturtium officinale</i>	300	0.25 \pm 0.01	88.68 \pm 5.60a	0.0060 \pm 0.04a	0.024 \pm 0.005a	82.09	0	0	100
	200	0.25 \pm 0.01	63.37 \pm 8.77a	0.0106 \pm 0.05a	0.042 \pm 0.001a	68.36	0	0	100
	125	0.25 \pm 0.02	50.82 \pm 7.44a	0.0115 \pm 0.05a	0.046 \pm 0.002a	65.67	0	0	100
	100	0.25 \pm 0.02	20.66 \pm 2.66a	0.02 \pm 0.06a	0.08 \pm 0.004a	40.30	80	46	53.61
<i>Nasturtium officinale</i> + <i>Carthamus tinctorius</i> Combination (1:1)	300	0.25 \pm 0.02	100.00 \pm 0.00a	0.000 \pm 0.00a	0.00 \pm 0.00a	100	0	0	100
	200	0.25 \pm 0.02	100.00 \pm 0.00a	0.000 \pm 0.00a	0.00 \pm 0.00a	100	0	0	100
	125	0.24 \pm 0.02	58.69 \pm 5.13a	0.0100 \pm 0.02a	0.042 \pm 0.005a	68.91	0	0	100
	100	0.25 \pm 0.02	47.11 \pm 6.12a	0.0114 \pm 0.06a	0.046 \pm 0.002a	65.97	0	0	100
<i>Amitraz</i> (Positive control)	12.5	0.25 \pm 0.02	82.10 \pm 3.66	0.00 \pm 0.000	0.00 \pm 0.00	100	0	0	100

(FW): Mean of female weights; (SE): Standard error; (MA15): Mean % adult mortality within 15 days; (EW): Egg weights; (REI): Reproductive efficiency index; (EP): Efficacy of the product. Three replicates each having 15 adult females ticks were used for each treatment.

^aSignificant difference in relation to the negative control. (ANOVA one way $p \leq 0.0001$).

(LC50 = 120.11 mg/ml) and the oil combination (LC50 = 117.40 mg/ml) have the highest oviposition inhibition rate. However, at the highest dose, individual *N. officinale* oil showed only 82.09% IO. The results were significantly different from the control group (2% Tween 80) ($p < 0.001$). In all concentrations tested, all treatments used in this study could affect tick reproduction *in vitro* by inhibiting oviposition and hatchability. However, at a low concentration of 100 mg/ml, seed oil from *N. officinale* only showed a 46% reproductive efficiency.

Larval packet test (LPT)

The LPT was used to investigate the efficacy of *C. tinctorius* and *N. officinale* seed oils against *H. scupense* larvae (Table 3). Both plants showed larvicide properties. Separately, the lowest mortality value for *N. officinale* seed oil was observed at 25 mg/ml concentration with 4.57% mortality, while 83.36% tick mortality was achieved at 300 mg/ml concentration (LC50 = 84.16 mg/ml). Meanwhile, *C. tinctorius* seed oil had a stronger larvicidal activity, with 91.88% tick mortality at 300 mg/ml (LC50 = 61.78 mg/ml) and 25.31% tick mortality at a considerably lower dosage of 25 mg/ml (Tables 3 and 4). Tick mortality was 99.69% and 100% at 200 and 300 mg/ml concentrations, respectively, when mixed seed oils. Two seed oils showed a concentration-dependent mortality effect. All of these outcomes differed significantly from the negative control ($p < 0.001$). No mortality rate was

shown for the larvae in the negative control; however, within 24 hours of exposure to amitraz, larvae died at a rate of 77.47%. Additionally, results showed that combined use of these seed oils was highly effective and showed higher acaricidal effects with LC50 value at 24 hours of 47.96 mg/ml (Table 4).

The SF value from mixing these seed oils at a 1:1 ratio is presented in Table 5. SFs against *C. tinctorius* larvae and female adults exhibited a synergistic effect at 1.28 and 1.02, respectively. In addition, a synergistic effect was observed against *N. officinale* larvae and female adults at 1.75 and 1.05, respectively. This revealed that the interaction of the two plants had a synergistic impact (SF > 1).

Discussion

As demonstrated in Table 1, linoleic acid is the most abundant fatty acid in *C. tinctorius* seed oil, accounting for 84.48%. Also, safflower seed oil contains appreciable saturated fatty acids, especially palmitic (6.54%) and stearic (3.77%). The oleic acid content in the Tunisian sample was low (0.33%).

This finding was completely different from the previous study, which detected the same chemical components in different amounts. Our results differed from those of Carvalho *et al.* (2006), who obtained 42.80% linoleic acid and 29.09% oleic acid, and Sabzalian *et al.* (2008) who obtained 45.43% linoleic acid and 28.75% oleic acid. It was also determined that oleic

Table 3. The activity of seed oils from *N. officinale*, *C. tinctorius* and in association on larval mortality of *H. scupense*.

Concentration (mg/ml)	Mortality (%) ^{a,b}		
	<i>Carthamus tinctorius</i> (%)	<i>Nasturtium officinale</i> (%)	Association (%)
(2%) Tween 80 (negative control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	25.31 ± 3.58**	4.57 ± 1.44	36.33 ± 6.81**
50	44.51 ± 5.73*	40.72 ± 2.99*	52.57 ± 3.10*
100	66.05 ± 3.07*	57.48 ± 5.72*	69.33 ± 4.58*
125	73.46 ± 2.14*	64.02 ± 4.24*	83.13 ± 3.38*
200	87.71 ± 5.23*	75.89 ± 4.37*	99.69 ± 0.53*
300	91.88 ± 0.00*	83.36 ± 3.50*	100 ± 0.00*

^aMortality in control groups was calculated as dead larvae/total larvae × 100;

^bMortality in groups treated with seed oils was calculated as abbott.

* $p < 0.0001$ and ** $p < 0.01$ compared with negative control.

Table 4. Lethal concentrations required to cause 50% or 90% mortality (LC50 and LC90) of *H. scupense* engorged females and larvae after exposure to *C. tinctorius*, *N. officinale* and their combination with a 95% confidence interval.

Acaricidal test	Treatment	LC ₅₀	IC	LC ₉₀	IC	R ²
Mortality of engorged females (%) (AIT)	<i>Carthamus tinctorius</i>	120.11	108.17–123.10	210.37	191.10–228.53	0.91
	<i>Nasturtium officinale</i>	124.08	115.26–134.64	341.16	339.21–365.44	0.97
	Association	117.40	95.22–120.33	152.08	151.02–166.36	0.95
Mortality larvae (%) (LPT)	<i>Carthamus tinctorius</i>	61.78	60.68–82.72	280.09	238.84–291.03	0.98
	<i>Nasturtium officinale</i>	84.16	81.71–97.59	318.37	311.08–322.81	0.97
	Association	47.96	44.77–66.11	132.01	128.11–1471	0.93

Table 5. SF for plant seed oils.

Plants	Seed oil combination	SF against <i>C. tinctorius</i>		SF against <i>N. officinale</i>	
		Engorged females	Larvae	Engorged females	Larvae
<i>Nasturtium officinale</i> and <i>C. tinctorius</i>	1:1	1.02 ^a	1.28 ^a	1.05 ^a	1.75 ^a

(SF): Synergistic factor.

(SF > 1): additive; (SF < 1): antagonism; (SF = LC50): individual extract/LC50 combined extract.

^aSynergism.

acid (8.0%–21.0%) and linoleic acid (68.0%–83.0%) were the main fatty acids in safflower Turkey oils, according to Orhan *et al.* (2021). The fatty acid profile of *N. officinale* analysis by GC/MS in this study showed as major-compounds linolenic acid (50.78%), gondoic acid (13.57%), linoleic acid (10.58%), palmitic acid (8.02%), and erucic acid (6.62%). The GC-MS analysis of commercial Egyptian watercress revealed two fatty acids: oleic acid (46.44%) and palmitic acid (10.10%) (Alagawany *et al.*, 2018), while oleic acid was the second most abundant fatty acid (17.11%), and palmitic acid was the third most abundant fatty acid (12.89%) in Saudi watercress (Al bratty *et al.*, 2021). According to Ben Moumen *et al.* (2015), the quality of vegetable oil is determined by several elements, including plant variety, maturity level, pedoclimatic factors, agricultural techniques, oil extraction technology, and storage conditions.

To the authors' knowledge, no research on the acaricidal effects of combining *C. tinctorius* and *N. officinale* seed oils on cow ticks have been reported. The current study revealed the acaricidal effect of *C. tinctorius* and *N. officinale* seed oils on different stages of *H. scupense*. The tick mortality rate was dose-dependent (Tables 2 and 3).

Safflower oil had a greater mortality rate of engorged females, attaining 95% mortality at 300 mg/ml and 100% egg hatching at all doses examined than seed oil from *N. officinale*. *Carthamus tinctorius* had LC50 and LC90 values of 120.11 and 210.37 mg/ml, respectively. The LC50 of *N. officinale* was 124.08%, while the LC90 was 341.16%. In addition, The anti-tick action of *C. tinctorius* oil against larval *H. scupense* (LC50 = 61.78 mg/ml) was significantly higher than that of *N. officinale* oil (LC50 = 84.16 mg/ml). It is worth noting that there are various papers on the acaricidal activities of volatile oils and extracts from diverse plants for controlling tick populations, all of which are thoroughly reported (Figueiredo *et al.* 2018; Alimi *et al.* 2021; Sbhatu *et al.* 2021). However, reports about the acaricidal properties of fixed oils, to which our results can be compared, are scarce. Nevertheless, Santos *et al.* (2021) investigated the effects of *Mauritia flexuosa* and *Mauritiella armata* seed oils on engorged females and larvae *Rhipicephalus (Boophilus) microplus* cattle tick; the *in vitro* tests revealed a typical reduction of the hatched larvae as well as inducing high larval mortality above 80% and reduction in the laying capacity of

R. microplus at 5% and 10% concentrations. However, according to Villarreal *et al.* (2017), the fixed oils of *Bertholletia excelsa* (Brazil nut) and *Helianthus annuus* (sunflower seed) had a low effect *in vitro* in cattle against engorged females of *R. microplus*, indicating that both fixed oils had low acaricidal activity (39.39% and 58.75%, respectively, at 200 mg/ml). Another study found that the oil from *Jatropha curcas* seeds had high acaricidal action against *R. microplus* larvae, with effectiveness levels above 90% (Rizo-Borrego *et al.*, 2019).

The fatty acid content of a vegetable oil determines its appropriateness for a certain application, such as nutritional, industrial, or medicinal (Sabzalian *et al.*, 2008). Otherwise, due to their pharmacological usefulness, fatty acids contained in vegetable oils (oleic, linoleic, lignoceric, elaidic, palmitic, palmitelaidic, and stearic) have significant biological activity in animal and human medicine (Asgarpanah and Kazemivash 2013; Orsavova *et al.*, 2015; Jeong *et al.*, 2020).

In this study, safflower showed higher effects on ticks than watercress (which contains 10.58% linoleic acids); hence, the anti-tick action (95% mortality) on *H. scupense* can be associated with this polyunsaturated fatty acids mechanism of action.

Many scientific studies have confirmed the effect of seed oils and their major compounds against microbial infections. Khémiri *et al.* (2020) reported that the safflower seed oil had high antibacterial action against the pathogenic bacterial strains studied (*Enterobacter cloacae*, *Escherichia coli*, and *Streptococcus agalactiae*) with inhibition diameters of 3 and 5 mm. Furthermore, the scientists speculated that safflower seed oil had an antifungal impact on spore germination. Fatty acids have been shown to block certain membrane enzymes, thus killing or inhibiting the development of bacteria (bactericidal activity) (bacteriostatic action). Furthermore, these antimicrobial actions of fatty acids might be associated with those of phytosterols found in safflower oil to improve its efficiency against pathogenic bacteria (Khémiri *et al.*, 2020). Fatty acids have recently been found to block various membrane enzymes, such as glucosyl transferase and stimulate autolytic cell wall enzymes, leading to apoptosis. Furthermore, fatty acids are bacteriostatic and fungistatic action by reducing energy pathway synthesis in mitochondria (Khémiri *et al.*, 2020). According to Marques *et al.* (2004), each vegetable oil possessing a high content of linoleic

acid can be recommended as a therapeutic option in veterinary medicine.

On the other hand, multiple studies have shown that the antibacterial properties of safflower are related to its phenolic component, which disrupts cell membranes (Salem *et al.*, 2014). Other investigations have found that secondary metabolites have physiological functions in humans, animals, and microbes (Salem *et al.*, 2011, 2014; Khémiri *et al.*, 2020).

Compared to oils tested separately, the combined oil proved to be more toxic to ticks. The main goals of deploying synergistic combinations, according to reports, are to minimize the concentration of each chemical while increasing biological activity against the target organism (Jyoti *et al.*, 2019). Furthermore, the greater the chemical complexity of combinations, the less probable resistant populations are to develop (Arajo *et al.*, 2016a).

According to the results, the oil combination showed a significant adulticide action, reaching 100% mortality (LC90 = 152.08 mg/ml) (Table 4), at a slightly higher concentration than the *C. tinctorius* seed oil. Also, even at low concentrations, the mixture of oils showed higher larvicide action against *H. scupense* larvae (LC90 = 47.96 mg/ml) compared to *C. tinctorius* (LC90 = 61.87 mg/ml) and *N. officinale* (LC90 = 84.16 mg/ml).

Plant mixtures have been shown to have synergistic effects on numerous species, such as bacteria (Didry *et al.*, 1994), fungus (Mugnaini *et al.*, 2012), gastrointestinal parasites (Ntalli *et al.*, 2011), and insects (Ntalli *et al.*, 2011). Furthermore, the associations are viable for controlling bovine ticks (Vinturelle *et al.*, 2017; De Carvalho Castro *et al.*, 2019; Shezryna *et al.*, 2020). Overall, researchers found that mixing volatile oils showed good action on *R. (B.) microplus* than testing them alone.

Yessinou *et al.* (2016) studied the use of essential oils in combination as a way to improve efficacy. According to the authors, the combination of *Syzygium aromaticum* and *C. citratus* essential oils had higher potential against *R. microplus* than the oils studied separately. Moreover, the impact of a mixture of main elements of plant-derived essential oils on ticks has been proven (Novato *et al.*, 2015; Arajo *et al.*, 2016b).

In addition to concentration, the ratio combination appears to have a role in the efficacy of mixed seed oils. A 1:1 ratio of *C. tinctorius* and *N. officinale* exhibited a synergistic effect. Several further studies on different ratios have been published; combining *Alpinia galanga* with *Cymbopogon citratus* volatile oils at different ratios revealed that the 3:7 ratio was the most toxic (Shezryna *et al.*, 2020). Poonia and Kaushik (2013) employed *Pongamia pinnata* and *Kigelia africana* plant extracts in three different ratios on *Aedes aegypti*: 1:1, 1:2, and 2:1. The individual usage of *P. pinnata* has been proven to have greater toxicity than *K. africana*. A 2:1 ratio of combination (*P. pinnata*: *K. africana*) produced

an increased effect, but the 1:2 ratio combination (*P. pinnata*: *K. africana*) generated antagonism.

Due to reported tick resistance to commercial acaricides and the risks these drugs cause negative impact humans, animals, and the environment, the combination of *C. tinctorius* and *N. officinale* is both environmentally-friendly (green pesticide) and cost-effective could be used to control cattle tick infestations. Further study is needed to conduct experiments by isolating the bioactive components to discover which one(s) could produce acaricide activity to better understand the action mechanisms.

Both *C. tinctorius* and *N. officinale* seed oils were effective against *H. scupense* larvae and engorged females in the current investigation. A 1:1 mixture of these seed oils, on the other hand, had a synergistic effect (SF > 1). It may be concluded that *C. tinctorius* and *N. officinale* in individual usage and a 1:1 mixture of these seed oils can be recommended as a feasible synthetic acaricide alternative. Isolation and purification of bioactive chemicals might be important in developing alternative acaricidal drugs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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