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Research Paper

Preparation and optimization nanoemulsion of Tarragon (*Artemisia dracunculus*) essential oil as effective herbal larvicide against *Anopheles stephensi*

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ABSTRACT

Continuous use of chemical larvicides causes resistance in many species around the world and also environmental pollution. Nanoemulsions of herbal larvicides (such as essential oil based larvicides) are suitable alternatives for this purpose. However, no work so far has investigated the stability of a nanoemulsion after 100–200 times dilution and its effect on larvae of *Anopheles stephensi*, a major vector spreading malaria. Tarragon is a common medicinal plants and its essential oil had effective larvicidal activity. Different nanoemulsions of Tarragon essential oil were prepared using various amounts of surfactants and co-surfactants. For the first time, a comparison of larvicidal activity between two optimized preparations (particle sizes of 15.6 & 14.5 nm) with tarragon essential oil against *Anopheles stephensi* was performed. The components of Tarragon essential oil were identified by GC–MS analysis. Forty eight components were determined, with 5 major components including P-Ally anisole (67.623%), *cis*-Ocimene (8.691%), beta-Ocimene Y (7.577%), Limonene (4.338%) & 3-Methoxy cinnam aldehyde (1.49%). The bioassay of essential oil was performed, LC at 50 or 90% with values of 11.36 or 17.54 ppm, were calculated using probit analysis. No larvicidal effect was observed from nanoemulsion inactive ingredients (i.e. Tween 20 and isopropyl alcohol). Larvicidal activity of the most stable formulation after dilution (particle size 14.5 nm), showed significant increase compared to bulk of the oil and nanoemulsions with lesser stability profile after dilution. By designing and preparing a stable essential oil nanoformulation, against dilution, which takes the advantage of lesser volatile properties. And also, achieving to effective and environmentally friendly larvicides with low cost will be possible.

1. Introduction

Mosquitoes play a significant role in spreading vector borne diseases such as malaria, dengue fever, yellow fever, encephalitis and filariasis (Sedaghat et al., 2016). According to a report by world health organization (WHO), 2 billion people in the world, live in high risk regions (> 1 in 1000 chance of getting malaria in a year). Malaria imposes substantial cost to both individuals and governments. In 2014, international funding for control of malaria around the world was US$ 1.9 billion (WHO, 2014, 2015). *Anopheles stephensi* is an important vector spreading malaria with wide distribution in the Arabian Peninsula, the Indian subcontinent, Afghanistan and Iran (Vatandoost et al., 2006; Hanafi-Bojd et al., 2012; WHO, 2015).

Several methods have been applied to control vector borne diseases, including spraying of chemicals, herbal insecticides, vaccines and prevention of mosquito bite by using insects repellents. Controlling mosquito larva is probably the easiest and the most cost effective way to control vector borne diseases (Bellan, 2010; Sumitha and Thoppil, 2016). Continuous use of chemical larvicides cause resistance in many species around the world (Mittal et al., 1991; Vatandoost and Borhani, 2004), thus, many scientists worldwide are looking for active natural products such as essential oils to control mosquitoes in an environmentally friendly approach (Kyarimpa et al., 2014; Liu et al., 2015).

Components of essential oils are generally volatile (Singh et al., 2002; Edris, 2007; Pavela, 2015), therefore, they are not appropriate

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for us, as practical mosquito larvicides. A novel approach to stabilize essential oils and increase their efficacy, is formulating them into nanoemulsions, which are translucent or transparent submicron oil-in-water or water-in-oil emulsions with a nanoscale droplet diameter (Wu et al., 2001; Anjali et al., 2012; Cheong et al., 2016, 2017). In the literature, there are few reports about preparation of nanoemulsions of essential oils of *Rosmarinus officinali*, *Eucalyptus globulus*, *Ocimum basilicum*, *Copaifera duckei* and *Azadirachta indica* and evaluating their larviciding effects (Anjali et al., 2012; Ghosh et al., 2013; Rodrigues da CR et al., 2014; Sugumar et al., 2014; Duarte et al., 2015). However, no work so far has evaluated the effect of a nanoemulsion on *Anopheles stephensi*. Additionally, according to WHO guidelines for laboratory testing of mosquito larvicides, such larvicides should be diluted in the ratio 1:100 or 1:200 in water to perform the related tests (WHO, 2005). In line with the WHO guidelines, none of the above mentioned literatures, stability of the diluted nanoemulsions has been evaluated and physical properties of the diluted nanoemulsions have not been reported. In this study, we first reported larvicidal activity of Tarragon essential oil (TEO), Tarragon is a common medicinal plant with a widespread distribution (Eisenman and Struwe, 2011; Obolskiy et al., 2011). Then we prepared different formulations of tarragon nanoemulsions (TNE); using different amounts of surfactants and co-surfactants. For the first time, a comparison of larvicidal activity between two optimized preparations with tarragon essential oil against *Anopheles stephensi* was performed.

2. Materials and methods

2.1. Materials

TEO was purchased from Zardband Pharmaceuticals Co (Iran), stored at 4–8 °C away from sunlight. Tween 20 (TW) and Isopropyl alcohol (IPA) were purchased from Merck (Germany). All volumetric tools were supplied in A grade.

2.2. Analysis of the essential oil

Components of the Tarragon essential oil were identified by gas chromatography–mass spectrometry (GC–MS). GC–MS was performed on HP (Agilent Technology) 6890 Network GC System (gas chromatograph) connected with a 5973 mass selective detector. The oil was analyzed using HP-5MS Fused silica column (Length: 60m, I.D.: 0.25 mm & Film: 0.25 μm) and temperature programmed as below. The percentage of each component is presented in Table 1. The GC–MS settings were programmed as follows; initial oven temperature was held at 40 °C for 1 min, rising to 250 °C at 3C/min. The injector temperature was maintained at 250 °C. Detector temperature was at 230 °C. Carrier gas used was helium (He 99.999%) at a flow rate of 1 mL/min.

2.3. Essential oil larvicidal bioassay

In this study third and fourth instars larvae of *Anopheles stephensi* were used, obtained from the Department of Medical Entomology, Tehran University Medical Sciences (TUMS). Colonies were maintained at 28 ± 1 °C with 12:12 light and dark photo periods and 65% ± 5% relative humidity. Investigated larvicidal activity in line with WHO guidelines for laboratory testing of mosquito larvicides, with some modifications (WHO, 2005).

Components of TEO have hydrophobic behavior, thus, was dissolved in IPA to determine its larvicidal activity. TEO stock solution (20 μL/mL) was prepared, and diluted with IPA to prepare working standard solution. By adding 1 mL from working standards solution in each test container, containing 199 mL of no chlorine water (with 8 cm depth), different concentrations of essential oil were prepared. Each container was mixed by exclusive plastic probe to prevent changing in concentration of solution.

Table 1
List of components identified in Tarragon essential oil using GC–MS analysis.

No.	RT[a]	Compound	Peak area	%	RI[b]
1	6.141	Tricyclene	1551424	0.004	
2	6.35	alpha-Thujene	5230036	0.015	
3	6.664	alpha-Pinene	513847405	1.466	
4	7.171	Camphene	36376876	0.104	
5	8.211	Sabinene	39661740	0.113	
6	8.341	beta-Pinene	217125236	0.620	
7	8.979	beta-Myrcene	81623487	0.233	605
8	9.504	Phellandrene	27173227	0.078	625
9	10.448	o-Cymene	143777034	0.410	662
10	10.727	Limonene	1520194907	4.338	673
11	11.321	cis-Ocimene	3045372978	8.691	696
12	11.899	beta-Ocimene Y	2654890107	7.577	712
13	12.166	gamma-Terpinene	338598016	0.966	719
14	13.36	alpha-Terpinolene	91988314	0.263	749
15	14.214	Linalool	100804203	0.288	770
16	15.853	Allocimene	153590024	0.438	809
17	19.176	p-Allylanisole	23695362067	67.623	876
18	21.66	Cuminic aldehyde	389019146	1.110	924
19	21.698	Carvone	16960150	0.048	925
20	21.855	p-Allylphenol	19717087	0.056	928
21	21.947	Anisaldehyde	43431241	0.124	930
22	22.365	Geranial	41184671	0.118	938
23	22.69	Nerol	8812108	0.025	944
24	22.929	Bornyl acetate	181055212	0.517	948
25	23.132	Benzenemethanol, .alpha. – 2-propenyl-	65325726	0.186	952
26	24.827	alpha-Terpinene	33391272	0.095	984
27	25.265	Cyclohexylmorpholine	27948634	0.080	992
28	25.711	Eugenol	103221898	0.295	1001
29	26.362	alpha-Copaene	33352538	0.095	1013
30	26.698	Cinnamic acid methyl ester	55253155	0.158	1019
31	27.007	beta-Elemene	10571314	0.030	1025
32	27.687	Methyleugenol	269269779	0.768	1038
33	28.119	trans-Caryophyllene	55810389	0.159	1047
34	28.742	alpha-Bergamotene	14295464	0.041	1058
35	29.15	Cinnamyl acetate	4956966	0.014	1066
36	29.456	alpha-Humulene	7004369	0.020	1072
37	29.582	trans-beta-Farnesene	10123459	0.029	1074
38	30.095	2(3H)-Furanone, 5-hexyldihydro-	18753954	0.054	1084
39	30.314	Acoradiene	89347554	0.255	1088
40	30.561	Germacrene D	23644363	0.067	1093
41	31.131	cis-*trans*-alpha-Farnesene	39918948	0.114	1104
42	31.242	peri-Ethylenenaphthalene	83391636	0.238	1106
43	31.617	trans-*trans*-alpha-Farnesene	18456048	0.053	1114
44	32.246	beta-Sesquiphellandrene	43067473	0.123	1126
45	34.245	3-Methoxycinnamaldehyde	522580078	1.491	1166
46	34.537	Spathulenol	99969871	0.285	1172
47	39.807	7-Methoxycoumarin	28349494	0.081	1278
48	45.615	Nonadecane	15292173	0.044	1411

[a] Retention time.
[b] Retention index.

Batches of larvae (containing 25 larvae) were transferred by means of strainers to each test cup. After 24 h exposure, the number of death was noted, moribund larvae, incapable of rising to the surface, were counted and added to dead larvae. Larvae that were pupated during the test period were also negated. For each concentration, such as TEO or TNE, the test repeated 12 times, in 3 different replicates. Two control groups were also taken in each replicate and 1 mL IPA was added to each samples.

The test were repeated, if more than 10% of the control larvae pupated or mortality was over 20% in the experiment. If the mortality of control was between 5% and 20%, the mortalities of treated groups were corrected by Abbott's formula:

$$\text{Mortality (\%)} = ((X - Y)/X)\ (100)$$

Where X, is% survival in the untreated control and Y, is% survival in the treated sample (WHO, 2005). Results from all larvicidal bioassay replicates were subjected to SPSS software v.22 for probit analysis to