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*Original article*

# Response of *Aloysia citriodora* L. to treatments of titanium dioxide nanoparticle and salt stress

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***Extended abstract***

**Introduction**

As medicinal plant, *Aloysia citriodora* (Family, Verbenaceae) is applied in nutritional and pharmacological purposes. According to the findings, many parameters relating to cultivation conditions may influence on the different factors of the herb and result in different percentage of concerned constituents. Among various stresses, salinity is a major threat to modern agriculture causing inhibition and impairment of crop growth and development. Salinity impairs plant growth and development via water stress, cytotoxicity due to excessive uptake of ions such as sodium ($Na^+$) and chloride ($Cl^-$), and nutritional imbalance. Additionally, salinity is typically accompanied by oxidative stress due to generation of reactive oxygen species (ROS). Finding the method to overcome this issue is of great importance. Utilization of nano technologies to increase of medicinal plant production is one of significant aims of plant efficiency, which affects plant growth processes. Nanomaterials offer a wider specific surface area to fertilizers and pesticides. In addition, nanomaterials as unique carriers of agrochemicals facilitate the site-targeted controlled delivery of nutrients with increased crop protection. Among different nano materials, application of titanium nanoparticle in agricultural and nutritional industries is suitable due to its role in conservation, increase of photosynthesis and increment of tolerance to different kinds of stresses.

**Matherial sand methods**

In this study, the completely randomized factorial design was performed with two factors including titanium nano particle (0, 50, 100, 200, 400 ppm) and salt stress (0, 75, 159 mM of NaCl) with three replications in order to assess some of morphological and phytochemical properties of medicinal plant Aloysia citriodora L. The three months transplants were prepared from the greenhouse of the Iranian Institute of Medicinal Plant. After transferring the transplants to Sana Institute of Higher Education, they were cultivated into plastic vases containing perlit and pitmoss with light/dark period of 16/8 h, humidity percentage of 60% and temperature of 25°C. After the last treatment, the whole plant was harvested, washed and the plant height was measured by ruler. In order to measure the dry weight of shoot and root, these parts were separated and placed into paper envelope to be dried in oven at 121°C

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for 12 h. for calculating the wet and dry weights of shoot and root, we used the weigh machine (Sartatius) with accuracy of 0.1 g. In order to assess the physiological traits, the leaves and roots of three plants in each replication were collected conserving at -20°C. Then, the physiological properties such as chlrorophyll a, chlorophyll b, total chlorophyll, carotenoid and anthocyanin contents were measured. The analysis of variance, mean comparison, simple correlation and step-by-step regression were performed based on SAS software version 9.4.

**Result**

The result showed that salt stress caused decrease of wet and dry weight of root and shoot. While, the increase trend of these traits was indicated based on titanium nanoparticle effect. The obtained result from correlation and regression analysis of the studied traits showed that the shoot dry weight had the most direct effect on shoot dry weight yield with coefficient of determination of 0.82 ($R^2$). The mean comparison of chlorophyll contents (a, b and total) were increased by increment of titanium nanoparticle concentration. However, the chlorophyll contents were decreased by increase of salt stress levels. In addition, the interaction effect of elicitor and salt stress showed that carotenoid and anthocyanin contents were increased by 400 ppm of titanium under 75 and 150 mM of salt stress.

***Keywords*****:** Abiotic stress, Morphological traits, Phyto-chemical properties

**Table 1. The variance analysis of wet and dry weight of root and shoot of *Aloysia citriodora* L. under salt stress and titanium dioxide**

| S.O.V | df | Shoot dry weight | Root wet weight | Shoot wet weight | Root dry weight |
|---|---|---|---|---|---|
| **Titanium (T)** | 4 | 12.8491$^{**}$ | 3.0531$^{*}$ | 14.1523$^{**}$ | 2.1505$^{**}$ |
| **Salt (S)** | 2 | 16.3208$^{**}$ | 48.4606$^{**}$ | 47.4922$^{**}$ | 15.2322$^{**}$ |
| **T × S** | 8 | 0.6756$^{ns}$ | 2.5426$^{ns}$ | 1.3746$^{ns}$ | 0.2821$^{ns}$ |
| **Error** | 30 | 0.5875 | 1.4044 | 1.4395 | 0.3411 |
| **CV%** | | *7.0051* | *7.7660* | *4.4408* | *8.0502* |

⁎ and ⁎⁎ showe the significance level of 0.05 and 0.01 respectively and ns indicates non significance level

**Table 2. The variance analysis of photosynthesis and anthocyanin traits of *Aloysia citriodora* L. under salt stress and titanium dioxide**

| S.O.V | df | Chlorophyll a | Chlorophyll b | Total chlorophyll | Carotenoid | anthocyanin |
|---|---|---|---|---|---|---|
| **Titanium(T)** | 4 | 0.0168$^{**}$ | 0.0617$^{**}$ | 0.1344$^{**}$ | 0.0096$^{**}$ | 0.1538$^{**}$ |
| **Salt(S)** | 2 | 0.0174$^{**}$ | 0.0351$^{**}$ | 0.0969$^{**}$ | 0.0008$^{**}$ | 0.0567$^{**}$ |
| **T × S** | 8 | 0.0007$^{**}$ | 0.0011$^{**}$ | 0.0031$^{**}$ | 0.0001$^{**}$ | 0.0012$^{**}$ |
| **Error** | 30 | 0.0001 | 0.0002 | 0.0003 | 0.0000 | 0.0005 |
| **CV%** | | *5.2596* | *3.0948* | *2.5571* | *8.6423* | *3.6207* |

$^{*}$, $^{**}$ showed the significance level of 0.05 and 0.01 respectively and $^{ns}$ indicates non significance level

**Table 3. Simple correlation coefficient between studied traits in *Aloysia citriodora* L. under salt stress and nano particle titanium dioxide**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **Chlorophyll a** | 1 | | | | | | | | |
| **2** | **Chlorophyll b** | 0.911** | 1 | | | | | | | |
| **3** | **Total chlorophyll** | 0.965** | 0.984** | 1 | | | | | | |
| **4** | **Carotenoid** | 0.629** | 0.662** | 0.669** | 1 | | | | | |
| **5** | **Anthocyanin** | 0.487** | 0.529** | 0.533** | 0.914** | 1 | | | | |
| **6** | **Root wet weight** | 0.541** | 0.408** | 0.470** | -0.003 | -0.199 | 1 | | | |
| **7** | **Shoot wet weight** | 0.745** | 0.715** | 0.745** | 0.262ns | 0.148 ns | 0.585** | 1 | | |
| **8** | **Root dry weight** | 0.662** | 0.562** | 0.613** | 0.165 ns | -0.070 | 0.929** | 0.635** | 1 | |
| **9** | **Shoot dry weight** | 0.825** | 0.830** | 0.847** | 0.455** | 0.336* | 0.433** | 0.872** | 0.528** | 1 |

*, ** and ns shows the probability level of 1%, 5% and no significant difference, respectively

**Table 4. The linear regression for shoot wet weight as dependent variable and other variables as dependent ones.**

| Variables | Constant | Regression coefficient | Coefficient of determination | Regression equation |
|---|---|---|---|---|
| **Root wet weight** | 15.54 | 0.69 | 0.44** | $Y = 15.54 + 0.69 X_1$ |
| **Total chlorophyll** | 13.55 | 0.90 | 0.79** | $Y = 13.55 + 0.90 X_1$ |
| **Root dry weight** | 17.87 | 0.77 | 0.58** | $Y = 17.87 + 0.77 X_1$ |
| **Chlorophyll a** | 14.62 | 0.91 | 0.81** | $Y = 14.62 + 0.91 X_1$ |
| **Shoot dry weight** | 14.62 | 0.91 | 0.82** | $Y = 14.62 + 0.91 X_1$ |
| **Anthocyanin** | 32.34 | -0.57 | 0.27* | $Y = 32.34 - 0.57X_3$ |
| **Chlorophyll b** | 21.1 | 0.81 | 0.63** | $Y = 21.1 + 0.81 X_4$ |
| **Carotenoid** | 20.58 | 0.69 | 0.42** | $Y = 20.58 + 0.69 X_5$ |

** and * shows the significant difference level at 1% and 5%, respectively

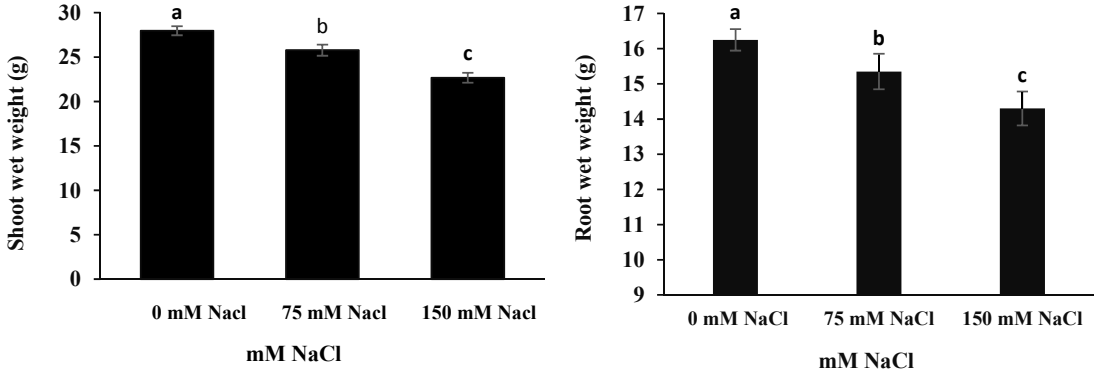


**Fig.1. the effect of salt treatment on dry and wet weight of root and shoot of *Aloysia citriodora* L. under salt stress**

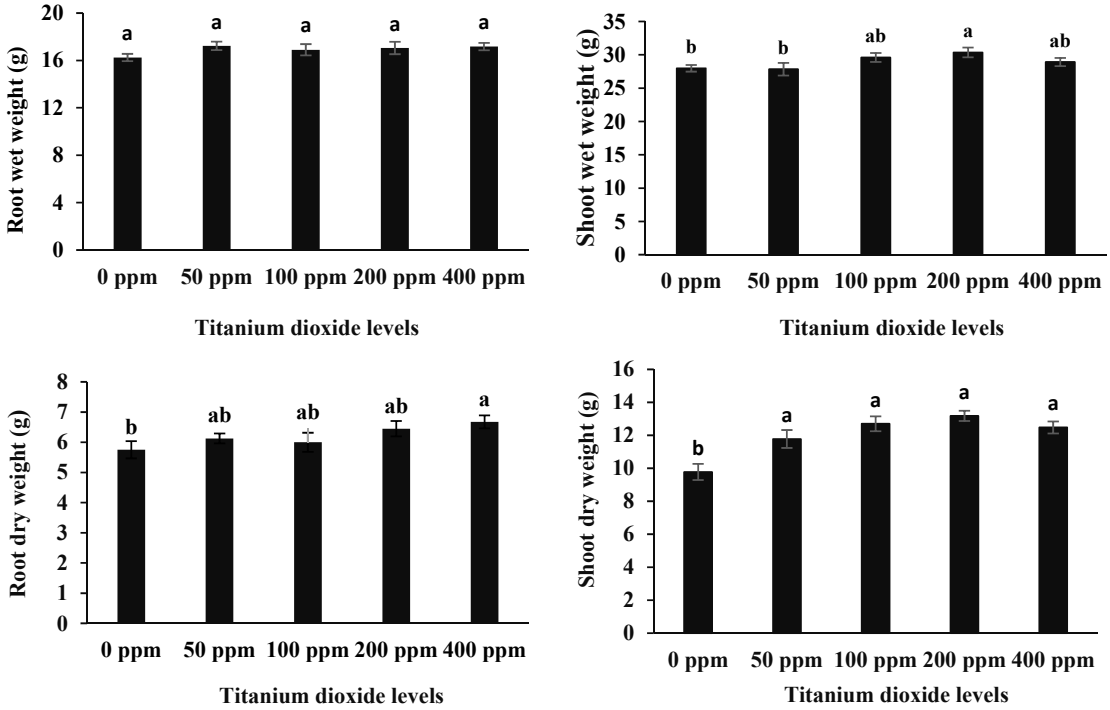


**Fig. 2. The titanium dioxide treatment effect on wet and dry weight of root and shoot in *Aloysia citriodora* L. under salt stress**

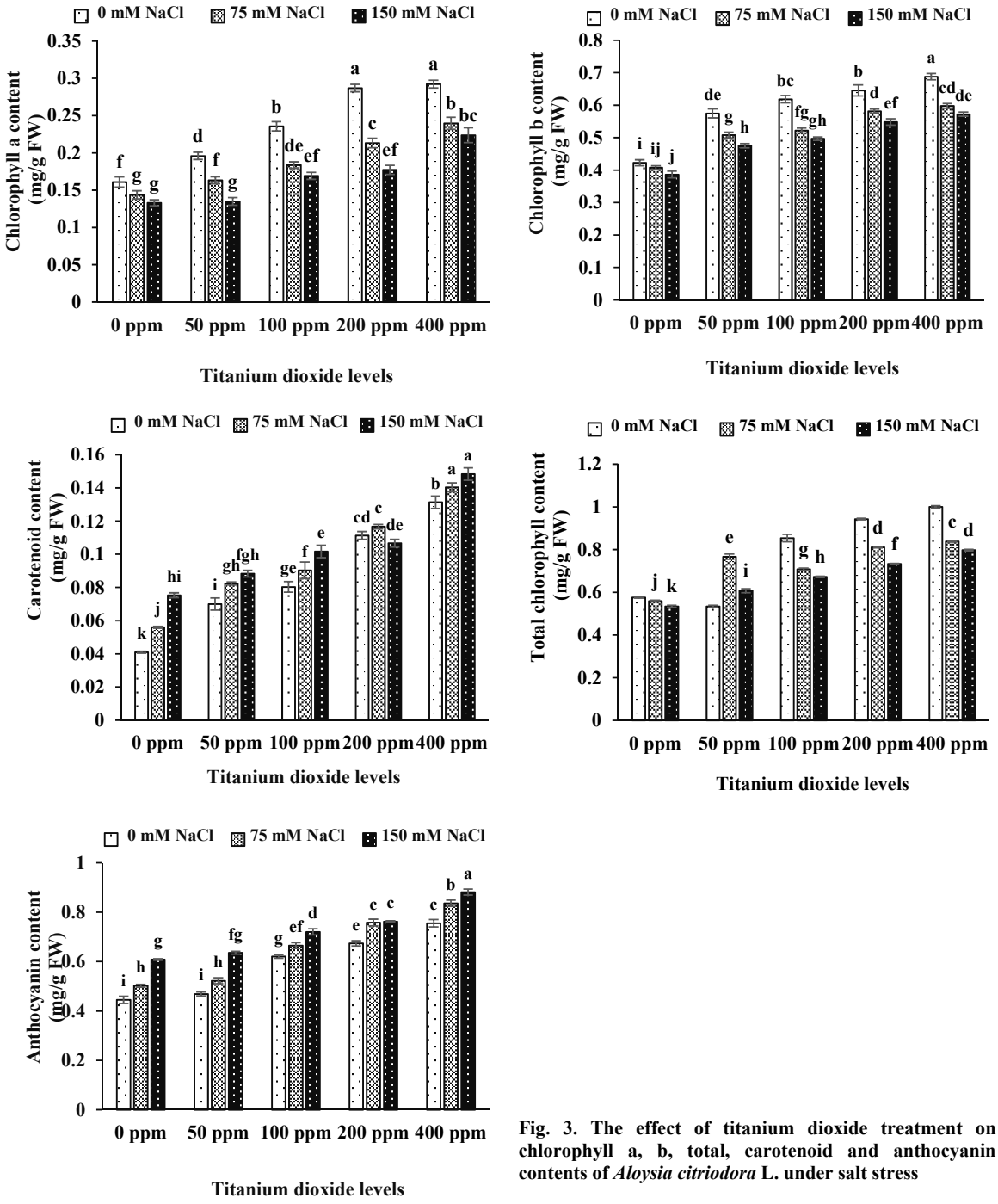


**Fig. 3. The effect of titanium dioxide treatment on chlorophyll a, b, total, carotenoid and anthocyanin contents of *Aloysia citriodora* L. under salt stress**