Experimental effects of sand-dust storm on tolerance index, percentage phototoxicity and chlorophyll a fluorescence of *Vigna radiata* L.

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**Abstract**
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. In this way, we studied the role of sand-dust accumulation on various aspects of productivity of *Vigna radiata* L. including dry mass (DM), chlorophyll (Chl) *a, b*, Chlorophyll *a* fluorescence (effective quantum yield of PSII photochemistry (ΦPSII), maximal quantum yield of PSII photochemistry (Fv/Fm) and electron transport rate (ETR)). *V. radiata* was exposed to a gradient of dust concentrations in a dust chamber (0.5 (T1), 1(T2) and 1.5 g/m³ (T3)) simulated by a dust generator for a period of 60 days. Results of this experiment indicate that DM and Chl content of shoot are negatively correlated with the intensity of the dust exposure. Exposure of *V. radiata* to dust compared with the control was caused 5% (T1), 14% (T2) and 27% (T3) reduction in leaf DM (p≤0.05, ANOVA). Also, exposure to the dust induced a significant (p≤0.05) reduction in the Total Chl content in (T3) 25%. Also, we showed that ΦPSII, ETR and Fv/Fm were affected by increasing of the dust concentrations. Exposure to the dust resulted in a significant reduction in ETR of 15%, 22%, and 43%.

**Keywords** sand and dust storm; *Vigna radiata* L; effective quantum yield of PSII photochemistry (ΦPSII).

1 Introduction
In arid and semi-arid parts of the world, excessive mineral aerosols which are 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. Chemically inert dust particles can physically affect photosynthesis and transpiration when dusts accumulate on leaf surfaces (Naidoo and 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 (Borka, 1984) 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 (Hirano et al., 1995). 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 (Sharifi et al., 1997).

Unlike climatic provision through increased rainfall and temperature, 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, Ilam and Khuzestan Provinces up to southeastern in 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.

2 Materials and Methods

2.1 Plant medium

We used 12 PVC (80 × 30 × 25cm) containers for planting Mung Bean (V. radiat) at ecology laboratory, Department of Biology, Razi University, Kermanshah, Iran. Soil of a mixture of fine sand and compost (50:50) was used over a 15 cm layer of cobles. Two hundred seeds of V. radiata were planted in three replicate containers. Following plantation, every container was covered by a black plastic sheet for 48 h. The seedlings grow in control condition at average daily temperature of 27° C. Light was supplied by 12 metal halide lamps (3 for three containers), attached to wooden sheets and placed over PVC containers at the height of 50cm over the plants. These lamps provided a broad spectrum of photosynthetically available irradiance. Quantum flux density (QFD) was (90µm m⁻² s⁻¹) when measured at the soil surface in the containers. Plants have been irrigated by tap water every other day up to the wilting point acknowledged by finger touch.

2.2 Dust generator

The dust used in this experiment was a typical heavy eutric combisol formed by alluvial process and collected at the bank of River Gharasou in Kermanshah Province. This heavy textured soil was grinded and passed through sieve (200 opening/inches) in order to provide a fine texture dust. For simulation and calibration of dust storm over the PVC containers planted with V. radiata, we used a dust chamber and a dust generator (Fig. 1). Using transparent plastic sheet a dust chamber was made. Dimension of the dust chamber was (1 × 1 × 1m) and could conveniently cover three replicates of the containers every time dust was generated into the chamber. Available information on dust concentration in western and southern provinces of Iran including Kermanshah and Khuzistan which experience aeolian dust from neighboring countries indicates that average annual frequency of critical dusty days (days with visibility less than 1000 meters) varies greatly. Data collected by IMO (Iranian Metrological Organization) indicates that in five years from 2001 to 2005 average dusty days in Dezfoo and Abadan in Khuzistan Province were 87.8 and 58.2 days respectively (Atai, 2010). Similar values for Kermanshah dusty days are 73 days (DOE-Kermanshah). We, therefore, selected the measured amount of dust in the dust chamber as 0.5, 1 and 1.5 g/m³ per exposure with a four day intervals between every exposure for a period of 60 days.
2.3 Biomass
In order to assess productivity of planted *V. radiata* under exposure of varying amounts of dust, dry and wet mass of leaf and roots were measured. At every sampling (every four day intervals), four plants unearth completely from each vessel and cleaned thoroughly by tap water for removing debris. In order to obtain the DM, fresh root and leaf of the plant have been incubated at 60º C for 48 h and weighted to get DM.

2.4 Chlorophyll content
Content of Chl \(a\), \(b\) and total obtained using Arnon (1949) method. At end of experiment, four individual leaf were collected from each container and cleaned thoroughly by water, then 0.2g fresh leaf from each sample was separated, grinded in a mortar with 5ml of (80%) acetone (acetone: water 80:20 v:v) and 15ml of (100%) acetone. After, the absorbance at A645 and A663 was read in the spectrophotometer instrument. For calculation, Arnon’s equation “Eq. (1)” was used to convert absorbance measurements to mg Chl g\(^{-1}\) leaf tissue:

\[
\text{Chl } a \ (\text{mg/g}) = [(12.7 \times A663) - (2.6 \times A645)] \times \text{ml acetone/mg} \text{g leaf tissue}
\]

\[
\text{Chl } b \ (\text{mg/g}) = [(22.9 \times A645) - (4.68 \times A663)] \times \text{ml acetone/mg} \text{g leaf tissue}
\]

\[
\text{Chl T}=\text{Chl } a + \text{Chl } b
\]

Growth parameters like vigor index (VI), tolerance index and percentage of phototoxicity (Bewly and Black, 1982) were evaluated. Also, biochemical parameters such as total sugar (Nelson, 1944) were measured and recorded.

2.5 Chlorophyll \(a\) fluorescence
Chl \(a\) fluorescence was determined with portable, pulse amplitude, modulated fluorometer (MINI-PAM, S/N: PYAA0421). \(\Phi\)PSII were calculated as \((\text{Fm'}-\text{F})/\text{Fm'}\) (Genty et al., 1989). Measurements of Chl \(a\) fluorescence was made under laboratory conditions at saturating on the same leaves. ETR through PSII was calculated as \(\Phi\)PSII \(\times\) PFDa \(\times\) 0.5 assuming that (84%) of incidental light is absorbed by leaves (PFDa) and those photons are equally distributed between PSII and PSI (Schreiber et al., 1995). Fv/Fm of electron transport through photosystem II (PS II) was specified from Chl \(a\) fluorescence induction kinetic. It was measured after 30 min dark period in black room.

3 Results and Discussion
3.1 Biomass
3.1.1 Leaf dry mass
The effect of different amounts of dust exposure on leaf DM after the course of the experiment (60 days) is illustrated in Fig. 2a. At this stage, leaf DM showed a significant difference in T3 at 0.05 confidence level using single factor analysis of variance (ANOVA). Average reductions in the amount of leaf DM at the end of experiment for the three treatments (0.5, 1 and 1.5 g/m³) were 5%, 14% and 27%. However, the degree of leaf DM reduction varies between the treatments. Average reduction in DM at the end of the experiment in T1 compare to T2 was not significant at 0.05 level, but T1 compare with T3 was significant (p≤ 0.05). Also, the amount of DM in T2 compare to T3 did not show a significant difference.

3.1.2 Root dry mass
In spite of the impact of sand-dust concentrations on plant leaf, plant root performed a slow and random reaction to the sand-dust exposure. After the end of the experiment, plant exposure to the highest concentration of dust (1.5 g/m³), the amounts of root DM illustrated a reduction compared to the control. In total, while plants in control perform 0.89% growth in root DM, exposure to 0.5, 1 and 1.5 g/m³ causes about 39% reduction in root DM (Fig. 2b).

3.2 Chlorophyll a, b
The impact of sand-dust amounts at 0.5, 1 and 1.5 g/m³ on Chl a content in V. radiata is presented in Fig. 3a. It is clear that the exposure to sand-dust amounts has caused a reduction in Chl a content as shown in T1, T2 and T3 compared to control. Statistical analysis illustrates no significant difference (p≤0.001) for all treatments. These reductions were 4% (T1), 8% (T2) and 17% (T3). Differences among treatments were not considerable as the reduction in Chl a content in T1 compare to T2 was not significantly different. Also, T2 compare with T3 had not a significant difference (p≤0.05).
Fig. 3 The impact of simulated sand-dust storm on Chl a (a), Chl b (b), Total chl (c), Total sugar (d), Vigour index (e), Tolerance index (f) and Percentage phototoxicity (g) in *V. radiata* exposed to concentration of 0.5 (T1), 1 (T2) and 1.5 g/m³ (T3) of dust in a dust chamber. Error bars indicate one standard error of the mean. Significant difference are shown by (*), (***) and (****) at 0.05 and 0.01 and less probability levels using single factor analysis of variance.
In Fig. 3b, the reduction in Chl b content of the plant exposed to 0.5 g/m³ dust per cubic meter was significant. As, higher concentration of dust amounts (1.5 g/m³) has caused significant differences in Chl b content of the leaf (Fig. 3b). Also, similar to Chl b, Chl T, Growth parameters like vigor index, tolerance index and percentage of phototoxicity and total sugar illustrated reclined respond to sand-dust exposure in third treatment (Fig. 3c, d, e, f and g). This reduction for Chl T was significant in (T3) 25%.

3.3 Chlorophyll fluorescence

Fig. 4 provides changes in the amount of ΦPSII, ETR and Fv/Fm compare to control sample. Despite of decline in all treatment, ΦPSII of Control samples compared to treatments had not the significant difference at the 0.05 level (p≤0.05). Exposure to dust resulted in a reduction in ETR of 15%, 22%, and 43%. However, control samples than to third treatment had the significant difference at 0.05 levels. Also, there was not the significant difference at 0.05 level within three treatments. Similarity, Fv/Fm was reduced by increasing of the exposure of sand-dust concentration but, control compare to T1, T2 and T3 has no significance difference at 0.05 level (p≤0.05). Also, T1 compare to T2 and T3 had no significant difference at 0.05 level.

4 Conclusions

Present study provides information indicating that both Chl content and chlorophyll a fluorescence are affected by exposure to sand-dust. Anthropogenic dust pollution result in the decrease in physiological characteristics of seed progeny, germinability, and root length (Prokopiev et al., 2012). The reduction in Chl content of the shoot exposed to dust compared to that of the control leaf may be attributed to the alkaline condition developed by solubilization of chemicals present in the dust particulates in cell sap which is believed to be responsible for Chl degradation (Prusty et al., 2005). Another factor that may cause a reduction in the synthesis of Chl a is dust deposition on leaf surfaces (Chaurasia et al., 2013). Inhibition of enzymes essential for Chl biosynthesis might be caused by the interference of dust particles which, it is a potential factor in leading to a reduction in Chl content (Vijaywargiya and Pandey, 1996). Similar reduction in the total Chl content of leaves exposed to polluted air was reported by various authors (Anthony, 2001). The extent of reduction of Chl pigments under the influence of dust deposition in present study is similar to several studies. Chl a and b contents in the leaf samples of Ficus religiosa under the influence of industrial dust have shown 38.13% and 42.73% reductions respectively (Prusty et al., 2005). In a similar study Rao (1971) has reported 20.13 and 19.70% decreasing in Chl a and b for Mangifera indica. The reduction decrease of 38.13% in Chl a was recorded at polluted site in comparisons to control site, whereas a decrease of 42.73% in Chl b was recorded at polluted site in comparison to control site (Chauhan, 2010).
This study has clearly demonstrated that the experimental simulated sand-dust storm has significantly reduced various parts of plant productivity including shoot and root DM and stem and root length. Similarly, Chl a fluorescence data indicated that dust covered leaves exhibited significantly lower \( \Phi_{\text{PSII}} \), lower ETR through PSII and reduced quantum efficiency of PSII. Reduced photosynthetic performance in this experiment is also associated with significant decrease in DM and Chl content of the \( V. \text{radiata} \) shoot. Dusts of different types affect plants in different ways and dust deposition occurred from a wide range of sources including industrial aerosol, cement industry, road dust and natural dust storm. Physiological responses of plant species or ecological outcome of a plant community exposed to mineral dust have many different direct routes. Exposure to dust may intensify secondary stresses such as drought or vulnerability to insects and pathogens or to the grazers. Dust may also facilitate penetration of toxic metals or gaseous pollutants. Effects of dust on natural communities is less known and may alter the interactive balance between species in a community and bring about changes in species composition or vegetation structure. Soil as a substrate for plant community may under influence of excessive dust exposure experiences changes in several factors including soil reaction which may cause changes in species composition according to their adaptation to soil alkalinity. Finally these changes in the vegetation cover may also affect animal communities and transfer a
community of mainly vertebrate grazers to a community of mainly soil invertebrates or microbial consumers (McTainsh and Strong, 2006).

*V. radiata* is a major legume crop in western Iran. Seeds of *V. radiata* are rich in amino acids and protein and serve as a valuable protein source for human consumption. Also, sprouts of this plant are eaten as a vegetable and are a source of mineral elements and vitamins (Somta et al., 2008). It is not easy to extrapolate the results of present study to agricultural products in the general study area. However, this study provides several major impacts upon agricultural products. Most direct impact of sand-dust storm is the loss of crop and possibly on the livestock resulted from tissue damage. There is a direct loss of plant productivity resulted from damaged caused to plant tissue as a result of sandblasting. With this loss of plant leaves, there is a reduction in photosynthetic activity and therefore reduced energy for the plant to utilize for growth, reproduction. Additionally, the loss of energy for plant growth would also delay plant development and in regions with short growing seasons. If the sand and dust storms occur later in the season, the plant damage will reduce yield during grain development and if it occurs at maturity but before harvest, there will be a direct harvest loss.

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