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Nanoemulsions formation from essential oil of *Thymus capitatus* and *Majorana hortensis* and their use in weed control

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ABSTRACT

Essential oil formulation of *Thymus capitatus* L. (wild and cultivated thyme) and *Majorana hortensis* L. (marjoram) were investigated for allelopathic activity against *Convolvulus arvensis* and *Setaria viridis* seeds and seedling growth. Thymol, camphor, carvacrol, thujone, ä-terpinene, borneol, p-cymene, carvacrol, 1,8-cineole, caryophyllene oxide, á-humulene á-pinene, borneol, â-pinene, caryophyllene, caryophyllene oxide, linalol and phellandrene were detected by GC-Ms analysis from the oil contents. Macroemulsion (Mac-E) and nanoemulsion (Nano-E) were formulated from oil by adding co-surfactant and surfactant. These formulations were subjected to stability stresses and were tested for herbicidal activity against *C. arvensis* seeds germination and seedling growth. Nanoemulsion had particle size of 5.3, 12.0 and 22.1 nm for *M. hortensis*, *T. capitatus* wild and *T. capitatus* cultivated, respectively. Depending on ED_{50} *M. hortensis* (oils, Mac-E and Nano-E) exhibited strong allelopathic activity on *C. arvensis*, however, the lowest activity was achieved from *T. capitatus* cultivated followed by *T. capitatus* wild. The Nano-E exhibited pronounced post-emergence properties on (5-7 leaves stage) than others formulation on *C. arvensis* in greenhouse conditions.

Key words: Allelopathy, Constituents, Macro emulsion, Nanoemulsion, *Majoran*a *hortensis* L., *Thymus capitatus* L., Volatile oils

Thyme, *Thymus capitatus* L. (wild and cultivated) and marjoram (*Majorana hortensis* L.) belonging to Family Lamiaceae are native to Mediterranean countries (Harley *et al.* 2004), which are used for medicinal and spice purposes (Morales 2002). The major essential oil constituents of *Majorana hortensis* have been reported as cis-sabinene hydrate (37.05–47.49%), terpinen-4-ol (14.45–16.22%) and trans-sabinene hydrate (5.81–6.97%) (Verma, *et al.* 2010). Other components detected in lower amounts in all oil samples were sabinene and p-cymene (up to 7.4% and 13.9% in autumn), and á-terpinene (up to 13.3% in summer) in *Majorana hortensis* (Soliman *et al.* 2009). The volatile extract compositions of marjoram estimated by GC–MS were terpinen-4-ol, ã-terpinene, trans-sabinene hydrate, linalool, trans-sabinene hydrate acetate, thujanol, terpinolene and thymol (El-Ghorab *et al.* 2004).

Bindweed (*Convolvulus arvensis*) is known to reduce crop value and provide a breeding site for insects attacking adjacent crops (Tamaki *et al.* 1975) and serves as an alternative host for plant viruses. The control of bindweed is difficult because of its vigorous regeneration capacity. The phytotoxic effects of aromatic plants volatile oils have increased the interest in exploring for potential weed management (Dayan *et al.* 2009). Lamiaceae, Myrtaceae, Asteraceae and Anacardiaceae are the most cited plant families with promising essential oils used as herbicide. Individual compounds present in these mixtures with high activity include á-pinene, limonene, 1, 8-cineole, carvacrol, camphor and thymol (Amri *et al.* 2013). The allelopathic effects of thyme essential oil have been tested in vitro on germination percentage (GP), hypocotyl (HL) and radicle (RL) length of *Citrullus colocynthis* L., *Lepidium sativum* L. and *Trigonella foenum-graecum* L. at 20 mg/l (Soliman 2013). In the present study, phytotoxicity of essential oils extracted from wild thyme, cultivated thyme and *Majorana hortensis* and their formulated macroemulsion (Mac-E) and nanoemulation (Nano-E) were investigated.

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MATRIALS AND METHODES

Characterization of plant soil and water: The wild *Thymus capitatus* (thyme) shoots were collected from Wadi Habbes, about 18 km of Matrouh city. Cutting (shoots 6-8 cm) of half-ripe wood were taken on March 2014 for cultivation in greenhouse. The seedling of *Majorana hortensis* (marjoram) were obtained from the Experimental Farm of Medicinal and Aromatic plants, Faculty of Pharmacy, Cairo

University, Egypt. Both seedlings of thyme and marjoram were planted in May and April 2014 in Matrouh Research Station, respectively. The field was incorporated with sheep manure 15 m^3/Feddan and calcium superphosphate 30 kg (P_2O_4)/Feddan before planting. The physical and chemical properties of soil and water of experimental station and sheep manure are presented (Table 1).

Sampling and determination of volatile oil: The shoots of both wild and cultivated thyme and marjoram plants were harvest by cutting in April 2015. Essential oil of air dried canopy samples was extracted by hydro distillation and was subjected to GC-Ms analysis at Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications: a Trace GC Ultra Gas Chromatographs (Thermo Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed; 60-200 °C (8/min.), injection temperature 150 and 220 °C (20 min.). Helium was the carrier gas with flow rate of 1 ml/min; detection was by (EI, 70 eV. Interface 230). Qualitative identification of the oil constituents was carried out by comparing the retention times and mass fragmentation with computer matching of authentic samples and of published data.

Emulsions preparation: Macro-emulsions (Mac-E) of essential oils were prepared by mixing oil volume (2.5%) with two volumes of surfactant [polyethylene glycol dioleate (nonionic surfactant) + toximol (ionic surfactant)] and water. After that, the mixture was vortex several times and visually evaluated at room temperature, then subjected for stability testing and specifications. Nanoemulations (Nano-E) were prepared by mixing one volume of oil (2.5%) with one volume of chloroform (co-surfactant) and ten volume of surfactant (Tween 20 plus Tween 80) and vortex several time with adding deionized water to the final volume. The obtained formulation was subjected to sonication in ultrasonic bath for two hours and stored at laboratory condition for testing.

Physical and chemical tests of prepared formulation: Stability was studied according to Sinha *et al.* (2015) for thermal and mechanical stress. Formulations (5 ml) was stored at elevated temperature (40 ± 2 °C, 25 ± 2 °C and 4 ± 2 °C) and centrifuged at 2000 rpm for different intervals (20,40,60 and 120 min.) then, visually inspected (phase separation). The pH values of the samples were measured by a pH meter (JENCO, 6010N, USA), at 25 ± 2 °C Electrical conductivity by EC Meter (Orion 150 A of Thermo Electron Corporation, USA) at ambient temperature. The formulation transparency was determined by measuring percentage transmittance at scan mode with purified water taken as blank using UV-VIS spectrophotometers (Thermo, Nicolet evolution 300) according to Date and Nagarsenker (2008). Droplets size of formulations was measured by puting in droplet size analyzer (PSS NICOMP, N3000, Dynamic light scattering, Particle Size Systems, Inc. Santa Barbara, Calif., USA) without diluting the formulation.

Pre-emergence activity under laboratory conditions: *Convolvulus arvensis* and *Staria viridis* (Foxtail, Poaceae) were collected from wheat crop in El-Frafra Oasis, Egypt. Bioassay of *T. capitatus* (wild and cultivation) and *M. hortensis* oils were carried out on *C. arvensis* and *S. viridis*. Seeds were surface-

Table 1. Physical and chemical analysis of soil and water at experiment site

Particles size distribution of the experimental soil

	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil texture
Wadi Habbes	9.87	72.90	16.10	1.13	Sandy loamy
Matrouh	8.83	58.88	28.11	4.18	Sandy loamy

Chemical properties of the experimental soil

	pH	E.C. (dSm)	Soluble cations (meq./l)				Soluble anions (meq./l)			
			K^+	Na^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
Wadi habbes	7.53	4.16	0.20	4.10	1.00	2.50	-	1.75	3.45	2.60
Matrouh	7.30	4.99	2.7	5.9	4.7	36.6	-	3.00	37.10	9.80

Irrigation water analysis

	pH	E.C. (dSm)	Soluble cations (meq./l)				Soluble anions (meq./l)			
			K^+	Na^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
Matrouh	7.01	55.9	0.03	0.48	0.24	0.15	-	0.11	0.60	0.19

Sheep manure analysis

pH	Organic carbon %	N %	C/N ration
7.50	20.10	1.50	13.56

sterilized with 0.3% sodium hypochlorite and washed many times with sterile waters. Ten seeds were placed on filter paper in a sterilized Petridish (9 cm diameter). The concentrations of volatile oil of *M. hortensis* were; 0, 1.0, 2.0, 4.0, 8.0 µl/ml for *C. arvensis* and 0.5, 1, 2.5,5 µl/ml for *S. viridis*, while, *T. capitates* cultivated and *T. capitatus* wild were evaluated with 0, 5, 10, 20, 40 µl/ml concentrations. However, the formulated Mac-E and Nano-E were evaluated at 0.1, 0.2, 0.4, 0.8 µl/ml (*M. hortensis*) and 1.0, 2.5, 5.0, 10.0 µl/ml (*T. capitates* wild and cultivated) on *C. arvensis* seeds and seedling growth. Treated Petri-dishes with oil, Mac-E and Nano-E were sealed with parafilm and kept at 25 ± 2 °C. Seed germination and seedling growth (radical and hypocotyl) were measured after 7 days.

Post-emergence activity of prepared formulations: Bindweed *C. arvensis* seeds were sown in plastic pots filled with sandy soil and watered two times weekly in the greenhouse. The desired concentration of 10 ml solution was sprayed with the help of glass hand sprayer on each petridish. Survival of seedlings and dry weight were recorded after one week of spraying.

Statistical analysis: Treatment means were compared by Duncan and LSD test at 5% level of probability (Snedecor and Cochran 1990) and the effective dose (ED_{50} values) were calculated by signing the point in a semi-log graph paper. Finally, the reduction percentage was obtained from the below equations. R%=C-T/C*100 [C=Control] [T=Treatment].

RESULTS AND DISCUSSION

Chemical composition of essential oils: Content of essential oils from the dry herb of *T. capitatus* wild, *T. capitatus* cultivated and *M. hortensis* were obtained by 1.85, 0.8 and 2.1% (v/w), respectively. The major compounds identified in *T. capitatus* wild were thymol (34.40%) and á-terpinene (14.67%), followed by1-4-terpineol (9.65%). Compounds of *T. capitatus* cultivated were thymol (23.74%), o-cymene (18.74%), trans caryophyllene (9.82%) followed by á-terpinene (9.13%). Major constituents in oils of *M. hortensis* were trans-sabinene hydrate (19.23%), cis-sabinene hydrate (17.55), terpinen-4-ol (15.66%) followed by á-terpinene (12.06%) as determined by GC/MS (Table 2).

Formulated emulsions: Formulated Nano-E exposed to ultra sonication for 2 hours had minimum dispersion. It was stabilized in the final stage due to decreasing of the hydrodynamic droplet diameters which improved the dispersion quality.

Table 2. GC-mass analysis of *T. capitatus*, *T. capitatus* cultivated and *M. hortensis* essential oils

IUPAC name	*T. capitatus* Wild % (V/W)	*T. capitatus* cultivated % (V/W)	*M. hortensis* % (V/W)	Molecular weight
Thujene	1.13	0.68	-	136
α – pinene	0.90	1.92	0.46	136
Camphene	0.92	1.77	-	136
Sabinene	4.47	-	10.24	136
1-octen-3-ol	0.31	-	-	128
α –myrcene	1.61	1.19	1.03	136
3-octanol	0.40	-	-	130
Phellandrene	0.33	-	3.18	136
α –terpinene	14.67	9.13	12.06	136
o-cymene	5.50	18.74	1.65	134
d-limonene	0.89	-	-	136
trans-sabinene hydrate	1.61	-	19.23	155
α –terpinolene	1.86	-	0.71	154
L- linalool	0.78	3.71	-	154
Cis-sabinene hydrate	4.09	-	17.55	154
1-Terpineol	1.06	-	0.61	154
Borneol	5.00	1.59	-	154
1-4-terpineol	9.65	1.20	-	154
α –terpineol	2.07	-	-	154
Thymol	34.40	23.74	-	150
Iso-thymol	0.34	-	-	150
Carvacryl acetate	3.39	-	-	150
Trans-caryophyllene	3.41	9.82	4.24	204
4-isopropylidene	0.57	-	-	204
Caryophllene oxide	0.63	4.12	-	220
Geraniol	-	1.47	-	154
Geraniol isovalerate	-	3.35	-	138
α –citronellol	-	1.68	-	156
camphor	-	3.05	-	152
1,8-cineole	-	3.87	5.09	154
Humulene	-	0.59	-	204
Iso caryophyllene	-	1.71	-	204
α –cadinol	-	1.36	-	222
pogostol	-	0.49	-	236
1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl	-	3.83	-	122
Terpinen-4-ol	-	-	15.66	154
Linalyl acetate	-	-	2.88	138
Geranyl actate	-	-	1.81	182

Mac-E had higher pH than 7, while *T. capitatus* cultivated had maximum pH value. The EC value ranged from 0.184 to 0.142 m mols/cm (Mac-E) and 0.125 to 0.112 m mols/cm (Nano-E). There was less variation in EC value within Nano-E and Mac-E. These values explained the high steady state of establishing water continuous phase (Table 3).

The nanoemulsions (Nano-E) was characterized with excellent transparency and higher (UV/VIS) transmission percentage as compared with Mac-E, and had milky color and lower transmission percentage ranged from 92, 90 and 88.4% for *T. capitatus* wild, *T. capitatus* cultivated and *M. hortensis* for Nano-E, respectively. In formulated Mac-E, transmittance percentage was 0.71, 11.2 and 0.55% for *T. capitatus* wild and *T. capitatus* cultivated, respectively. On the other hand, λ_{max} in formulated Nano-E was recorded by 222, 247, 243,

Table 3. Characterization of the formulated Mac-E and Nano-E

	Emulsion type	Nano particles (nm)	pH	λ_{max}	EC (m mols/cm)	Transmittance
T. capitatus wild	Macro-E	1468.6±0.54	7.7±0.1	222, 247, 243, 253 260,	0.165±0.1	0.71%±0.53
	Nano –E	12±0.525	6.61± 0.11	272, 282	0.118±0.1	92%±1.3
T. capitatus cultivated	Macro-E	15840±1.01	7.71±0.13	225,235,262,	0.184±0.1	11.2%±2.6
	Nano –E	22.4±0.357	6.49±0.05	266,273,383, 479,486	0.125±0.1	90%±1
M. hortensis	Mac-E	90.7± 0.63	7.59±0.1	248, 262, 272, 486,	0.142±0.1	0.55%±0.5
	Nano –E	5.3± 0.680	5.64±0.15	568, 575, 586	0.112±0.1	88.43%±2.6

253 260, 272, 282 (*T. capitatus* wild), 225,235,262, 266, 273, 383,479,486 (*T. capitatus* cultivated) and 248, 262, 272, 486, 568, 575, 586 (*M. hortensis*) (Table 3).

Particle size of the formulated emulsion: Particle size is the important determiner which influences the characterization of *M. hortensis*, *T. capitatus* wild and *T. capitatus* cultivated formulations. The particle size of Nano-E was measured by 5.3, 12.0 and 22.1 nm for *M. hortensis*, *T. capitatus* wild and *T. capitatus* cultivated, respectively. The particle size of Mac-E was 90.7, 15840 and 1468.6 nm for *M. hortensis*, *T. capitatus* wild and cultivated, respectively. It revealed that *M. hortensis* had nano size only in the presence of its milky color and without any sonication exposure while other formulation appeared in macrosize (Fig. 1).

Mechanical stability of the formulation: The prepared emulsions (Mac-E and Nano-E) were subjected to the stability assessment of centrifugation at 2000 rpm during 20, 40, 60 and 120 min. (Table 4), while thermal stability was implemented at 4±1, 25±1 and 50±1 °C after 1, 5, 10, 20, 30 days. The results showed the stability of Nano-E toward precipitation after 20, 40, 60 minutes centrifugation. However, higher trace precipitation was recorded in *T. capitatus* cultivated at 120 min, followed with *T. capitatus* wild, while it was lowest in *M. hortensis* (Fig. 1). Thermal stability in Mac-E and Nano-E was stable at 4±1, 25±1 and 50±1 °C for thirty days (Table 5), while at laboratory temperature, stability exceeded upto five month without any aggregation and separation.

Biological activity against weeds: The volatile oil at lowest and highest concentration, completely suppressed weeds germination (Table 6). Based on EC_{50}, it had more inhibitory effects upon shoot length of *C. arvensis* and *S. viridis* compared to root length and germination. Phytotoxicity on *C. arvensis* seed germination and seedling growth showed that most sensitive weed parts were root length and shoot length for Mac-E and Nano-E, respectively. The inhibitory effect of *M. hortensis* was highest against

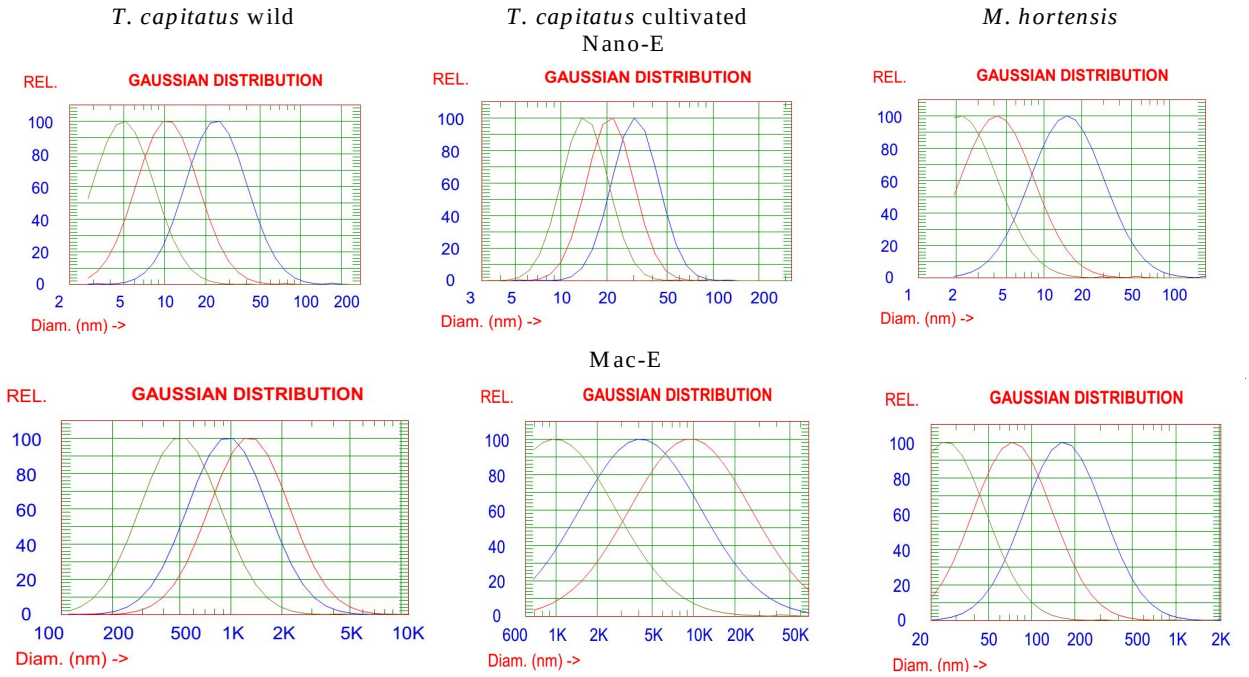

Fig. 1. Emulsions distribution analysis (particle size)

Table 4. Separated phase of emulsions after centrifugation at 2000 rpm

Centrifugation Time min.	*T. capitatus* wild		*T. capitatus* cultivated		*M. hortensis*	
	Mac-E	Nano-E	Mac-E	Nano-E	Mac-E	Nano-E
20	-	-	-	-	-	-
40	-	-	-	-	-	-
60	-	-	-	-	-	-
120	2.25	0.5	4.5%	1.2%	1.5%	0.5

Table 5. Separated phase after thermal exposure of formulated volatile oils emulsion

Temp. °C	days	*T. capitatus* wild		*T. capitatus* cultivated		*M. hortensis*	
		Mac-E	Nano-E	Mac-E	Nano-E	Mac-E	Nano-E
4 ±1,	1	-	-	-	-	-	-
25±1	5	-	-	-	-	-	-
40 ±1	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-

C. arvensis and *S. viridis* compared to *T. capitatus* wild and *T. capitatus* cultivated. It was concluded that concentration and emulsion types played considerable role in achieving complete weed suppression. Nano-E exhibited highest inhibition followed by Mac-E. Nevertheless, volatile oils exhibited lowest suppression on germination and growth. This indicates that volatile oils contained growth inhibiting allelochemicals but their effect depended on type of oils and formulation.

Post-emergence herbicidal activities of formulated Mac-E and Nano-E against *C. arvensis* have been shown in Fig. 2. *M. hortensis* Mac-E at 5 and 10 µg/ml concentration caused inhibition of 73.7, 81.7 and 54.3, 60.6 for fresh and dry weight, respectively. Compared with the control, Nano-E caused significant inhibition at 5 and 10 µg/ml concentration amounted to 63.6, 82.9% and 75.3, 62.6% for fresh and dry weight, respectively. *T. capitatus* wild showed its post-emergence activity on total biomass of fresh and dry weight over control by 46.9,70.4% and 22.0,41.0% (Mac-E); 50.5, 77.5% and 37.6, 50.3% (Nano-E), respectively. *T. capitatus* cultivated at 5 and 10 µg/ml caused inhibition in *C. arvensis* fresh and dry weight by18.3,49.9% and 12.8,38.9 (Mac-E); 29.9,64.1% and 20.15, 41.9% (Nano-E), respectively than the control.

The result revealed that Nano-E of *M. hortensis* caused inhibition by 49.0, 59.29% (fresh weight) and 26.4, 38.3% (dry weight) at 5 and 10 µg/ml of *C. arvensis*, respectively. *T. capitatus* wild exhibited 59.54, 65.8% (fresh weight) and 16.38, 38.6% (dry weight) reduction and finally *T. capitatus* cultivated showed inhibition by 45.0, 57.8% (fresh weigh), 17.0 and 33.4% (dry weigh) of *C. arvensis* at 5 and 10 µg/ml, respectively in comparison to control.

The herbicidal activity of essential oils revealed that *M. hortensis* had the greatest inhibitory effects followed by *T. capitatus* wild and *T. capitatus* cultivated on both *C. arvensis* and *S. viridis*. The formulated Mac-E and Nano-E showed post-emergence activity in the early stage of *C. arvensis* (2-3 leaves) on total biomass of fresh and dry weight while Nano-E showed inhibitary effect on *C. arvensis* at 5-7 leaves stage (Table 7). The nano formulation exhibited faster release of active ingredients after application on weed leaves surface and weed seeds (under laboratory) due to pronounced surface properties. Selecting the suitable mixture (type and amount) from oils, water and surfactants macro-emulsion (Mac-E) plus co-surfactant for nano-emulsion (Nano-E) were the critical points that control the prepared formulation suitability to deliver their function. The formulated nanoemulsions size were found close to the standard Nano-E between 1 to 100 nm (Casanova *et al.* (2005) to deliver their

Table 6. Dos response relationship of volatile oils and emulsions (ED_{50}) µg/ml

	Volatile oils *C.arvensis*	Volatile oils *S. viridis*	Mac-E *C. arvensis*	Nano-E *C. arvensis*
T. capitatus wild				
Shoot length	5.764±0.11	7.133±1.31	2.314±0.41	2.514±0.21
Root length	6.115±0.17	10.954±1. 32	2.116±0.51	2.623±0.27
Germination	8.100±0.10	8.215±1.34	2.490±0.34	2.616±0.21
T. capitatus cultivated				
Shoot length	8.516±0.20	7.436±0.44	3.855±0.12	4.013±0.34
Root length	8.632±0.18	12.713±0.12	2.463±0.18	2.577±0.21
Germination	10.211±0.42	8.891±0.87	3.143±0.16	3.465±0.22
M. hortensis				
Shoot length	1.693±0.27	0.863±0.11	0.286±0.08	0.324±0.06
Root length	1.745±0.15	1.788±0.02	0.196±0.09	0.367±0.04
Germination	1.713±0.21	1.536±0.1	0.573±0.12	0.392±0.05

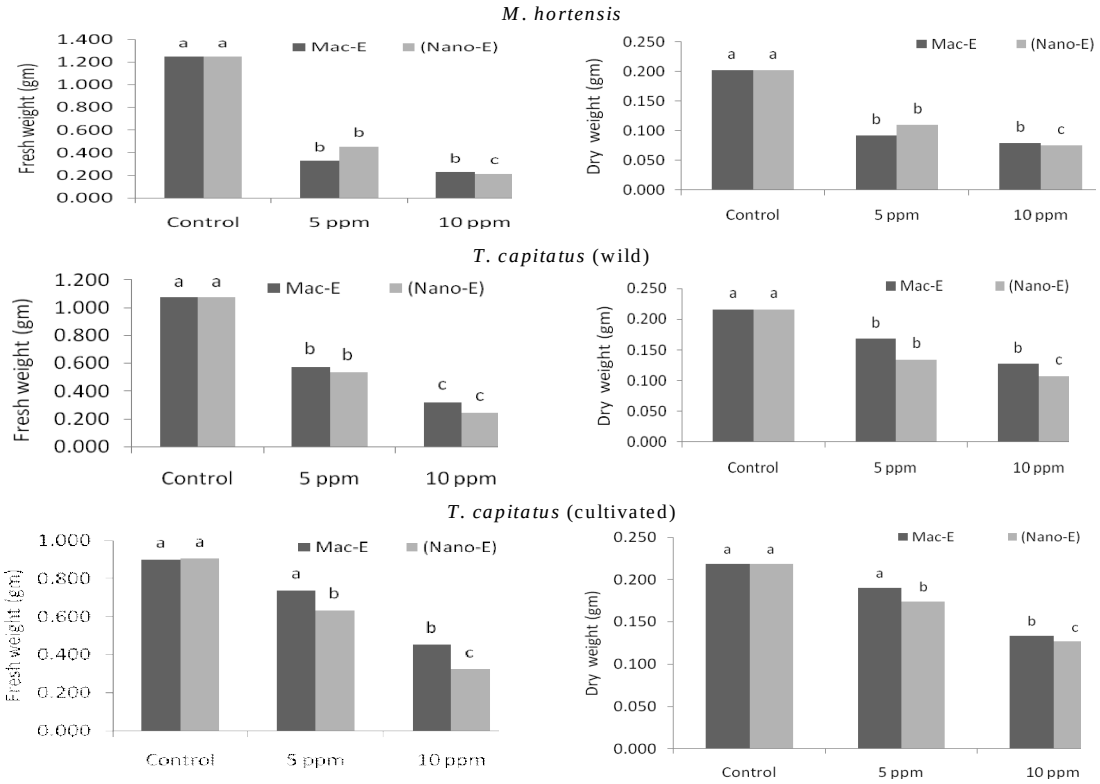

Fig. 2. Post-emergence activity of macroemulsion (Macro-E) and nanoemulsion (Nano-E) on fresh and dry weight of *C. arvensis* (2-4 leaves stage)

Table 7. Post-emergence activity of nano-E on *C. arvensis* at 5-7 leaves stage fresh and dry weight

Treatment	Fresh weight (gm)			Dry weight (gm)		
	T. capitatus wild	*T. capitatus* cultivated	*M. hortensis*	*T. capitatus* wild	*T. capitatus* cultivated	*M. hortensis*
Control	1.13	1.02	1.02	0.26	0.25	0.26
5 ppm	0.46	0.55	0.52	0.22	0.23	0.19
10 ppm	0.38	0.43	0.42	0.16	0.16	0.16
LSD (0.05)						
Plant Oils	0.26	0.27	0.22	0.12	0.10	0.13
Conc.	0.37	0.42	0.35	0.13	0.11	0.13
Interactions	0.52	0.62	0.55	0.141	0.09	0.14

function. Whereas, Nano-E size was not exceeded from 22 nm, while in *M. hortensis* Mac-E was less than 100 nm. These results supported by Owolade *et al.* (2008). Nanoparticles loaded with garlic essential oil were effective against *Tribolium castaneum* Herbst (Yang *et al*. 2009). The phytotoxicity of volatile constituents in *M. hortensis*, *T. capitatus* wild and *T. capitatus* cultivated was previously reported due to ä-terpinene, p-cymene, carvacrol, 1,8-cineole (Angelini *et al.* 2003, Grosso *et al.* 2010), caryophyllene oxide (Macini *et al.* 2009a), thymol, p-cymene, ä-terpinene (Almeida *et al.* 2010; Grosso *et al.* 2010) á-humulene (Tellez *et al.* 2000), á-pinene, 1,8-cineole, borneol (Angelini *et al.* 2003), carvacrol, p-cymene (Kordali *et al.* 2008), â-pinene (Almeida *et al.* 2010), 1,8-cineole (Mucciarelli *et al.* 2001), (Z)- caryophyllene, caryophyllene oxide (De Martino *et al.* 2010), (Z)-caryophyllene, caryophyllene oxide (De Martino *et al.* 2010), linalol, 1.8-cineole, â-phellandrene, á-pinene (Almeida *et al*. 2010), linalol, 1.8-cineole, â-phellandrene, á-pinene (Almeida *et al.* 2010), cis-thujone, 1,8-cineole, camphor (De Martino *et al.* 2010), camphor, 1,8-cineole, borneol (Kordali *et al.* 2008, Salmaci *et al.* 2007), thymol (Marandino *et al.* 2011) and carvacrol, linalol (Almeida *et al.* 2010). A-pinene, limonene, 1.8.cineole and camphor affected the, respiratory activity of mithochondria of maize and soybean hypocotyl axes and á-pinene has been shown to be most active among the all tested monoterpenes (Abrahim *et al.* 2003). 1,8-cineole inhibited the germination, speed of germination, seedling growth, chlorophyll content and respiratory

activity of *Ageratum conyzoides* (Singh *el al.* 2002). The present results showed potential useful role of essential oils for herbicidal constituents. Formulated emulsions may be a good alternative means to synthetic herbicides in weed control.

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