Response of chlorophyll *a* fluorescence, chlorophyll content, and biomass to dust accumulation stress in the medicinal plant, *Plantago lanceolata* L.

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Abstract

In arid and semi-arid areas of the world, excessive mineral aerosol carried by air parcels is a common climatic incident with well-known environmental side effects. In this study, the role of sand-dust accumulation was investigated on various aspects of photosynthetic yield of *Plantago lanceolata* including chlorophyll (Chl) *a*, *b*, effective quantum yield of PSII photosystem (ΦPSII), maximal quantum yield of PSII photosystem (Fv/Fm), electron transport rate (ETR), and biomass. *P. lanceolata* was exposed to a gradient of dust concentrations (0.5 (T1), 1 (T2), and 1.5 g/m^3 (T3)) in a dust chamber for a period of 60 days. Results of this experiment indicated that chlorophyll content of shoot is negatively correlated with the intensity of the dust exposure. Exposure of plant in T1, T2 and T3 treatments of dust caused a reduction in ΔF/Fm´ at 4%, 21%, and 26%, respectively. At the same time a reduction of 19%, 22%, and 46% in three treatments was observed for ETR. However, dust accumulation on the plant had not a significant reduction in Fv/Fm. Chlorophyll content had a significant reduction in the chlorophyll *b*. Also, the amounts of reduction in shoot dry mass of T1, T2, and T3 treatments were 26%, 29%, and 32%, respectively, as compared with their respective control.

Keywords: sand-dust storm, *Plantago lanceolata*, dust chamber, ΔF/Fm´, ETR, Fv/Fm, biomass

Abbreviations:

Chl: Chlorophyll; ΦPSII: Effective quantum yield of PSII photochemistry; Fv/Fm: maximal quantum yield of PSII photochemistry; ETR: electron transport rate

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Introduction

In arid and semi-arid parts of the world excessive mineral aerosol carried by air parcels is a common climatic incident with well-known environmental side effects. Both dust and sand storms are known to have profound effects on human health and on the environment. Chemical and physical properties of dust could produce a number of plant responses due to the direct effects on plant shoots or through indirect effects on the soil. Dust particles can physically affect photosynthesis and transpiration when they accumulate on leaf surfaces (Naidoo and

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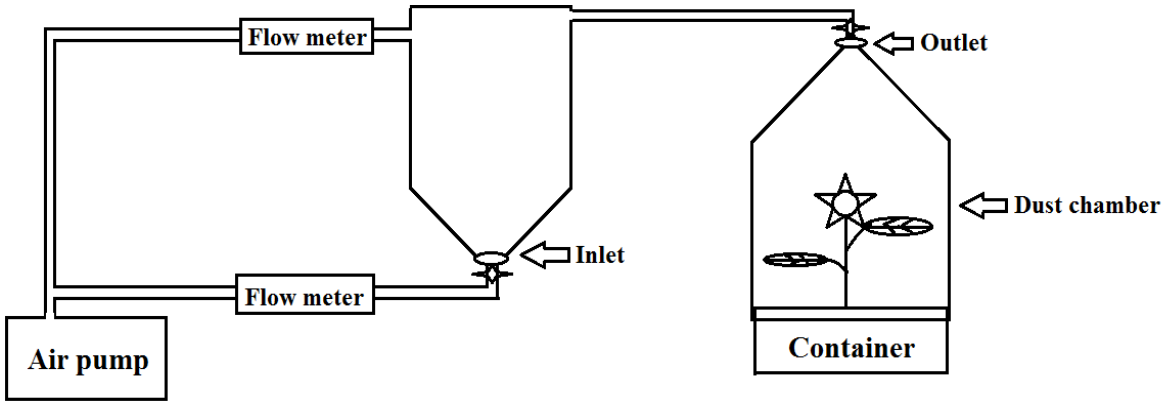

Fig. I. Schematic presentation of the dust chamber used in present study

Chirkoot, 2004). In extreme cases, leaf stomata can be plugged by mineral particles (Paling et al., 2001). Such cementing effects of dust accumulation on aerial shoots can affect temperature balance by increasing leaf temperature (Anthony, 2001), or some times by shading (Paling et al., 2001). Increasing leaf temperature by dust coverage and a corresponding increase in the rate of transpiration and photosynthesis have been documented for several plant species. Increase in dust deposition or accumulation on leaves is known to increase absorbance of solar radiation, which in turn may cause increase in leaf temperature by up to 3 °C (Grantz et al., 2003).

There are reports on a sudden increase in frequencies and intensities of dust storm in Iran which is thought to be associated with the land use practices in North Africa and Middle East (Gerivan et al., 2011). Recent dust storms have affected human health and the environment in the western and southern provinces of Iran such as Kermanshah, Illam, and Khuzestan Provinces up to southeastern Sistan and Baluchistan Provinces (Misconi and Navi, 2010). Until recently the downwind impacts of dust had received little attention compared with the impacts at source. This may be because the visible evidence of long distance transported dusts, called dust plumes, is often subtle, in contrast to dust storms which are visually more impressive. Therefore, we hypothesized that with increasing exposure to sand and dust, various aspects of plant productivity would decrease.

Material and Methods

Dust generator

We used a typical heavy combisol formed by alluvial process and collected at the bank of River Gharasou in Kermanshah Province as dust in this study. For providing suitable fine particles, soil was grinded and passed through sieve sized 200 opening/inches. We used a dust chamber and a dust generator in order to simulate and calibrate dust storm over the PVC containers planted with *P. lanceolata* (Hirano et al., 1995). A dust chamber was made by plastic sheet. Dimension of the dust chamber was 2× 1× 1 meters (Fig. I). Information about dust concentration in western and southern provinces of Iran including Kermanshah and Khuzestan which experience aeolian dust from neighboring countries illustrated that average annual frequency of critical dusty days (days with visibility less than 1000 meters) was various. IMO (Iranian Metrological Organization) provided information showing that in five years from 2001 to 2005 average dusty days in Abadan and Dezfool (Khuzistan Province) were 58.2 and 87.8 days, respectively (Atai, 2010). Also, in Kermanshah dusty days were 73 days (DOE, Kermanshah). But we used dust exposure in maximal concentrations for measurement of plant response in critical condition. We selected the dust concentration for a period of 2 months in the dust chamber as 0.5, 1, and 1.5 grams/m^3 with four day intervals between every exposure.

In this study, twelve PVC (80×30×25 cm) containers were used for planting *P. lanceolata*. A mixture of soil and sand compost (50:50) over a 15 cm layer of cobles was used. Ninety seeds of

plant were planted in three replicate containers. Afterwards, every container was covered by a black plastic sheet for 48 h. The seedlings growth was conducted in control condition at average daily temperature of 25 °C. Light was supplied by 12 fluorescent lamps, which provided quantum flux density (QFD) around 90 µm m^{-2} s^{-1}. The wilting point by finger touch was used for plant irrigation.

Measurement of chlorophyll content

Chlorophyll content of *P. lanceolata* leaves was quantified using Arnon (1949) method. At the end of experiment, four plants from each vessel were collected and cleaned thoroughly by water, then 0.2 gr of fresh leaf from each sample was separated, grinded in a mortar with 5 ml of 80% acetone, and 15 ml of acetone (100%). The homogenate was filtered through filter paper (Whatman No.1) and was made a volume of 25 ml with 80% cold acetone. The optical density of each solution was measured at 663 and 645 nm against 80% acetone blank in 1.5 cm cell. The content of the photosynthetic pigments was calculated with the following formula:

Chl *a* (mg/g) = [(12.7 × A663) - (2.6 × A645)] × ml acetone/mg leaf tissue

Chl *b* (mg/g) = [(22.9 × A645) - (4.68 × A663)] × ml acetone/mg leaf tissue

Chl T= Chl *a* + Chl *b*

Fluorescence measurements

Chlorophyll *a* fluorescence parameters of developed penultimate leaves were recorded following Force et al (2003) with a portable pulse amplitude modulated fluorometer (MINI-PAM, S/N:PYAA0421) connected to an optical leaf clip holder. Quantum yields of photosystem II (PS II) (ΔF/Fm´) were calculated as (Fm´-F)/Fm´ (Genty et al., 1989). ETR was calculated as (0.5 × 0.84 × PFD × ΔF/Fm´) assuming that 84% of incidental light is absorbed by leaves and that photons are equally distributed between PSII and PSI (Force et al., 2003). Fv/Fm was assessed after 30 min dark period in black room.

Biomass and root and shoot length

For productivity measurement of the planted *P. lanceolata*, four plants were unearthed completely from each vessel and cleaned thoroughly by water for removing debris after two months of the experiment. Shoot and root length were measured by caliper and to obtain dry mass, each parts were incubated at 60 °C for 48 h and weighed by an electronic balance (SCALTEC-SPB42 model).

Statistical analysis

Data were subjected to one-way analysis of variance and Tukey-Kramer multiple comparison test ($p<0.05$).

Results

Chlorophyll content

Chlorophyll content response to sand-dust exposure is illustrated in Figure (II). It is clear that the exposure to sand-dust concentrations has caused a reduction in chlorophyll *a* content as shown in T1, T2, and T3 compared to control. However, statistical analysis (ANOVA) indicated no significant difference for all treatments. These reductions were 1%, 9%, and 16% for T1, T2, and T3, respectively (Fig. II-a). In spite of reduction in all treatments in chlorophyll *b*, this pigment had only significant reduction in T3 ($p\leq0.05$). Also, total chlorophyll did show this significant reduction in T2 and T3.

Chlorophyll fluorescence

ΔF/Fm´ of control samples compared to T3 and T2 was significantly different ($p\leq0.05$) and compare with T1 did not show the significant difference at the 0.05 level ($p\leq0.05$). The reductions were 4%, 21%, and 26% for T1, T2, and T3, respectively (Fig. III-a). T1 compared to T3

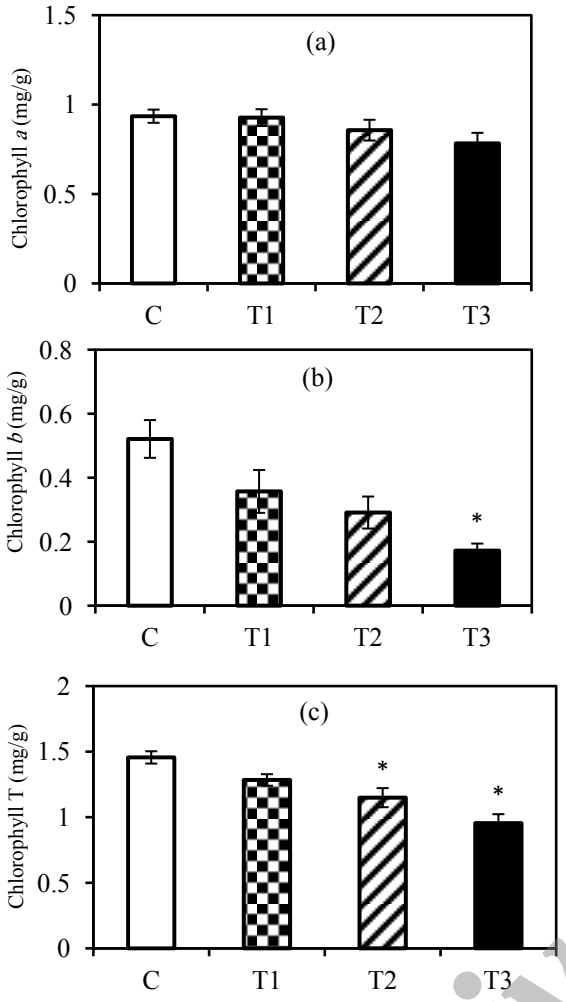

Fig. II. The impact of simulated sand-dust storm on Chl *a* (top), Chl *b* (middle), and Total Chl (down) in *P. lanceolata* exposed to concentration of 0.5 (T1), 1 (T2), and 1.5 g/m^3 (T3) of dust in a dust chamber; error bars indicate one standard error of the mean. Significant differences are shown by (*) at 0.05 level.

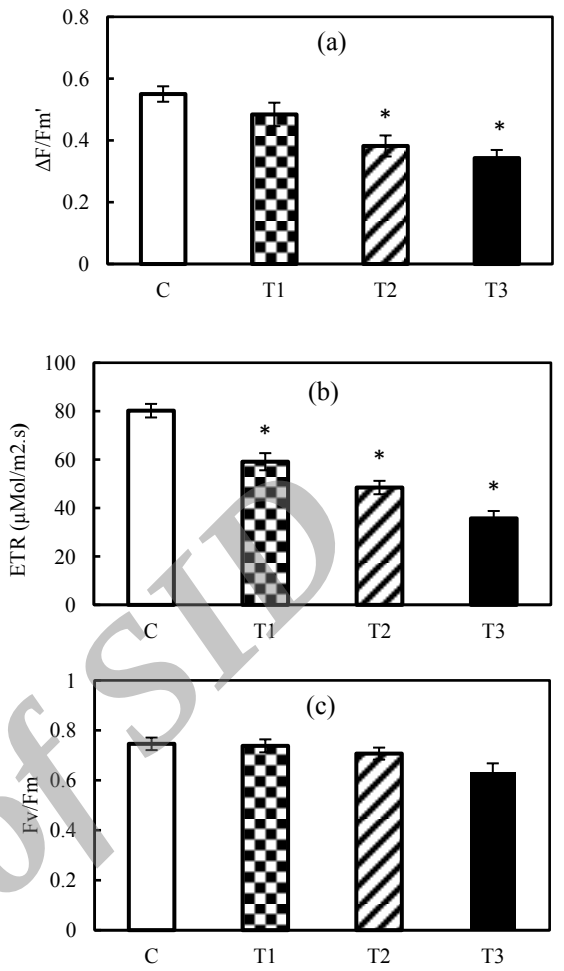

Fig. III. The impact of induced sand-dust storm on ΔF/Fm´ (a), ETR (b), and Fv/Fm (c) in *P. lanceolata* exposed to 0.5, 1, and 1.5 g/m^3 concentrations of dust in a dust chamber; error bars indicate one standard error of the mean. Significant difference are shown by (*) at 0.05 level using single analysis of variance and Tukey-Kramer multiple comparison test.

was not significantly different at 0.05 level. Similarly, T2 compared to T3 did not show significant difference at 0.05 level. Also, in case of ETR, the three treatments in comparison with control were significantly different at 0.05 level. T1 compared to T2 was not significantly different, but there was a significant difference within treatments T1 and T3. The amounts of reduction were 19%, 22%, and 46% for T1, T2, and T3, respectively (Fig. III-b). Similarly, Fv/Fm reduced by increase in sand-dust concentration; however, the control samples compared to treatments were not significantly different (p≤0.05). The amounts of decreasing were 1%, 5%, and 15% for T1, T2, and T3, respectively (Fig. III-c).

Biomass and root and shoot length

Various concentrations of dust had a reducing effect on shoot biomass (Fig. IV-a). At the end of the experiment, there was a significant difference in T2 and T3 compared with control at 0.05 level (p≤0.05). However, this parameter showed no meaningful difference within treatments. The amounts of reduction in treatments T1, T2, and T3 after two months of the experiment as compared with the control were 26%, 29%, and 32%, respectively. In contrast to shoot dry mass, root dry mass experienced no meaningful reduction in all treatments (Fig. IV-b).

Also with exposure to sand-dust concentrations at 0.5, 1, and, 1.5 grams/m3, shoot length decreased. After 60 days of study period, all treatments compared with the control had no significant difference ($p \leq 0.05$) and the amounts of reductions were 10%, 16%, and 21% for T1, T2, and T3, respectively (Fig. IV-c). In spite of regular impact of sand-dust concentrations on shoot length, root length reaction to sand-dust exposure was irregular (Fig. IV-d).

Discussion

Total chlorophyll content in the present study decreased by exposure to sand-dust. These reductions were 11%, 25%, and 36% for T1, T2, and T3, respectively (Fig. II-a). The application of dust on olive leaves decreased total Chl content by 23% compared to the control (Nanos and Ilias, 2007). Investigation has showed that as light intensity decreased, the leaf number and size increased and chlorophyll content decreased in *Tetrastigma hemsleyanum* plants (Dai et al., 2009). Lignified tissues are more intensively stained in the needles of pines growing in the dust polluted areas. Lignin content in the needles of *Pinus sylvestris* on the polluted areas were 13.65 ± 0.4, 14.98 ± 0.8, and 16.60 ± 1.1 with a mean of 15.08 while these measures in unpolluted areas were 12.77 ± 1.6, 13.76 ± 0.9, and 14.05 ± 0.8 with a mean of 13.53 lignin content. Also, the amounts of chlorophylls (Chl *a* + Chl *b*) were 2.639 ± 0.06, 2.660 ± 0.05, and 2.980 ± 0.05 in dust polluted areas with a mean of 2.76 compared to unpolluted areas with 3.04 ± 0.09, 3.21 ± 0.1, and 3.58 ± 0.06 with a mean of 3.28 (Lukjanova and Mandre, 2010).

Similarly, Chl *a* fluorescence data demonstrated that dust covered leaves exhibited significantly lower ETR through PSII. As the amounts of reduction were 19%, 22%, and 46% for T1, T2, and T3, respectively (Fig. II-b). Deposition of dust on olive leaves decreased photosynthetic rate by 23% and quantum yield by 31% compared to the control (Nanos and Ilias, 2007). The chlorophyll *a* fluorescence data demonstrated that dust-covered leaves exhibited significantly lower quantum yield of PSII and lower ETR through PSII reducing quantum efficiency of PSII (Naidoo and Chirkoot, 2004).

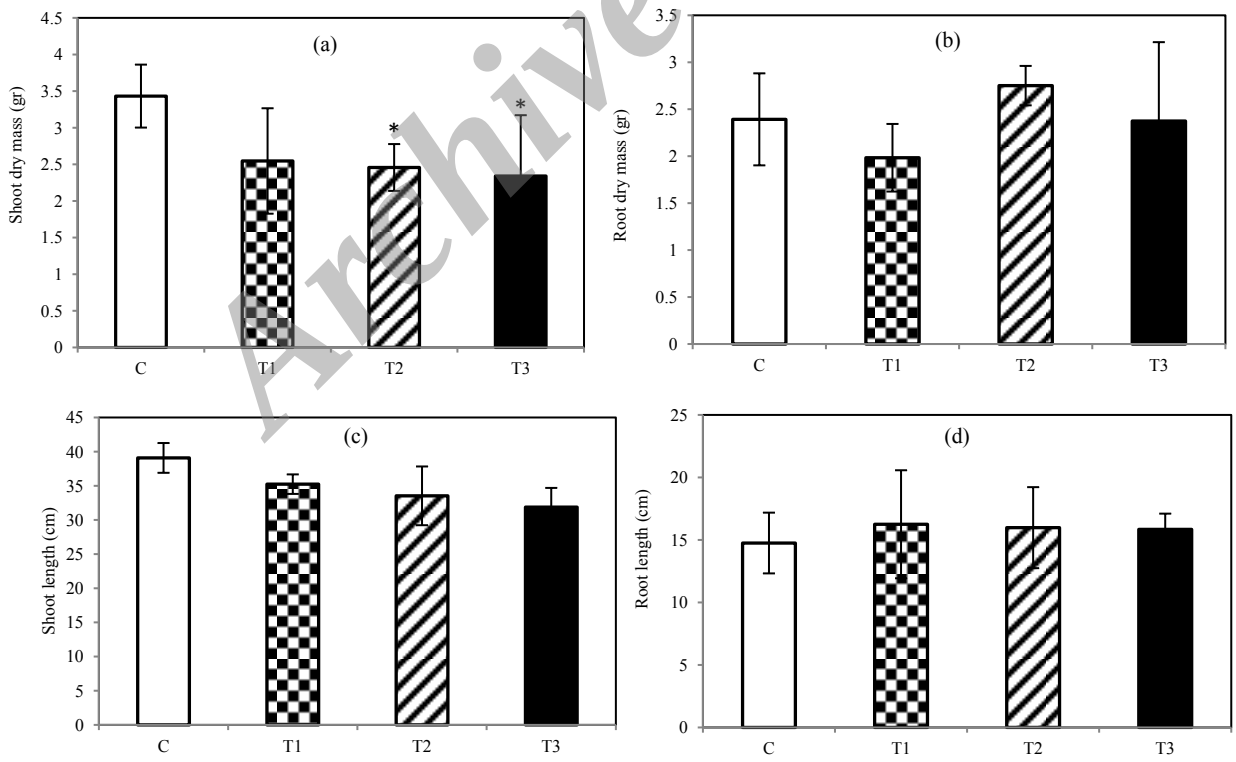

Figure IV. The impact of induced sand-dust storm on dry mass of shoot (a) and root (b), length of shoot (c) and root (d) in *P. lanceolata* exposed to 0.5, 1, and 1.5 grams/m^3 concentrations of dust in a dust chamber. Error bars indicate one standard error of the mean. Significant differences are shown by (*) at 0.05 level using single analysis of variance and Tukey-Kramer multiple comparison test.

Dust particles deposited on *P. lanceolata* leaves reduced shoot dry mass by 32% for T3 compared to the control (Fig. IV-a). This reduction resulted from the reduction in photosynthesis and an increase in respiration (Armbrust, 1986; Naidoo and Chirkoot, 2004; Prokopiev et al., 2012).

Conclusion

Damaging impact of sand-dust exposure on the *P. lanceolata* as medicinal plant was evident. This study has clearly demonstrated that the induced sand-dust storm significantly could decrease photosynthesis yield. Reduced pigments and chlorophyll *a* fluorescence can be due to the drop in pigment synthesis as a result of the shading effect. Also, the alkaline condition caused by solubilization of dust particles in cell sap may lead to pigment degradation and the inhibition of enzymes essential for biosynthesis of pigments (Lepeduš et al., 2003). The reduction of light under the dust crust formed during the experiment and the changes in the availability of elements needed for biosynthesis are other reasons for a depression of the biosynthesis of photosynthetically active pigments in this plant.

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