*J. Plant Prot. and Path., Mansoura Univ., Vol.7 (10), 641– 648, 2016*

# Emulsions and Nanoemulsions Formation from Wild and Cultivated Thyme and Marjoram Essential Oils for Weeds Control

**Balah, M. A. [1] and W. M. Abd El Azim [2]**
**[1]Plant Protection Dept., Desert Res. Center, El-Mataria, Cairo, Egypt**
**[1]Email: mbaziz1974@gmail.com**
**[2] Medicinal Plant Dept., Desert Res. Center, El-Mataria, Cairo, Egypt**
**[2]Email: dr.w_elzearly@yahoo.com**



## ABSTRACT

Essential oils and its formulation macroemulsion (Mac-E) and nanoemulsion (Nano-E) of *Thymus capitatus* L. (wild and cultivated thyme) and *Majorana hortensis* L. (marjoram) herbicidal activity were investigated against *Convolvulus arvensis* and *Setaria viridis* seeds and seedlings growth. The suitable systems from oils, water and surfactants for macroemulsion (Mac-E), with addition to co-surfactant for nanoemulsion (Nano-E) were chosen and optimizing based on the stability tests and biological properties. Nanoemulsions seems transparent or translucent and had particle size reached 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) had been exhibited strong herbicidal activity on *C. arvensis*, however, the lowest activity was achieved from *T. capitatus* cultivated followed by *T. capitatus* wild. The Nano-E exhibited post emergence properties pronounced than others formulation on *C. arvensis* at 5-7 leaves stage under the greenhouse. The herbicidal activity based on types of oils, concentration and formulations as well as the weed stage. These result showed that nano-formulations can be contribute in perennial weeds control programs.

**Keywords**: Herbicidal activity, Macroemulsion, Nanoemulsion, Volatile oils, *Thymus capitatus*, *Majorana hortensis*.

## INTRODUCTION

Thyme *Thymus capitatus* L. (wild and cultivated) and marjoram *Majorana hortensis* L. with others species belonging to Family Lamiaceae are native to Mediterranean countries (Harley *et al*. 2004). *Thymus sp* (thyme) is used for medicinal and spice purposes (Morales, 2002). This plants species are scattered around the world, but particularly gathered in the Mediterranean areas (Ghahreman, 1994). The major *Majorana hortensis* essential oil constituents, *viz*., cis-sabinene hydrate (37.05–47.49 %), terpinen-4-ol (14.45–16.22 %) and trans-sabinene hydrate (5.81–6.97 %) showed considerable variation in their concentrations in relation to crop age (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 α-terpinene (up to 13.3% in summer) in *Majorana hortensis* (Soliman *et al*. 2009). The volatile extract compositions of marjoram (*Majorana hortensis*) leaves were estimated by GC–MS; 26 components were identified. The major compounds in both extracts were terpinen-4-ol, γ-terpinene, trans-sabinene hydrate, linalool, trans-sabinene hydrate acetate, thujanol, terpinolene and thymol (El-Ghorab *et al*. 2004).

Bindweeds (*Convolvulus arvensis*) reduce the 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 (Feldman and Gracia, 1977). The control of bindweed is difficult because of its vigorous regeneration capacity. Some control is obtained with chemical herbicides but not eradication (Westra *et al*. 1992). The phytotoxic effects of aromatic plants volatile oils have increased the interest in exploring for potential weed management (Dayan *et al*. 2009). Among the plant families with promising essential oils used as herbicide, Lamiaceae, Myrtaceae, Asteraceae and Anacardiaceae are the most cited. Individual compounds present in these mixtures with high activity include α-pinene, limonene, 1,8-cineole, carvacrol, camphor and thymol (Amri *et al*. 2013). The allelopathic effects of thyme essential oil were 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 the maximum oil concentration (Soliman, 2013).

In this study, wild thyme, cultivated thyme and *Majorana hortensis* essential oils and their formulations herbicidal activities were evaluated. The prepared macroemulsion (Mac-E) and nanoemulsion (Nano-E) formulations characterizing and its utility for weed control were investigated.

## MATERIALS AND METHODES

### Collection and cultivation

The wild *Thymus capitatus* (thyme) shoots collected from Wadi Habbes about 18 km of Matrouh city and identified by plant specialist according to (Boulos, 2009). Whereas cutting (young shoots 6-8) cm and half-ripe wood were taken in March 2014 for cultivation in the greenhouse. The seedling of *Majorana hortensis* (marjoram) were obtained from the Experimental Farm of Medicinal and Aromatic plants, Faculty of Pharmacy, Cairo University. Both seedlings of thyme and marjoram were cultivated during May and April 2014 in Matrouh Research Station in the open field respectively, after land preparation as follow; sheep manure $15m^3$/Feddan with addition calcium superphosphate at rate of 30kg ($P_2O_4$)/Fed. were mixed with top soil before planting. The physical and chemical analysis of soil of Wadi Habbes & Matrouh Experimental Station, irrigation water in experimental station and sheep manure are presented in Tables 1 (A, B, C, D).

**Table 1. Physical and chemical analysis of soil and water.**

**(A): Particles size distribution of the experimental soil.**

| Experimental | 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 |

**(B). Chemical properties of the experimental soil.**

| Experimental | pH | E.C. ($dS\,m^{-1}$) | 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 |

**(C) Irrigation water analysis.**

| Experimental | pH | E.C. ($dS\,m^{-1}$) | Soluble cations (meq./l) | | | | Soluble anions (meq./l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $K^+$ | $Na^+$ | $Mg^{++}$ | $Ca^{++}$ | $CO_3^{--}$ | $HCO_3^-$ | $Cl^-$ | $SO_4^{--}$ |
| Matrouh | 7.01 | 5.59 | 0.03 | 0.48 | 0.24 | 0.15 | - | 0.11 | 0.60 | 0.19 |

**(D)Sheep manure analysis.**

| pH | O.C. % | N % | C/N ration |
|---|---|---|---|
| 7.50 | 20.10 | 1.50 | 13.56 |

**O.C. organic carbon**

**Extraction and determination of essential oils.**

The shoots of both wild & cultivated Thyme and Marjoram plants were harvest by cutting in April 2015. Essential oil of air dried canopy samples was extracted by hydro distillation (Grosso *et al*. 2010) and subjected to GC-Ms analysis at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: 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 $min^{-1}$), injection temperature 150 ºC 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 with published data (Adams, 2001).

**Emulsions preparation**

Macro-emulsions (Mac-E) of essential oils were prepared by mixing oils volume (2.5%) with two volumes of surfactant [polyethylene glycol dioleate (non ionic 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.

The 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 times with adding deionized water to the final volume. The obtained formulation subject to sonication in ultrasonic bath for two hours and stored at laboratory condition for specifications testing (Sinha *et al*. 2015).

**Physicochemical properties of prepared emulsions**

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 and120 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 (EC Meter, Orion 150 A+, Thermo Electron Corporation, USA) of formulations was measured at ambient temperature by m mols/cm. 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 the formulation were measured by put the samples into the sample chamber of the droplet size analyzer (PSS NICOMP, N3000, Dynamic light scattering, Particle Size Systems, Inc. Santa Barbara, Calif., USA) whereas the measurement conducted without diluting formulation.

**Pre and Post-emergence activity**

The recipient species was bindweeds *Convolvulus arvensis* (Convolvulaceae) and Foxtail *Staria viridis* (Poaceae) 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*, while the prepared formulation used *C. arvensis* seeds after surface- sterilization with 0.3% sodium hypochlorite (NaClO) and washed many times with sterile waters. Ten seeds were placed on filter paper in a sterilized petri-dish (9 cm). The tested concentrations from the volatile oils 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 by 0, 5,10,20,40 µl $ml^{-1}$ against both *C. arvensis* and S. *viridis*. 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. Petri dishes were sealed with parafilm and kept in 25± 2°C and then after 7 days seed germination and seedling growth (radical and hypocotyl) were measured.

Bindweeds *C. arvensis* seeds were sown in plastic pots filled with sandy soil and watered two times weekly until treated stage under the greenhouse. Formulation sprays were applied with a glass sprayer to provide 10 ml of liquid solution to each pot with control, 5 and 10 µg $ml^{-1}$ concentrations. The survival of seedlings and dry weight were recorded after one week of spraying of three replicates.

**Statistical analysis**

Treatment means were compared by Duncan and LSD test at 5%level of probability according to

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 obtained from the below equations. R%=C-T/C*100 [C=Control] [T=Treatment].

## RESULTS AND DISCUSSION

**Chemical composition of wild and cultivated *T. capitatus* and *M. hortensis* essential oils**

The 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 α-terpinene (14.67%), followed by1-4-terpineol (9.65%). Otherwise, the mean compounds of *T. capitatus* cultivated were thymol (23.74%), o-cymene (18.74%), trans caryophyllene (9.82%) followed by α-terpinene (9.13%). The major constituents of the third oils of *M. hortensis* were trans-sabinene hydrate (19.23%), cis-sabinene hydrate (17.55), terpinen-4-ol (15.66%) followed by α-terpinene (12.06%) as determined by GC/MS (Table 2).

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

| NO | Essential oils constituents | *T. capitatus* (Wild) % | *T. capitatus* (Cultivated) % | *M. hortensis* % | Molecular weight |
|---|---|---|---|---|---|
| 1 | Thujene | 1.13 | 0.68 | - | 136 |
| 2 | α – pinene | 0.90 | 1.92 | 0.46 | 136 |
| 3 | Camphene | 0.92 | 1.77 | - | 136 |
| 4 | Sabinene | 4.47 | - | 10.24 | 136 |
| 5 | 1-octen-3-ol | 0.31 | - | - | 128 |
| 6 | α –myrcene | 1.61 | 1.19 | 1.03 | 136 |
| 7 | 3-octanol | 0.40 | - | - | 130 |
| 8 | Phellandrene | 0.33 | - | 3.18 | 136 |
| 9 | α –terpinene | 14.67 | 9.13 | 12.06 | 136 |
| 10 | o-cymene | 5.50 | 18.74 | 1.65 | 134 |
| 11 | d-limonene | 0.89 | - | - | 136 |
| 12 | trans-sabinene hydrate | 1.61 | - | 19.23 | 155 |
| 13 | α –terpinolene | 1.86 | - | 0.71 | 154 |
| 14 | L- linalool | 0.78 | 3.71 | - | 154 |
| 15 | Cis-sabinene hydrate | 4.09 | - | 17.55 | 154 |
| 16 | 1-Terpineol | 1.06 | - | 0.61 | 154 |
| 17 | Borneol | 5.00 | 1.59 | - | 154 |
| 18 | 1-4-terpineol | 9.65 | 1.20 | - | 154 |
| 19 | α –terpineol | 2.07 | - | - | 154 |
| 20 | Thymol | 34.40 | 23.74 | - | 150 |
| 21 | Iso-thymol | 0.34 | - | - | 150 |
| 22 | Carvacryl acetate | 3.39 | - | - | 150 |
| 23 | Trans-caryophyllene | 3.41 | 9.82 | 4.24 | 204 |
| 24 | 4-isopropylidene | 0.57 | - | - | 204 |
| 25 | Caryophllene oxide | 0.63 | 4.12 | - | 220 |
| 26 | Geraniol | - | 1.47 | - | 154 |
| 27 | Geraniol isovalerate | - | 3.35 | - | 138 |
| 28 | α –citronellol | - | 1.68 | - | 156 |
| 29 | camphor | - | 3.05 | - | 152 |
| 30 | 1,8-cineole | - | 3.87 | 5.09 | 154 |
| 31 | Humulene | - | 0.59 | - | 204 |
| 32 | Iso caryophyllene | - | 1.71 | - | 204 |
| 33 | α –cadinol | - | 1.36 | - | 222 |
| 34 | pogostol | - | 0.49 | - | 236 |
| 35 | 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl | - | 3.83 | - | 122 |
| 36 | Terpinen-4-ol | - | - | 15.66 | 154 |
| 37 | Linalyl acetate | - | - | 2.88 | 138 |
| 38 | Geranyl actate | - | - | 1.81 | 182 |
| | Total | 25 | 22 | 15 | |

**(-) means not detected**

**Formulated emulsions**

The essential volatile oils were formulated by selected the suitable ternary quantity systems from oils, water and surfactants (Mac-E), plus co-surfactant (Nano-E). These materials and their variable quantity were mixed by vortex and the obtained formulation tested for stability which correlated the specification and desired response biological properties of herbicidal activity against bindweeds.

The nanoemulsion systems (2.5%) were markedly studied as a function of oil type and proper amount of the given co-surfactant/ surfactant: water. Indeed, the prepared Nano-E exposed to ultrasonication for 2 hours to optimizing the obtained Nano-E specific properties and has minimum dispersion. Otherwise, many compound from co-surfactant and surfactant and their amount were tested, the suitable materials were choosing depending on their $\lambda_{max}$ and visual observation of the formed Nano-E. Therefore, after emulsification and lamellar crystalline phase were crossed, it seems clearly and stabilized in the final stage due to the decreasing of the hydrodynamic droplet diameters that improving the dispersion quality. Thereby, the final practical Nano-E characterized after the stability test for biological activity using *C. arvensis*.

The formulated macroemulsion (Mac-E) consisting of the volatile oils 2.5% as dispersed phase and 2.5% emulsifying agent which is dispersed in

external water phase 95% ratios used for the preparation, then homogenization of the coarse emulsions was achieved with homogenizer and vortex the prepared emulsion inspected visually and subject to analysis for stability tests and others specific properties.

**Characterization of formulated emulsions**

The obtained formulated emulsions were assessed by PH and EC values. It's characterized with neutral or acidic pH properties in Nano-E. Otherwise, Mac-E had PH higher than 7, while *T. capitatus* cultivated at the prepared rate achieved the 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). In fact, 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. These experiments were performed three times (Table 3).

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

| | Emulsions Type | Nano particles (nm) | PH | $\lambda_{max}$ | EC (m mols/cm) | Transmittance |
|---|---|---|---|---|---|---|
| *T. capitatus* | Macro-E | 1468.6±0.54 | 7.7±0.1 | 222, 247, 243, 253 | 0.165±0.1 | 0.71%±0.53 |
| Wild | Nano –E | 12±0.525 | 6.61± 0.11 | 260, 272, 282 | 0.118±0.1 | 92%±1.3 |
| *T. capitatus* | Macro-E | 15840±1.01 | 7.71±0.13 | 225,235,262, | 0.184±0.1 | 11.2%±2.6 |
| Cultivated | 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 |

**The transparency and Transmittance percentage**

The obtained nanoemulsions characterized by excellent transparency and higher (UV/VIS) transmission percentage as compared with Mac-E which has milky color that reflected in their lowest transmission percentage. These values reached 92, 90 and 88.43% for *T. capitatus* wild, *T. capitatus* cultivated and *M. hortensis* for Nano-E respectively. In the other hand, lowest transmittance percentage described the formulated Mac-E by 0.71, 11.2 and 0.55% for *T. capitatus* wild, *T. capitatus* cultivated and *M. hortensis* respectively. On the other hands, the formulated Nano-E had $\lambda_{max}$ recorded by 222, 247, 243, 253 260, 272, 282 for *T. capitatus* wild, 225,235,262, 266, 273, 383,479,486 for *T. capitatus* cultivated and 248, 262, 272, 486, 568, 575, 586 for *M. hortensis* as show in 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 obtained particles size of Nano-E were measured by 5.3, 12.0 and 22.4 nm for *M. hortensis*, *T. capitatus* wild and *T. capitatus* cultivated respectively. On the others side, the particle sizes of Mac-E were 90.7, 15840 and 1468.6 nm for *M. hortensis*, *T. capitatus* wild and cultivated respectively. The studies revealed that *M. hortensis* only was found in the nano size in the present of his milky color and without any sonication exposure whiles the other formulations appeared in macrosize.

**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 implemented at 4±1, 25 ±1 and 50 ±1$^{o}$C during 1, 5, 10, 20, 30 days. The results showed the stability of the obtained nanoemulsions toward precipitation after 20, 40, 60 min. centrifugation. However, trace precipitation in most prepared formulations at 120min. was recorded in *T. capitatus* cultivated higher, followed with *T. capitatus* wild and lower in *M. hortensis*. These treatments will be giving the greatest precipitation amount in Mac-E than Nano-E (Fig 1). Thermal stability study of the tested Mac-E and Nano-E indicated that these emulsions were stable at 4±1, 25±1 and 50±1 $^{o}$C for thirty days. While, these emulsion at laboratory temperature stability were exceeded five month without any aggregation and separation (Table 5).

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

| Centrifugation | *T. capitatus* (Wild) | | *T. capitatus* (Cultivated) | | *M. hortensis* | |
|---|---|---|---|---|---|---|
| Time min. | Mac-E | Nano-E | Mac-E | Nano-E | Mac-E | Nano-E |
| 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 120 | 2.25 | 0.5 | 4.5 | 1.2 | 1.5 | 0.5 |

**Table 5. Separated phase after thermal exposure of formulated emulsions by %.**

| Temp. $^{o}$C | days | *T. capitatus* (Wild) | | *T. capitatus* (Cultivated) | | *M. hortensis* | |
|---|---|---|---|---|---|---|---|
| | | Mac-E | Nano-E | Mac-E | Nano-E | Mac-E | Nano-E |
| | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 ±1, | 5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25±1 | 10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 40 ±1 | 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

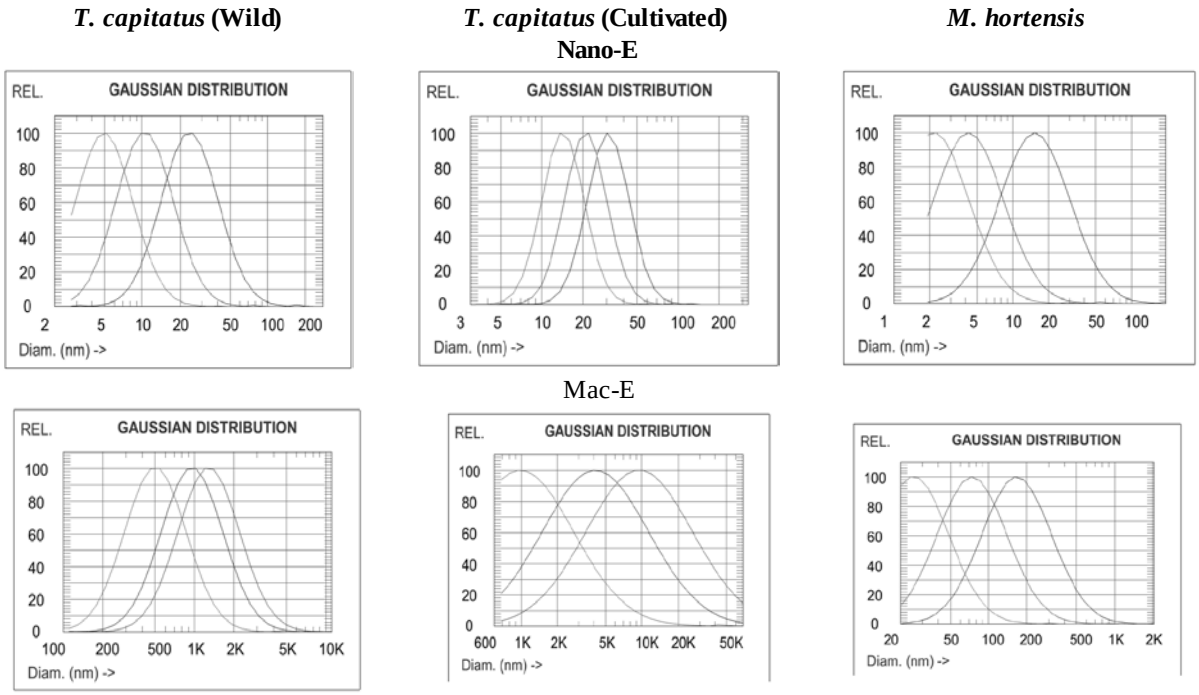


**Fig. 1: Emulsions distribution analysis (Particle Size Analysis).**

**Biological activity against weeds**

The essential oils were effective at the lowest concentrations and the highest concentration completely suppressed weeds germination. It had the greatest inhibitory effects upon shoot length of *C. arvensis* and *S. viridis* compared to root length and germination based on $EC_{50}$. The formulated emulsion phytotoxicity evaluated on *C. arvensis* seeds germination and seedling growth. The most sensitive weed parts were root length and shoot length for Mac-E and Nano-E respectively. The heights activity on *C. arvensis* seedling lengths recorded from Mac-E. While, most prepared Nano-E had slightly similar reduction activity against the tested weeds. The inhibitory effect of *M. hortensis* was extremely highest phytotoxic against both *C. arvensis* and *S. viridis* compared with *T. capitatus* wild and *T. capitatus* cultivated. It concluded that the concentration and emulsion types were played considerable role in achieving complete weeds suppression. Nano-E exhibited the greatest inhibition followed by Mac-E. Nevertheless, volatile oils exhibited a lowest suppression on the tested weed germination and growth.These indicates that the volatile oils contained growth inhibiting allelochemicals and their effects depended on the type of oils and formulation (Table 6).

**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 |

Post-emergence herbicidal activities of the formulated macroemulsion (Mac-E) and nanoemulsion (Nano-E) against *C. arvensis* are shown (Fig. 2). *M. hortensis* Mac-E at 5 and 10 µg/ml caused inhibition of 73.7, 81.7 and 54.3, 60.6 for fresh and dry weight respectively. Otherwise, Nano-E represent a significant inhibition amounted to 63.6, 82.9% and 75.3, 62.6% for fresh and dry weight respectively compared with the control. The result of *T. capitatus* wild clearly recorded its post emergence activity on total biomass fresh and dry weight by 46.9,70.4% and 22.0,41.0%(Mac-E), 50.5,77.5% and 37.6, 50.3%(Nano-E) respectively over the control. Spraying with the formulated *T. capitatus* cultivated at 5 and 10 µg/ml revealed inhibition in *C. arvensis* fresh and dry weight values 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.

*C. arvensis* seedling fresh and dry weight influenced by the formulated Nano-E under the stage of 5-7 leaves (Table, 7). The result revealed that *M. hortensis* caused inhibition by 49.0, 59.29 % (fresh weight) and 26.4, 38.3 %( dry weight), also, *T. capitatus* wild exhibited 59.54, 65.8 %( fresh weight) and 16.38, 38.6 %(dry weight) reduction, finally *T. capitatus* cultivated created inhibition value by 45.0, 57.8 %( fresh weigh), 17.0 and 33.4 %(dry weigh) compared to the control.

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

| Treatments | *T. capitatus* (Wild) | *T. capitatus* (Cultivated) Fresh weight (gm) | *M. hortensis* | *T. capitatus* (Wild) | *T. capitatus* (Cultivated) Dry weight (gm) | *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 |

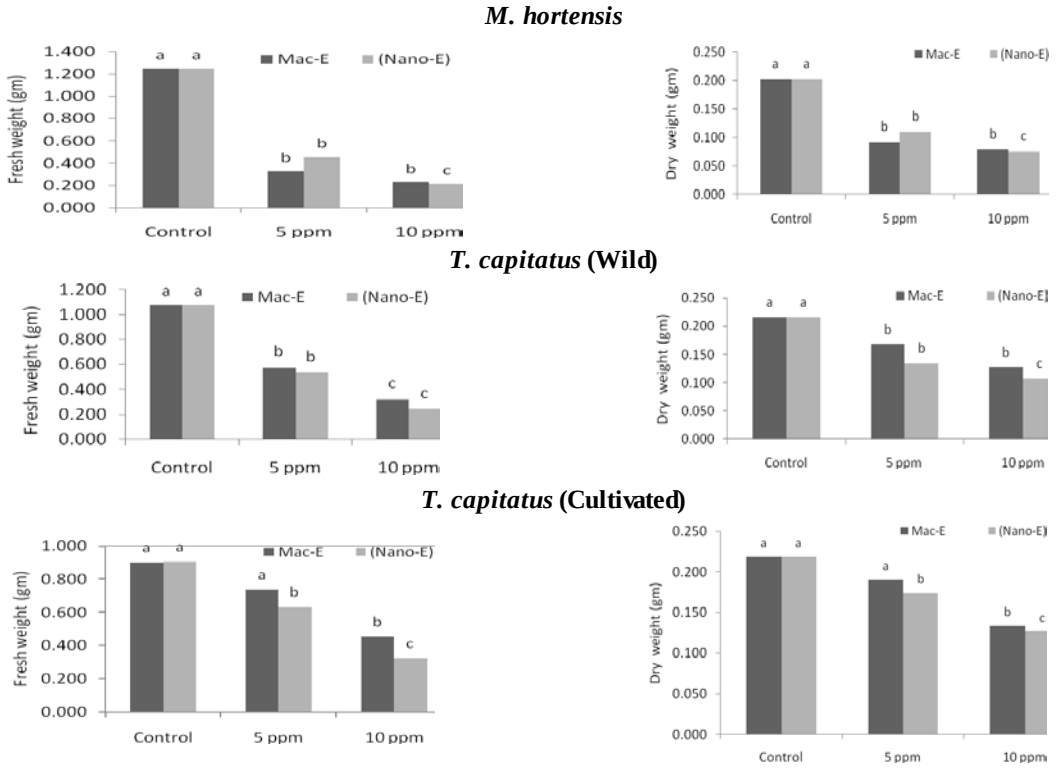


**Fig.2.Post-emergence activity of macroemulsion (Macro-E) and nanoemulsion (Nano-E) on *C. arvensis* fresh and dry weight at 2-4 leaves stage.**

The herbicidal activity of essential oils revealed that *M. hortensis* had the greatest inhibitory effects followed by *T. capitatus* wild and finally *T. capitatus* cultivated in both *C. arvensis* and *S. viridis.* Due to the application difficulties of the volatile oils, it's emulsified to Mac-E and Nano-E and re-bioassayed under laboratory condition before greenhouse investigation. The formulated Mac-E and Nano-E achieved post emergence activity in the early stage of *C. arvensis* (2-3 leaves) total biomass fresh and dry weight. Nevertheless, only Nano-E was in *C. arvensis* at 5-7 leaves stage than Mac-E. The differences were slightly lower within the tested nanoformulation under greenhouse. The nanoformulation may be of 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 macroemulsion (Mac-E) plus co-surfactant for nanoemulsion (Nano-E) are the critical point that control the prepared formulation suitability and deliver their function. The formulated nanoemulsions size were found close to the standard Nano-E between 1 to 100 nm (Casanova *et al*. 2005) for best deliver their function. Whereas, most prepared Nano-E size not exceeds 22 nm, on the other side only *M. hortensis* Mac-E less than 100 nm, which obtained without sonication and have milky colure similar other Mac-E and over them with higher herbicidal activity. These results supported by Owolade *et al*. (2008) found that nanopesticides, nanofungicides and nanoherbicides which are being used efficiently in agriculture. Nanoparticles loaded with garlic essential oil are efficacious against *Tribolium castaneum* Herbst

(Yang *et al*. 2009). The previous results indicate that the volatile oils contained growth inhibiting allelochemicals their seed germination and seedling growth. The phytotoxicity of volatile constituents in *M. hortensis, T. capitatus* wild and *T. capitatus* cultivated was previously reported; δ-terpinene, p-cymene, carvacrol, 1,8-cineole (Angelini *et al*. 2003; Grosso *et al*. 2010), caryophyllene oxide (Macini *et al*. 2009a), Thymol, p-cymene, δ-terpinene (Almeida *et al*. 2010; Grosso *et al*. 2010) α-humulene (Tellez *et al*. 2000), α-pinene, 1,8-cineole, borneol (Angelini *et al*. 2003), Carvacrol, p-cymene (Kordali *et al*. 2008), β-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, β-phellandrene, α-pinene(Almeida *et al*. 2010), Linalol, 1.8-cineole, β-phellandrene, α-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) was reported on many plants growth. α-pinene, limonene, 1.8.cineole and camphor affect the respiratory activity of mithochondria of maize and soybean hypocotyl axes and α-pinene has been shown to be the most active among the all tested monoterpenes (Abrahim *et al*. 2003), 1,8-cineole inhibits the germination, speed of germination, seedling growth, chlorophyll content and respiratory activity of *Ageratum conyzoides* Singh *el al*. (2002). The above results presented the potentially useful role of essential oils and their prepared formulations from natural sources to use for weed control. While nanoemulsions has a good and the highest inhibition properties towered the selected weeds than the others, that can be used a good in weed control as alternative means to synthetics herbicides. Also, recommended to develop a protocol based on this modern technique in order to provide a good source for weed control under Egyptian conditions.

## REFERENCES


Abrahim, D., L. Takahashi, A. M. Kelmer-Bracht and E.L. Ishii-iwamoto (2003). Effects of phenolic acids and monoterpenes on the mitochondrial respiration of soybean hypocotyls axes. Allelopathy J., 11: 21-20.

Adams, R.P., (2001). Identification of Essential Oil Components by Gas Chromatography – Quadrupole Mass Spectroscopy. Allured PublishingCorporation, Illinois, USA.

Almeida, R.L.F., F. Frei, E. Mancini, L. De Martino and V. De Feo (2010). Phytotoxic activities of Mediterranean essential oils. Molecules, 15: 4309-4323.

Amri, I., L. Hamrouni, M. Hanana and B. Jamoussi (2013). Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management. Int. J. of Appl. Biol.and Pharm.Technol., 2:69-114.

Angelini, L.G., G. Carpanese, P. L. Cioni, I. Morelli, M. Macchia and G.Flamini (2003). Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J. Agric. Food Chem., 51: 6158-6164.

Boulos, L.(2009). Flora of Egypt, Ckecklist. Al-Hadara Publishing, Cairo, Egypt, pp.401.

Casanova, H., P. Araque and C. Ortiz (2005). Nicotine carboxylate insecticide emulsions: Effect of the fatty acid chain length. J. Agric. Food Chem., 53: 9949-9953.

Date, A. A, and M.S. Nagarsenker (2008). Design and evaluation of microemulsions for improved parenteral delivery of propofol. AAPS Pharm. Sci. Tech., 9 (1):138-145.

Dayan, F. E., C. L. Cantrell and S.O. Duke (2009). Natural products in crop protection. Bioorg. Med. Chem. Lett., 17: 4022–4034.

De Martino, L., C. Formisano, E. Mancini, V. De Feo, F. Piozzi, D. Rigano and F. Senatore (2010). Chemical composition and phytotoxic effects of essential oils from four Teucrium species. Nat. Prod. Commun., 5: 1969-1976.

El-Ghorab, A. H., A. F. Mansour and K. F. El-Massry (2004). Effect of extraction methods on the chemical composition and antioxidant activity of Egyptian marjoram *(Majorana hortensis Moench)*. Flavour Fragr. J., 19: 54–61.

Feldman, J.M. and O. Gracia (1977) Studies of weed plants as sources of viruses. V. Occurrence of alfalfa mosaic virus on origanum crops and on some weeds in Argentina. Phytopathology Zeitschrift, 90:87–90.

Ghahreman, A. (1994). Cormophytes of Iran, 3rd edition, Tehran University Publications, 867.

Grosso, C., A. C. José, S. U. José, M. F. P. António and G. B. José (2010). Herbicidal Activity of volatiles from coriander, winter savory, cotton lavender, and thyme isolated by Hydrodistillation and Supercritical Fluid Extraction.. J. Agric. Food Chem., 58:11007-11013.

Harley, R. M., S.Atkins, A. L. Budantsev, P. D. Cantino, B. J. Conn, R. J. Grayer, M. M. Harley, R.P.J. de Kok, T. V. Krestovskaja, R. Morales, A. J. Paton and P. Olof Ryding (2004). "Labiatae" pages 167-275. In: Klaus Kubitzki (editor) and Joachim W. Kadereit (volume editor). The Families and Genera of Vascular Plants volume VII. Springer-Verlag: Berlin; Heidelberg, Germany.

Kordali, S., A. Cakir, H. Ozer, R. Cakmakci, M. Kesdek and E. Mete (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresour. Technol., 99: 8788-8795.

Mancini, E., N. A. Arnold, V. De Feo, C. Formisano, D. Rigano, F. Piozzi and F. Senatore (2009a). Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda* L. subsp. Albi flora growing wild in Lebanon, J. Plant Interact., 4:253- 259.

Marandino, A. L. De Martino, E. Mancini, L. Milella and V. De Feo (2011). Chemical composition and possible in vitro anti germination activity of three Hypericum essential oils. Nat. Prod. Comm., 6:1735-8.

Morales, 1. R. (2002). Medicinal and Aromatic Plants – Industrial Profiles, vol. 24-Thyme, E. Stahl-Biskup and F. Saez, eds., Taylor& Francis, pp. 16.

Mucciarelli M., W.Camusso, C.M. Bertez, S.Bossi and M. Maffei (2001). Effect of (+)-polegone and other oil components of *Mentha piperita* on cucumber respiration. Phytochem., 57: 91-98.

Owolade, O. F., D.O. Ogunleti and M.O. Adenekan (2008).Titanium dioxide affects disease development and yield of edible cowpea. Agri. Food. Chem., 7(50):2942-2947.

Salamci, E., S. Kordali, R. Kotan, A. Cakir and Y. Kaja (2007). Chemical composition, antimicrobial and herbicidal effects of essential oils isolated Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. chiliophyllum. Biochem. Syst. Ecol., 35: 569-581.

Sinha, M., 1. K. Balamurgan and N. Ganesh (2015).Preparation and characterization of nanoemulsion based on papaya seed oil. Vivo Scientia, 4(2):72-76.

Singh, H.P., D.R. Batish, S. Kaur, H. Ramezani and R.K. Kohli (2002). Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. Ann. Appl. Biol., 141:111- 116

Snedecor, G W. and W. G Cochran (1990). Statistical Methods 8th Ed. Iowa State Univ. Press, Ames, Iowa, U.S.A.

Soliman, A. (2013). Comparative study on composition and allelopathic effect of volatile oils extracted from two *Thymus species in* Egypt. Int. J. of Environ. & Water, 2:(3)59-66.

Soliman, F.M., M. F. Yousif, S. S. Zaghloul, and M. M. Okba (2009).Seasonal variation in the essential oil composition of *Origanum majorana* L. cultivated in Egypt . Z. Naturforsch. 64c:611–614.

Tamaki, G., H. R. Moffitt and J. E. Turner (1975). The influence of perennial weeds on the abundance of the redbacked cutworm on asparagi. Environmental Entomology, 4:274–276.

Tellez, M. R., F. E. Dayan, K. K. Schrader, D. E. Wedje and S.O. Duke (2000). Composition and some biological activities of the essential oil of *Callicarpa americana* (L.).J. Agric. Food Chem., 48: 3008-3012.

Yang, F. L., X.G. Li, F. Zhu and C. L. Lei (2009). Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Agric. Food Chem., 57(21): 10156-10162

Verma, R. S., R. K. Verma, A. Chauhan and A. K. Yadav (2010). Changes in the essential oil composition of *Majorana hortensis* Moench. cultivated in India during plant ontogeny. J. Serb. Chem. Soc. 75 (4) :441–447.

Westra, P., P. Chapman, P.W. Stahlman, S. D. Miller and P. K. Fay (1992). Field bindweed (*Convolvulus arvensis*) control with various herbicide combinations. Weed Technol., 6:949–55.

## تشكيل المستحلبات و المستحلبات النانومترية من زيوت الزعتر البرى والمنزرع والبردقوش لمكافحة الحشائش

**محمد عبد العزيز بلح[1] ووليد محمد عبد العظيم[2]**

**[1]قسم وقاية النبات – مركز بحوث الصحراء**

**[2]قسم النباتات الطبية – مركز بحوث الصحراء**

إستهدفت الدراسة تقيم الكفاءة الابادية العشبية للزيوت الاساسية ومستحضراتها من مستحلبات كبيرة ومستحلبات نانومترية لنبات الزعتر النامى بريا والمنزرع والبردقوش على إنبات ونمو حشائش العليق وديل الثعلب او للصيق. حيث إتضح من تحليل مكونات الزيوت الاساسية باستخدام جهاز الكروماتوجرافى الغازى المزود بمطياف الكتلة وجود المركبات الاتية: Thymol, camphor, thujone, δ-terpinene, Borneol, p-cymene, carvacrol, 1,8-cineole, caryophyllene oxide, α-humulene α-pinene, المعروفة بسميتها النباتية ضمن borneol, β-pinene, caryophyllene, caryophyllene oxide, linalol and phellandrene محتويات الزيوت. صيغة المستحضرات بواسطة إختيار أنسب نظم خلط بين الزيت والماء والمواد النشطة سطحيا بالاضافة للعوامل المساعدة للمواد النشطة سطحيا فى حالة المستحلبات النانومترية كما تم عمل تعظيم لها بالاعتماد على إختبارات الثبات والخصائص البيولوجية. كما تميزت المستحلبات النانومترية بالشفافية الكاملة ونصف الشفافية، حيث سجلت أحجام الجزيئات ٣.٥و١٢.٠و٢٢.١ نانومترلكل من مستحضرات البردقوش والزعتر البرى والمنزرع على التوالى. بناء على قيمة $ED_{50}$ للبردقوش ( زيت ومستحلب كبير ومستحلب نانومترى) أظهر أعلى كفاءة إبادية على حشائش العليق، وعلى العكس من ذلك اقل كفاءة إبادية نتجت من الزعتر المنزرع بينما توسطهم فى التأثير الزعتر البرى. أظهرت المستحلبات النانومترية كفاءة إبادية بعد الانبثاق على البادرات العليق فى عمر من ٥-٦ ورقات عن مستحضر ات المستحلب الكبيرفى الصوبة.أوضحت الدراسة ان الكفاءة الابادية إعتمدت على نوع الزيت، التركيز المستخدم ونوع المستحضر وكذلك طور نمو الحشائش اثناء المعاملة ، حيث أكدت النتائج إمكانية إستخدام المستحضرات النانومترية في برامج مكافحة الحشائش المعمرة.