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Full Length Article

Dust Particles Induce Stress, Reduce Various Photosynthetic Pigments and their Derivatives in *Ficus benjamina*: A Landscape Plant

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Abstract

Abiotic stress has a negative effect on plant growth and development. In current study *Ficus benjamina* plant was exposed to road dust to analyze the effect on plant stress, various photosynthetic pigments and their derivatives. Dry dust was sprinkled twice a week at T_0 (control), T_1, T_2 and T_3 (0, 1, 3 and 5 g plant^{-1} respectively) for a period of 4 months in green house. Stress hormone (abscisic acid) was found significantly higher in leaves and roots of treatment groups. Chlorophyll showed higher (P<0.05) trend in T_0 while lower was observed in T_3. Carotenoid contents showed inverse association (P<0.05) with dust deposition. Higher (P<0.05) porphyrin contents were observed in T_0, while lower in T_3 plants. Chlorophyllide contents were recorded maximum in T_0. Pheophytin contents were significantly higher in T_0. Dust induced abiotic stress and decreases photosynthetic pigments in treatment plants. Pattern of pigment expression is different in control and dusty environment; however, photosynthetic pigments and their derivatives respond inversely to dust deposition on plant leaves. This study suggests that roadside dust deposition induces stress in *F. benjamina* plant and degrade not only leaf chlorophyll but all the intermediate derivative pigments in chlorophyll biosynthesis pathway. © 2017 Friends Science Publishers

Keywords: Dry dust; *Ficus benjamina*; Leaf extracts; Leaf pigments; Roadside

Introduction

Ficus benjamina is a landscape plant belongs to family Moraceae which is most frequently planted on central medians along roadside throughout the world. It has tree like growth habit, graceful drooping branches, green glossy leaves (Starr *et al*., 2003). In the past few years, a considerable decrease in growth and development of plants were recorded along roadsides (Chaturvedi *et al*., 2013). The ability to absorb various trace elements has been frequently documented in *F. benjamina* by secretion of latex and morphological characteristic of leaf to accumulate metals and dust particulates on or in their tissues (Tang and Turner, 1999). This makes this plant as an excellent adsorbent of dust particles and hence fixes the atmosphere dust (Reyes *et al*., 2012). However, leaf surface morphology is an important factor governing foliar uptake of trace elements (Tang and Turner, 1999) which filter the atmosphere (Guzmán-Morales *et al*., 2011) and thus *F. benajmina* leaves were used as an indicator of atmosphere pollution (Reyes *et al*., 2012).

Fast movement of dense traffic vehicles blow a huge amount of roadside dust and consequently pollute our atmosphere. Dust has been reported as a big source of particulate pollution along central medians (Duong and Lee, 2011). The dust removed from earth surface is suspended in atmosphere and fall on plants leaves from nearby emission zone (Bagnold, 2012). Increase roadside dust deposition on plant leaves becomes an indirect threat for endangered plant species to sustain along central medians (Wijayratne *et al*., 2009). Leaves of delicate ornamental plants along road side are highly exposed to fine particulate matter and smoke from regular vehicle movement (Freer-Smith *et al*., 1997). On the other hand, plants like *F. benjamina* help to remove dust particles from atmosphere and clean the air quality in urban places (Beckett *et al*., 1998). Dust is considered to be a big challenge for environment, human health and plant physiology (Liu *et al*., 2013). Gas exchange and water uptake efficiency in plants is decreased when dust settled down and make layer on leaves of plants, which directly affect the photosynthesis. Absorbance of infrared radiation level is 2–3°C higher in dusted leaves as compared to leaves in dust-free areas which has a degradation effects on plant development (Sharifi *et al*., 1997).

Dust allows the penetration of phytotoxic gas pollutants in ornamental plants and affects the process of photosynthesis, respiration and transpiration which in response reduce the growth and productivity of plants (Gratani *et al*., 2000). In *Fagus sylvatica,* different levels of air pollution have affected stomatal conductance and functioning of various photosynthetic pigments (Taylor and Davies, 1990). Studies showed that air pollution such like

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cement (Iqbal and Shafiq, 2000), brick kiln (Skinder *et al*., 2014), vehicle exhaust emission (Honour *et al*., 2009), dust storm (Ibrahim and El-Gaely, 2012) and sand storm (Badarinath *et al*., 2007) have been found to be the most toxic environmental factor, which physically block stomata (Krajíčková and Mejstřik, 1984) and cause damage to plant growth and development (Prajapati and Tripathi, 2008). However, little is known about effect of roadside particulate dust deposition on chlorophyll contents and their derivatives in leaves of filtration plants. We predict that dust shading on leaf surface attenuate plant physiology and level of photosynthetic pigments can be affected. Thus the current study was carried out to determine the adverse effects of roadside dust deposition on photosynthetic pigments and their derivatives in *F. benjamina*.

Materials and Methods

Site Description

The experiment was conducted in green house under the uniform natural environmental condition at the Ornamental Horticulture Nursery Farm, Department of Horticulture, The University of Agricultural, Peshawar-Pak (34.01° N latitude, 71.6° E longitude; 350 m altitude), Peshawar (Zakirullah and Khalil, 2012).

Experimental Design

The experiment was laid out in completely randomized design. A total of 36 uniform sized pots of healthy and uniform sized *F. benjamina* of more than one-year-old plants were selected and divided in to four groups (n=9) and three replicates. Different dry dust treatment combinations were T_1 (1 g plant^{-1}), T_2 (3 g plant^{-1}) and T_3 (5 g plant^{-1}) while T_0 (0 g plant^{-1}) was set as control. All the plants were grown with three parts fine sand and one-part natural manure. Pots were 14 cm in diameter and 13 cm in depth. The plant pots were properly labeled and randomly divided in dust chamber partitions at green house. Dry dust was collected through vacuum cleaner from road sides and was passed through fine filters to get fine particles. That dust was sprinkled throughout the experiment with different level of treatment for the purpose of dusting twice a week for a period of 4 months. Leaves and root samples were collected in quintuplicate from each plant according to Herbarium (2013) and brought to lab on ice packs for further processing.

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS Assay)

Abscisic acid (ABA) contents of leaves and roots were analyzed by LC-MS/MS assay (Ma *et al*., 2008) with mild modification. In brief, fresh leaf and roots tissues were powdered in pestle and mortar by using liquid nitrogen and extracted with acetone/water/acetic acid at ratio of 80:19:1 (v/v/v) at -20°C. The extract was vortexed (BioCote, model SA8, United Kingdom) and centrifuged at 500×*g* for 5 min in centurion scientific centrifuge (model 1020 DE, West Sussex, United Kingdom) to save the supernatant (needs a couple of re-extraction), then dried using rota-vapour (BÜCHI Rota-vapor R-205, Switzerland) and then under nitrogen stream followed by extract reconstitution in the aceto-nitrile/water/acetic acid (90:10:0.05, v/v/v), centrifuged at 3024×*g* for 15 min and filtered. Finally, 10 µL of the filtrate was injected in to the LC-MS/MS system (Thermo Electron, San Jose, CA, USA). Different concentrations of standard ABA (Sigma, St. Louis, MO, USA) were run before the sample.

Determination of Leaves Pigments

Chlorophylls and their derivatives were determined by using the method of Yang e*t al*. (1998). The leaf samples were weight by digital balance (Shimadzu, model AY220, Japan) and 0.25 g sample was dried with liquid nitrogen and grinded into powder with motar and pestle. The extract dissolved in 5 mL of 80% acetone was centrifuged at 756×*g* for 5 min. Green colour supernatant was saved. Further the sample was put into cuvette and the absorbance was measured at 663.6, 646.6 and 440.5 nm using spectrophotometer (GENESYS 10 UV-VIS, Thermo Spectronic, Rochester, USA).

Chlorophyll-*a* (Chl-*a*), chlorophyll-*b* (Chl-*b*), total chlorophyll (TChl) and carotenoids (Car) were calculated by hiring mathematical expression of Holm (1954) and Porra *et al*. (1989) given below:

$$\text{Chl-}a\ (\mu g/mL) = 12.25A_{663.6} – 2.55A_{646.6}$$

$$\text{Chl-}b\ (\mu g/mL) = 20.31A_{646.6} – 4.91A_{663.6}$$

$$\text{TChl}\ (\mu g/mL) = 17.76A_{646.6} + 7.34A_{663.6}$$

$$\text{Car}\ (\mu g/mL) = 4.69A_{440.5} – 0.267\ \text{Chl-}a + \text{Chl-}b$$

The acetone solution was mixed with equal amount of hexane and centrifuged at 756×*g* for 3 min until interface appear. The upper phase containing less polar compounds dissolved in hexane and lower phase contains porphyrin, chlorophyllide and more polar compounds dissolved in acetone. Upper phase was collected in another test tube and lower fraction is measured at absorbance of 575, 590, 628, 667, 650 and 440.5 nm using spectrophotometer. The mathematical expression of Kahn *et al*. (1976) was used to calculate protoporphyrin (PPIX), magnesium protoporphyrin (MGPP) and protochlorophyllide (Pchlide).

$$\text{PPIX}\ (nM) = 196.25\ A_{575} – 46.6\ A_{590} – 58.68\ A_{628}$$

$$\text{MGPP}\ (nM) = 61.81\ A_{590} – 23.77\ A_{575} – 3.55\ A_{628}$$

$$\text{Pchlide}\ (nM) = 42.59\ A_{628} – 34.22\ A_{575} – 7.25\ A_{590}$$

The method of McFeeters *et al*. (1971) was used to calculate the content of chlorophyllide-*a* (Chlide-*a*) and chlorophyllide-*b* (Chlide-*b*) by using the following mathematical expressions:

$$\text{Chlide-}a\ (mM) = A_{667}/A_{74.9}$$

$$\text{Chlide-}b\ (mM) = A_{650}/A_{47.2}.$$

The more polar carotenoids (MP-Car) pigment was calculated according to mathematical expression used by Holm (1954) and Porra *et al*. (1989):

MP-Car (µg/mL^{-1}) $=4.69A_{440.5}–0.267$ TChl

The upper hexane fraction was dried with liquid nitrogen and pellets were dissolved in 80% acetone. Chlorophyll molecules were denatured by adding one drop or 50 µL of 12.5% Hydrochloric acid. The absorbance was measured at 665.4, 653.4 and 470 nm. The pheophytin-*a* (Phe-*a*), pheophytin-*b* (Phe-*b*) and less polar carotenoid (LP Car) contents were calculated by using the following mathematical expression (Lichtenthaler, 1987).

Phe-*a* (µg/mL) $= 22.42A_{665.4}–6.81A_{653.4}$

Phe-*b* (µg/mL) $= 40.17A_{653.4}–18.58A_{665.4}$

LP Car (µg/mL) $= (1000A_{470}–4.78A_{653.4}) /164$

Statistical Analysis

The Kolmogorov Smirnov test was employed to test the normal distribution of the data. The data recorded in different treatment groups of T_0, T_1, T_2 and T_3 was subjected to analysis of variance (ANOVA) suitable for completely randomized design using statistix 8.1 software pakage (Statistix®, Analytical Software Inc, Tallahassee FL, USA). Significant (P<0.05) findings were tested by least significant difference (LSD) (Steel and Torrey, 1980).

Results

Stress Hormone

The results revealed that dust deposition significantly increased the concentration of ABA in leaves of *F. Benjamina*. Similarly, in root samples a maximum abscisic acid (1.02 µg g^{-1}) was noted in T_3; however, minimum (0.12 µg g^{-1}) was noted in T_0 (Fig. 1).

Chlorophyll Contents

A negative association was found between dust and chlorophyll contents. Chlorophyll-*a* content (18.93 µg/mL) was recorded higher in T_0, while, lower (13.12 µg/mL) in T_3. Dust exposure caused a significant reduction of chlorophyll-*b* in T_3 (4.14 µg/mL), as compared to T_0 (8.73 µg/mL). Furthermore, higher trend of total chlorophyll (27.67 µg/mL) was observed in T_0, while, lower (17.26 µg/mL) in T_3 (Fig. 2).

Carotenoids Contents

The carotenoid contents were also reduced by dry dust deposition on leaves of *F. benjamina* (Fig. 3). In comparison with T_0 (3.84 µg/mL), a significant reduction in carotenoids contents was observed in T_3 (2.93 µg/mL). Statistical analysis depicted that more polar carotenoid

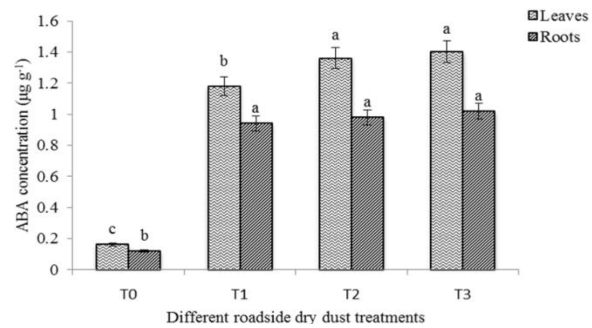

Fig. 1: ABA (µg g^{-1}) in leaves and roots of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other (P<0.05). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

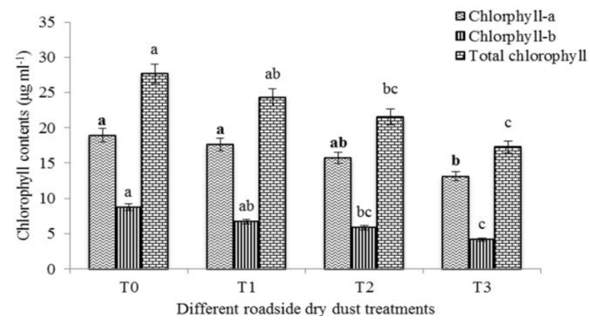

Fig. 2: Chlorophyll-*a*, chlorophyll-*b* and total chlorophyll contents (µg mL^{-1}) of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other (P<0.05). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

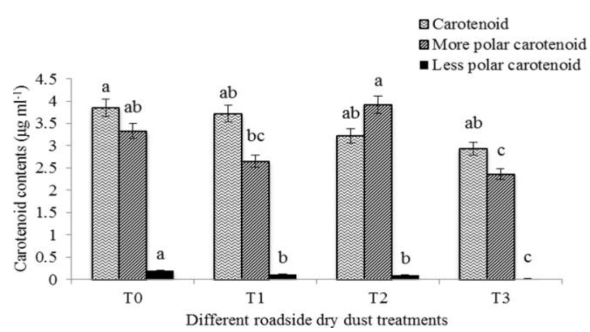

Fig. 3: Carotenoid, more polar carotenoid and less polar carotenoid contents (µg mL^{-1}) of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other (P<0.05). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

contents were significantly higher in T_2 (3.91 µg/mL), while lower level was recorded in T_3 (2.36 µg/mL). Data revealed that dust deposition significantly reduces less polar carotenoids contents, as compared to control. Less polar carotenoids contents were significantly higher in T_0 (0.20 µg/mL); whereas, lower level of polar carotenoid contents (0.02 µg/mL) were recorded in T_3 treated plants.

Porphyrin Contents

Protoporphyrin content was found higher in T_0 (17.062 n*M*) and T_2 (15.498 n*M*), while minimum in T_1 (12.13 n*M*) and T_3 (12.58 n*M*). Magnesium protoporphyrin contents were reduced as the dry dust deposition increased. Higher content of magnesium protoporphyrin (15.66 n*M*) were found in T_0, while lower (9.34 n*M*) content were observed in T_3 (9.34 n*M*). T_0 exhibited significantly higher protochlorophyllide contents (9.53 n*M*), as compared to other treatment, whereas, lower protochlorophyllide contents (4.99 n*M*) were found in T_3 treated (Fig. 4).

Chlorophyllide Contents

Data revealed that chlorophyillide-*a* content was reduced as the dry dust deposition on *F. benjamina* increased. T_0 exhibited higher chlorophyllide-*a* contents (0.02 m*M*), as compared to T_3 (0.01 m*M*). Similarly, dust deposition on leaves of *F. benjamina* plant has negative impact on chlorophyllide-*b* pigments (Fig. 5).

Pheophytin Contents

Pheophytin-*a* contents decreased significantly as the dry dust deposition increased. The higher trend was observed in T_0 (0.62 µg/mL), while, lower in T_3 (0.19 µg/mL). Furthermore, the highest ($P>0.05$) pheophytin-*b* contents were recorded in T_0 and T_1; and lowest in T_3 (0.39 µg/mL) (Fig. 6).

Discussion

ABA is a plant stress marker and was increased in leaves and shoots of *F. benjamina* with deposition of dry dust. Our findings support the results of Zhang and Davies (1989) who reported that ABA increases in roots of dehydrated plants. Leaves treated with dry dust deposition alters the osmotic potential of stomatal guard cells, initiating to shrink and close the stomata (Steuer *et al.*, 1988), which results in reduced transpiration (Munns and Cramer, 1996) and increase in ABA (Seo and Koshiba, 2002) which prevent production and development of leaf pigments. Our findings confirm the results of El-Khawas (2011) who reported that roadside dust deposition on leaves of certain medicinal plants increases ABA concentration. Monni *et al.* (2001) also found increase in ABA contents in

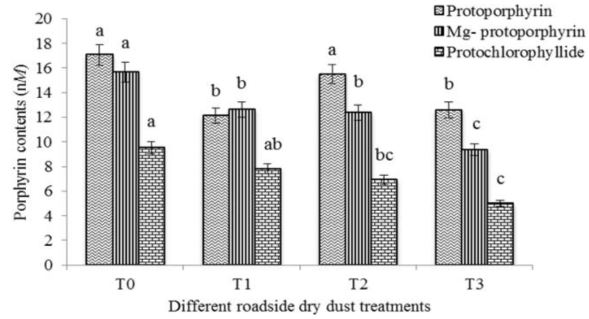

Fig. 4: Protoporphyrin, magnesium protoporphyrin and protochlorophyllide contents (n*M*) of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other ($P<0.05$). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

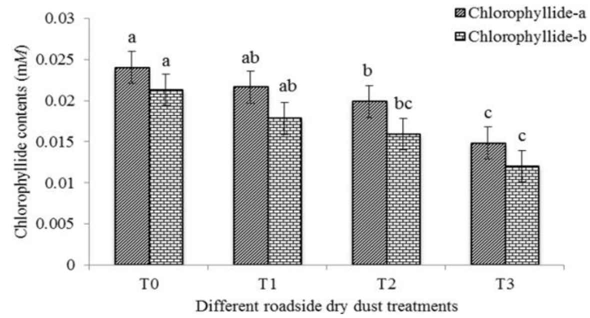

Fig. 5: Chlorophyllide-*a* and chlorophyllide-*b* contents (m*M*) of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other ($P<0.05$). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

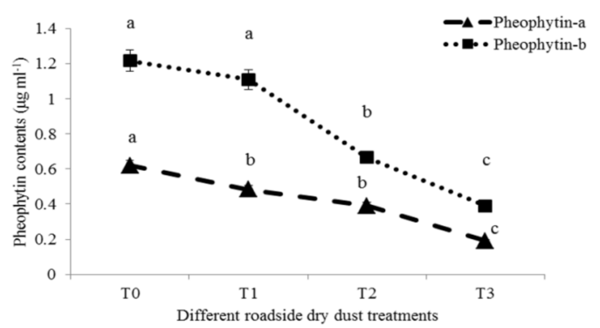

Fig. 6: Pheophytin-*a* and pheophytin-*b* contents (µg mL^{-1}) of *F. benjamina* affected by different roadside dry dust treatments. [a-c]Mean ±SEM with different superscript are significantly different from each other ($P<0.05$). Whereas, T_0 (0 g plant^{-1} dry dust), T_1 (1 g plant^{-1} dry dust), T_0 (3 g plant^{-1} dry dust) and T_0 (5 g plant^{-1} dry dust)

stems and leaves of *Empetrum nigrum* L. grown 0.5 km away, as compared to those grown at 8 km away from pollution source. Previous investigations showed that changes in leaf physiological parameters had direct effect on growth, yield and development of plants (Proietti and Famiani, 2002). Dust deposition on leave surface of plants cause reduction in quantity and intensity of light to reach the chloroplast that increases leaf temperature (Hirano *et al*., 1995), decreases the photosynthetic pigments (Thompson *et al*., 1984) and slow down photophosphorylation. Similarly, the presence of toxic heavy metals in dust have been reported to induce negative effect on chlorophyll-*a*, chlorophyll-*b*, total chlorophyll (Leghari *et al*., 2014), carotenoids and other derivatives (Prajapati and Tripathi, 2008).

The pathway of biosynthesis of photosynthetic pigments and their derivatives are linked together and have direct co-relationship with each other (Gross, 2012). The biosynthesis of protoporphyrin, magnesium protoporphyrin, protochlorophillide need light (Armstrong *et al*., 1995) as they are a strong photo-sensitizer (Woodward, 1961), which is followed by the chlorophyllide-*a* and chlorophyllide-*b*. Similar finding were also reported by Nanos and Ilias (2007) who found that decrease in photosynthetic pigments and their derivatives was due to the shading which indicate a similar effect roadside dust on the leaf surface which in return reduce green pigments such as chlorophyll. Due to dust stress, NADPH becomes lower which is required in the enzymatic conversion steps from protoporphyrin to protochlorophylilde and chlorophyllides in the presence of enzymes Mg-Chelatase, vinyl reductase, protochlorophyllide oxidoreductase and other accessory elements of light and oxygen (Reinbothe and Reinbothe, 1996). Furthermore, not only shading but low gaseous exchange and stomata clogging can also decline the biosynthetic pathway of chlorophyll and their derivatives (Taylor and Davies, 1990; Sharifi *et al*., 1997). Current results are in strong conformity with Prusty *et al*. (2005) who found significant decrease in protoporphyrin, magnesium protoporphyrin and protochlorophyllide contents in polluted plants.

Weak acidification of chlorophyll-*a* and chlorophyll-*b* is followed by pheophytin-*a* and pheophytin-*b* respectively in degradation pathway (Yang *et al*., 1998; Klimov, 2003). Lower pheophytins contents in dust deposited plants are due to the lower content of chlorophyll in dusted plants (Dissanayake *et al*., 2012) and acidic nature of dust particles which degrade the chlorophyll (Van Boekel, 1999).

Conclusions

We conclude from this study that accumulation of dry dust on leaves cause stress and reduce biosynthesis of photosynthetic pigments and their derivatives which inhibit the process of photosynthesis in these plants. Moreover, decrease in chlorophyll contents directly depend and interlinked with their derivative pigments. Furthermore, decrease in photosynthetic pigments and their derivatives leads to severe reduction in growth and development of plants along roadsides.

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