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ORIGINAL ARTICLE

Study of MWCNTs effects on growth rate,germination and morphological characteristics of two Salvia species seedlings, Salvia sclarea and Salvia macrosiphon

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Abstract. In order to study of MWCNTs on morphological characteristics of Salvia sclarea and Salvia macrosiphon seeds, different concentrations of MWCNTs,0,20,60,80,200 µg/ml were pepared,then with compeletly randomized design experiment,10 seeds of Salvia sclarea and Salvia macrosiphon were placed on filter papers in petri dishes in sterile in vitro conditions, in different concenterations and 4 replication.After 12 days rate and percentages of germination ,shoot and root length ,dry and wet weight were measured.Results showed that treatment of 80µg/ml MWCNTs($P \leq 0.05$) was maximum in germination percentage, germination rate, seedling length and dry weight, while comparing two species on seedling length showed that in S. sclarea concentration of 80µg/ml MWCNTs was maximum but in S.macrosiphon concentrations of 60,80 and 200 µg/ml MWCNTs had same value and high effect. Comparing of two Salvia species in wet weight showed that in S.sclarea treatment of 80µg/ml MWCNTs had higest effect while in S.macrosiphon concentrations of 0 and 80µg/ml MWCNTs had highest effect with same value.

Keywords: Salvia sclarea, Salvia macrosiphon, MWCNTs, Germination

INTRODUCTION

Salvia is an important genus of the Lamiaceae family that includes more than 700 species which are spread throughout the world (Ewans, 1996). Salvia species called Maryam-Goli in Persian (Mozaffarian, 1996) has been famous for its medicinal properties since ancient times. Most of salvia species are commonly, utilized for their essential oils in the foods, medicines and perfumery industries (Goren et al., 200; Ulubelen 1998). Salvia sclarea L. belonging t o t he family is also popularly known as 'Clary Sage'. The plants are 60-100 cm high with large hairy leaves and small blue, white or purple flowers . The plant is native to Mediterranean countries, southern France, Italy and Morocco, and is one of the most important plants cultivated worldwide as a source of essential oils and other perfumery products [Dzamic. et al 2008].

Salvia macrosiphon Boiss. is a quite-abundanand polymorphic plant in Iran and afghanistan. It is a perennial, herbaceous, strongly aromatic,lemon-scented and pale yellowish green plant. Its stems are few to several from a woody root stock, up to 60 cm, erect, sturdy, qudrangular, below eglandular pilose, above with a dense indumentum of short glandular hairs and sessile oil globules [Rechinger 1986].

New discoveries in nanotechnology provided knowledge and technological platforms for a number of applications in medical science, aerospace, electronics and defense industries. It is demonstrated that multi-walled carbon nanotubes (MWCNTs) can activate growth of tomato plants and affect the expression of genes that are essential for cell division and plant development. [Khodakovskaya, et al. 2013]. Rao and srivistana[2014] demonstrated that MWCNTs effects on Wheat, Corn, Peanut and Garlic increase size and number of leaves.and biomass. It is showed that cotton seedlings had highest growth in 60µg/ml MWCNTs treatment[Nalwade,et.al.2013].Tiwari,et.al.[2013]

introduced 20µg/ml MWCNts concentration can hasten water absorbanse and seedlinas growth. In this research with respect to application of two species of Salvia ; S.sclarea and S.macrosiphon in pharmacology, agriculture and perfume industry,we studied MWCNTs effects on germination, seedling length, shoot and root length , dry weight and wet weight of S.sclarea and S.macrosiphon.

MATERIAL AND METHOD

SOLUBILIZATION OF MWCNTS

MWCNTs of diameter 10-20 nm and length 3-8 µm were purchased from USNANO,USA. CNTs were made water soluble using H2SO4+HNO(3:2 by volume for 24 h [Rao. et.al,1996]. Excess of acids were removed and black mass washed with distilled water several times till it was neutral. Repeated adding of water and evaporation under boiling water bath removed all traces of acids. Acid free final wash was tested using Griess reagent [Roy.et.al,1994]. The black mass was vaccum dried and subjected to analysis. CNTs became water soluble after sonication [Huang.et.al,2002].

IN-VIVO TECHNIQUE

Seeds were surface sterilized in %70 ethanol for 1 minute, then rinsed three times with strile distilled water,then in 10% sodium hypochlorite solution and then rinsed three times with sterile distilled water; then placed on wetted Whatman No. 3 filter paper discs (Cerabolini et al., 2004) in Petri dishes containing 0 (control),20, 60,80 and 200 µg/ml of MWCNTs For each accession, seeds were allocated to four replicate Petri dishes, each containing 10 seeds in a completely randomized design. Seeds were incubated for 12 days in a germination chamber in the following environmental regime: 16/8 h light/dark cycle at 25±1°C. All Petri dishes were sealed to prevent from desiccating with parafilm and to ensure no systematic influences due to position within the chamber re-randomizing of Petri dishes was done every other day (Yang et al., 1999). Seeds with at least two millimeters radicle emergence were recorded daily as ' germinated'.

DATA ANALYSIS

Statistic analysis was carried out with SPSS software , version 16. It was used Tukey and Schefe assay in %5 level For mean comparison.

RESULTS AND DISCUSSION

GERMINATION PERCENTAGE

Highest germination percentage in S.sclarea was %51 relevent to 80µg/ml MWCNTs concentration. According to tukey and sheffeh analysis, treatments of 200 and 80µg/ml MWCNTs stand in one group with same value and 0 , 60 µg/ml MWCNTs treatments stand in another group and 60 µg/ml MWCNTs treatment stand in one another group (fig.1). in S.macrosiphon germination percentage is stood at highest level with 80µg/ml MWCNTs and then 0,200 and 60 µg/ml MWCNTs an at the end 20 µg/ml MWCNTs(fig.2).

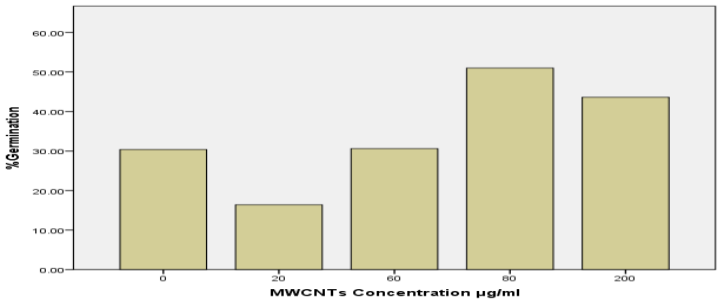

Figure1.Germination percentage of S. sclarea seeds in different MWCNTs concentrations

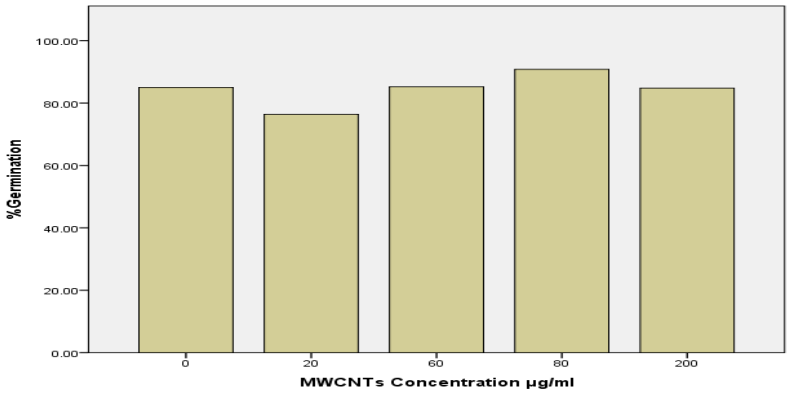

Figure 2.Germination percentage of S. macrosiphon seeds in different MWCNTs concentrations

GERMINATION RATE

Germination rate in S.sclarea in 80µg/ml MWCNTs concentration was highest and then 0 and 20µg/ml MWCNTs concentration and at the end is stood 200µg/ml MWCNTs concentration (fig.3).descending order of germination rate in S.macrosiphon was 80,200,0,60 and 20 µg/ml MWCNTs concentrations (fig.4).

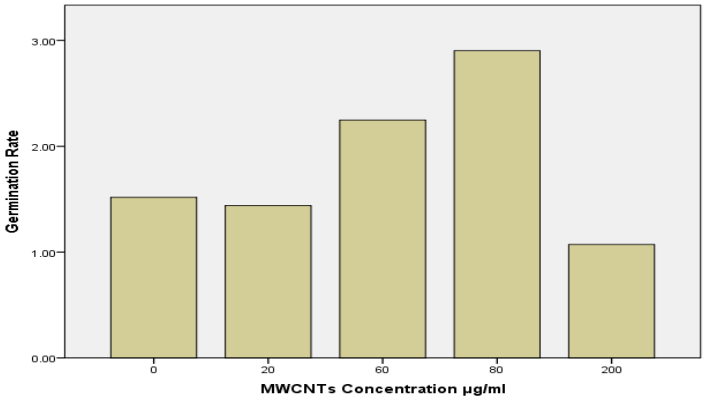

Figure 3.Germination rate of S. sclarea seeds in different MWCNTs concentrations

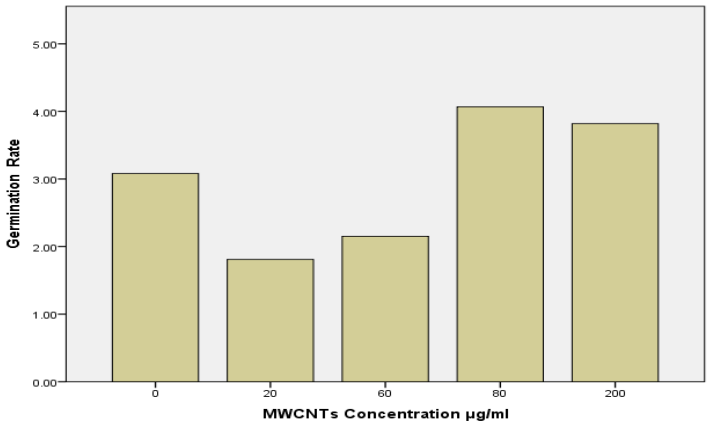

Figure 4.Germination rate of S.macrosiphon seeds in different MWCNTs concentrations

SEEDLING LENGTH

Maximum seedling length in S.sclarea was relevant to 80µg/ml MWCNTs concentration, then control(0) and then 60and 200 µg/ml MWCNTs concentration and finally 20µg/ml MWCNTs concentration (fig.5) while in S. macrosiphon 80µg/ml MWCNTs concentration was maximum and other concentrations was stood in next group with same value(fig.6).

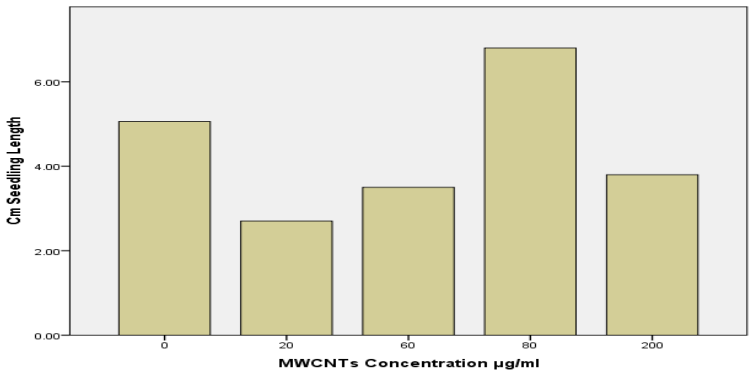

Figure 5.total seedling length of S.sclarea in different MWCNTs concentrations

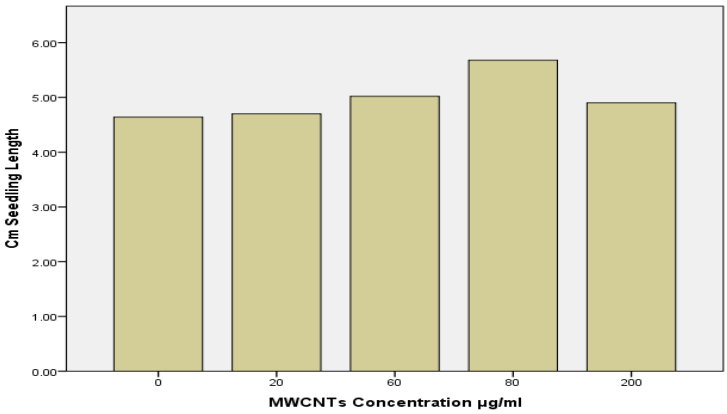

Figure 6.total seedling length of S.macrosiphon in different MWCNTs concentrations

SHOOT LENGTH

Data analysis from shoot length measurement of S. sclarea displayed that 80µg/ml MWCNTs concentration had maximum effect , then 60,200µg/ml MWCNTs concentration and at the end 0 and 20 µg/ml MWCNTs concentration had same value (fig.7) while in S. macrosiphon 200,80, 60µg/ml MWCNTs concentrations were maximum with same value and then 0and 20 µg/ml MWCNTs concentrations were stood in next group with same value (fig.8).

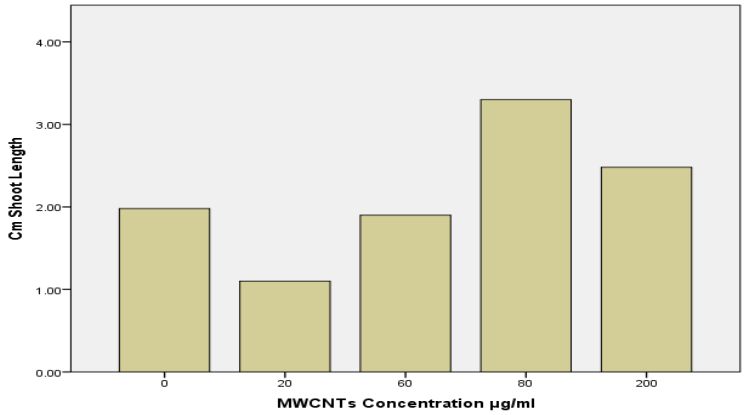

Figure 7. Shoot length of S.sclarea seedlings in different MWCNTs concentrations

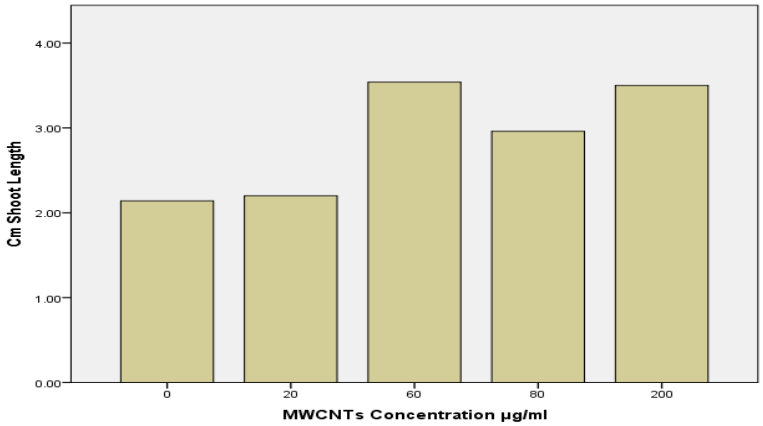

Figure 8. Shoot length of S.macrosiphon seedlings in different MWCNTs concentrations

ROOT LENGTH

0 and 80µg/ml MWCNTs concentrations had maximum effect on root length in S. sclarea whereas 200,20, 60µg/ml MWCNTs concentrations stand in other group with less and same effects (fig.9) but in S.macrosiphon 0,20,80µg/ml MWCNTs concentrations had maximum effect and 200 and 60µg/ml MWCNTs concentrations had less effects with same value (fig.10).

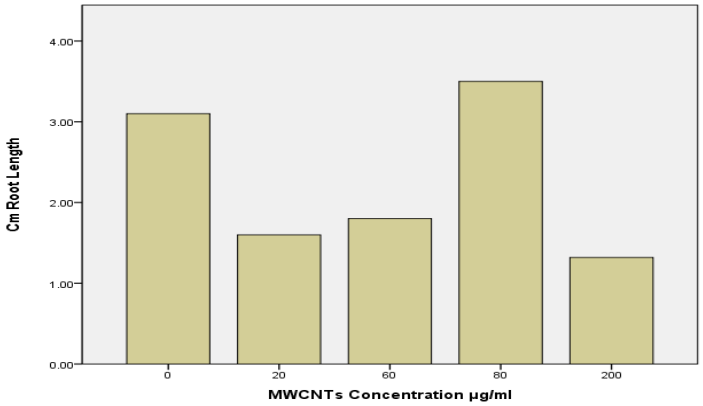

Figure .9. Root length of S.sclarea seedlings in different MWCNTs concentrations

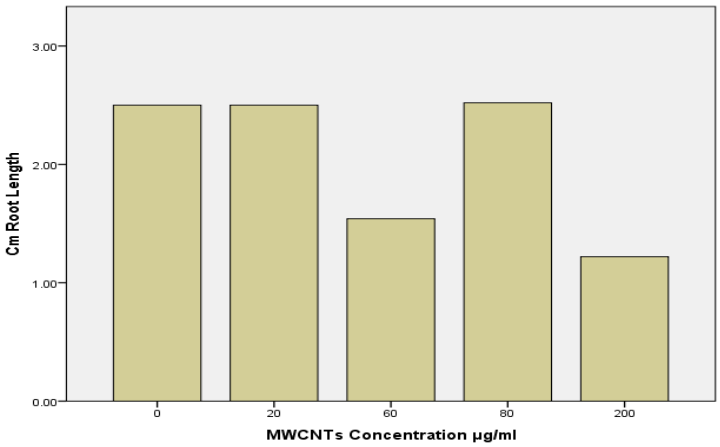

Figure 10. Root length of S.macrosiphon seedlings in different MWCNTs concentrations Wet weight

Results of wet weight of S.sclarea seedlings displayed that 80µg/ml MWCNTs concentration had maximum effect , then 60,20 and 0 µg/ml MWCNTs concentration and at the end was 200 µg/ml MWCNTs concentration (fig.11) while in S. macrosiphon 0,80, µg/ml MWCNTs concentrations had maximum effect with same value then were 200,20, 60µg/ml MWCNTs concentrations with same value (fig.12).

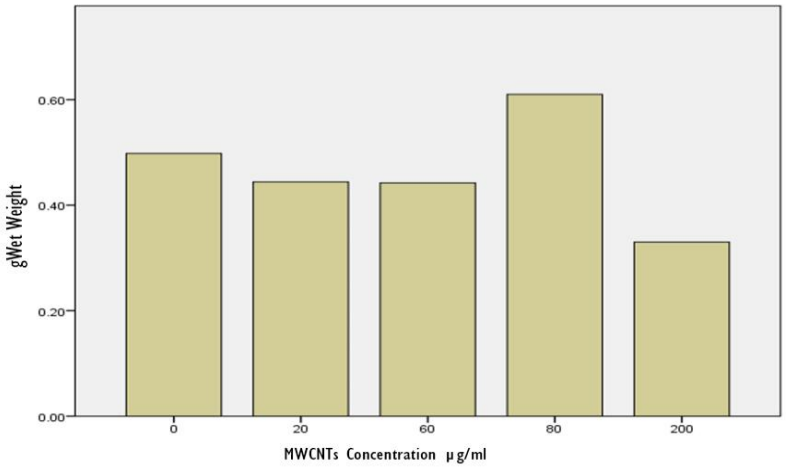

Figure 11. Wet weight of S.sclarea seedlings in different MWCNTs concentrations

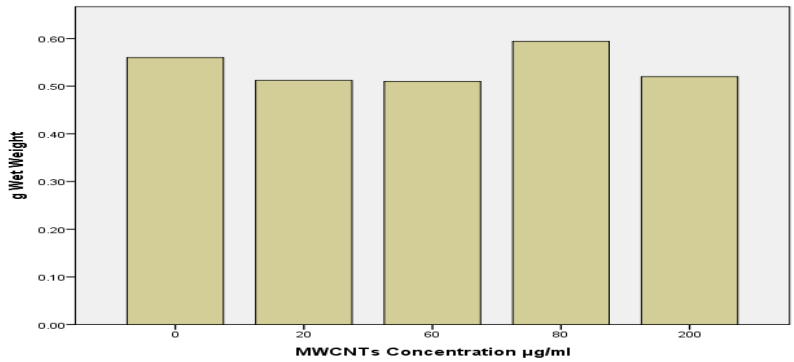

Figure 12. Wet weight of S.macrosiphon seedlings in different MWCNTs concentrations

DRY WEIGHT

Comparing of dry weight of S.sclarea seedlings displayed that 80µg/ml MWCNTs concentration had maximum effect then 60,20 and µg/ml MWCNTs concentration and at the end stood 0 and 200 µg/ml MWCNTs concentration (fig.13),also in S.macrosiphon ,80µg/ml MWCNTs concentration had maximum effect and at end were 0,200,20, 60µg/ml MWCNTs concentrations with same value (fig.14).

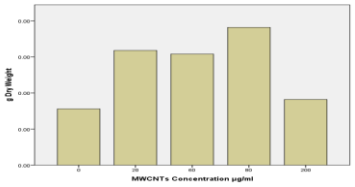

Figure 13. Dry weight of S.sclarea seedlings in different MWCNTs concentrations

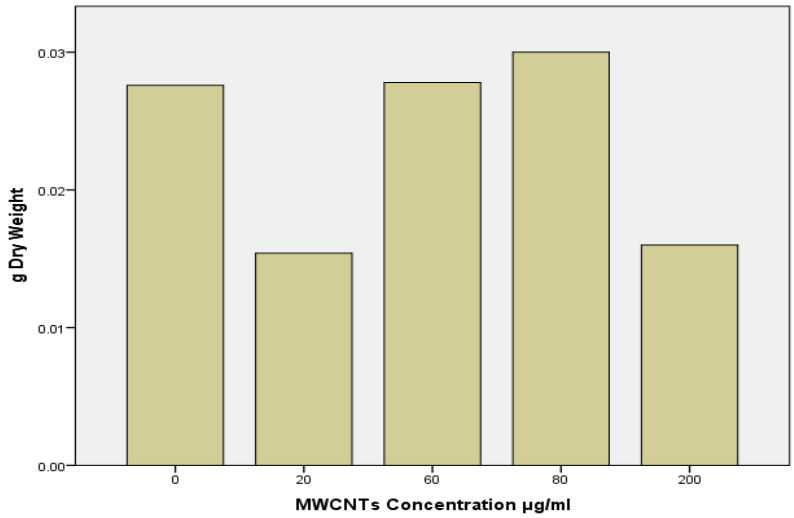

Figure 14. Dry weight of S.macrosiphon seedlings in different MWCNTs concentrations

CONCLUSION

MWCNTs induce unknown changes in root and leaf gene expression of tomato specially upstream regulation of stresses genes that induced with pathogens and induced aqua chanel gene of LeAqp2 [Vaiseman, et.al.2006]. Carbon nanotubes (CNT) also can intracellulary traffic through different cellular barriers and deliver biomolecules into living cells [Fouad ,et.al.2008]. In this research regard to importance of nanotubes effect on plants growth, we examine MWCNTs effects on percentage and rate of S.sclarea and S.macrosiphon germination, seedling length, shoot length , root length, wet weight and dry weight. Effect of different concentrations of MWCNTs on germination percentage of S.sclarea and S.macrosiphon displayed that 80µg/ml MWCNTs has significant effect in two species. also maximum effect on germination rate was 80 µg/ml MWCNTs and also at seedling total length was 80 µg/ml MWCNTs. Measuring of shoot length demonstrated that maximum effect in S.sclarea was 80 µg/ml MWCNTs and in S.macrosiphon was 60,80 and 200 µg/ml MWCNTs with same value. Coparing of root length displayed that maximum effect in S.sclarea was 0 and80 µg/ml MWCNTs while in S.macrosiphon was0 ,20 and 80 µg/ml MWCNTs. Treatment of MWCNTs on wet weight of S.sclarea demonstrated that 80 µg/ml MWCNTs concentration was maximum effect while in S.macrosiphon 80 and 0 µg/ml MWCNTs had maximum effect with same value. Comparing of dry weight in two species displayed that maximum effect was 80 µg/ml MWCNTs.

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