Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences 8 (8): 562-565, 2009
ISSN 1682-3974

The Effect of Air Pollution on Some Morphological and Biochemical Factors of *Callistemon citrinus* in Petrochemical Zone in South of Iran

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Abstract: In this study the main purpose is to study on some biological factors of a deciduous tree species *Callistemon citrinus* in polluted and high temperature condition in South of Iran. The location was selected because of high rate of industrial pollution that is caused by petrochemical companies. *Callistemon citrinus*, family Mirtaceae, is a kind of evergreen tree with alternate, linear leaf. The type and margin of leaves are simple and entire, round brown fruits with hard or dry cover and red flowers. The common name of *C. citrinus* is red bottlebrush that is describe it's flowers shape. The concentrations of chlorophyll A, B total chlorophyll, carotenoieed, soluble sugar, proline and morphological effects were examined in the leaves of tree species, growing in polluted area as compare to unpolluted condition. In the polluted regions higher concentrations of soluble carbohydrate, proline, chlorophyll A, B, carotenoieed were observed in comparison with trees in the unpolluted regions. The morphological characters affected by stress of pollution. Characters such as leaf area, length and breadth of blade, showed decrease.

Key words: Air pollution, *Callistemon citrinus*, soluble carbohydrate, chlorophyll, proline

INTRODUCTION

Natural air pollutants have always been a part of the earth's history. The natural composition of air is mostly nitrogen and oxygen, along with water droplets, fine particles and small amounts of other gases, such as carbon dioxide, nitrous oxide, methane, ammonia and argon. These gases can be either free in the air or associated with water vapor. Air pollution is any visible or invisible particle or gas found in the air that is not part of the normal composition of air. Natural air pollution has been around for millions of years, but during the last century, pollution created by humans started to become a major concern. We are most familiar with visible air pollution like smog; however, many other air pollutants, including some of the most dangerous, are totally invisible. Air pollution from man-made sources is the result of our increasing use of large quantities of fuel and high levels of industrial activity. Plant and animal life has adapted to most natural pollutants except for the rare catastrophic occurrences that create worldwide climate changes. The most serious air quality concerns are the additional, often harmful, pollutants that humans add to the air. Since little can be done by humans about natural pollution, our main concern has to be with the additional pollutants from human activity. Because of the increasing concern over toxic chemicals in the air we breathe, many laws have been passed to control emission sources.

Various stresses affect plants in different ways. The effects of air-borne pollutants are varied, such as the edges of the leaves dying, brown spotting on exposed surfaces, leaves fading, stunted growth, buds dying, flowers, fruits or leaves falling off or suppressed shoot growth. Determination of resistant plant to pollutant is one of the useful things that can be done. In this case many other workers have done various researches.

Plants fumigated with 40, 80 and 120 ppbv concentrations of O_3 exhibited significant reduction in total chlorophyll content, RuBP carboxylase activity and net photosynthesis (Chapla and Kamalakar, 2004). *Cassia siamea* plants growing at two different sites (polluted and non-polluted) on two important roads of Agra city exhibited significant differences in their flowering phonology and floral morphology (Chauhan *et al*., 2004). Inclusion of cobalt and molybdenum in nutrient medium brought about a substantial increase in pigments composition, total N content and protein content whereas proline accumulation was reduced in pea (*Pisum sativum* L. cv. *Arvenses*). Plants grown under NaCl salinity. Between the two micronutrients, the effect of cobalt was more pronounced and consistent and it was more so at lower (50 μM) concentration in saline as well no saline conditions (Singh *et al*., 2005).

According to previous studies plants response to air pollution in range of subcellular level to entire ecosystem, anatomy pathology and biochemistry (Mudd and Kozlowski, 1975). In 2001 researchers showed that stimulation of photosynthetic rates in elevated CO_2 was nullified by decreased total leaf area (Noormets *et al*., 2001). Light preconditioning caused increased ascorbic acid concentrations in the leaves of two

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tobacco (*Nicotiana tabacum* L.) varieties (Menser, 1963). The effect of NH_3 and SO_2 fumigations on cold stress and water stress of *Pinus svlvestris* were also examined. Bud burst and subsequent shoot growth was strongly reduced by NH_3 fumigations (Dueck *et al*., 1990). In closed-chamber fumigation experiments dry matter partitioning and chlorophyll fluorescence of wheat were studied, analyzing the effects of ozone during different stages of plant development (Soja and Soja, 1995). In this study, the air pollution effects on the content of soluble carbohydrate chlorophyll A, B, total chlorophyll, carotenoieed, soluble sugar, proline and morphological effects were investigated in *Callistemon citrinus* in Mahshahr City in Iran. In a previous record this species identified as suitable tree species for control of effect of air pollution and showed pollution tolerance (Santhoshkumar and Paulsamy, 2006).

MATERIALS AND METHODS

Site description: The investigation was carried out in damaged forests (situated around the industrial region of Mahshahr City) and in a forests from a relatively unpolluted region. The forests studied in two regions consisted of 10 to 15year-old. The study was conducted between 2 May 2009 to 5 Sep 2009. The climate in this land is warm and humid that is influenced by the Persian Gulf which is located in South of Iran.

The mean annual air temperature was about 35-40°C and about 30°C for the growing season. The average annual precipitation was about 500 mm (200-250 mm for the growing season), with a spring-summer maximum. Prolonged summer droughts and warmthness are typical.

Air pollution: The main toxicants in the industrial region of Pet zone are: nitrogen oxides (NO, NO_2), sulphur dioxide, carbon oxides, HF, NH_3, Cl, HCl, CaO, $CaCO_3$, solid or liquid aerosols and organic compounds, higher amounts of silicon, aluminum, sodium, Iron, cadmium and lower concentration of magnesium, calcium, nickel, lead, copper, zinc, manganese, as well as dust. To this add the dust and gases from more distant industrial establishments, from the city and from motor vehicles.

Biochemical analysis: Ten similarly aged trees of the species were sampled from damaged and control stands. Young leaves were collected from the each tree crown. In the morning of sunny days in spring. The leaves were analyzed after mechanical cleaning of the leaf blades. Measuring some morphological factors including (leaf area, length of petiole and etc.) in addition to contents of water soluble sugar, chlorophyll and proline were determined by the biochemical method. Absorption was measured at specific light wave with a spectrophotometer.

Analyze of soluble sugar were done after making hydro alcoholic extract of leaf powder total carbohydrate concentration was determined by the phenol-sulfuric acid procedure (Verma and Deby, 2001) chlorophyll A, B and total in addition to carotinoid was analyzed by use of acetone 85% (Lichtenthaler, 1987).

To determine Proline we used fresh leaves, ninhydrin and acetic acid (Irigoyen *et al*., 1992).

At least three readings of samples were taken, each sample was replicated twice and the entire experiment was repeated once. The results were analyzed statistically by using the standard MSTATC in order to determine the significant differences between polluted and control stands.

RESULTS

The trees in selected area expose to pollutant that is release in the environment by industrial activities of pet zone and pollution and high temperature cause some changes in plants. In morphological analysis length of vein, petiole, length and breadth of leaf and leaf area were examined. Length of vein and leaf, breath of leaf and leaf area showed reduction significantly. Length of petiole didn't showed changes (Table 1). Length of vein changed from 6.24 to 4.89 cm, length of leaf reduced from 6.01 to 4.83 cm, we had also reduction in breath of leaf from 0.74 to 0.49 cm the area of leaf decreased from 4.13 to 2.87 cm^2.

The investigation of biochemical effects the concentration of chlorophyll A, B, total chlorophyll, carotinoid, water soluble sugar and proline were determined. In all characters increase has been revealed except of content of proline (Table 2).

Table 1: Effect of air pollution on the leaf characteristics of *Callistemon citrinus*

Characteristic	Unpolluted	Polluted
Length of vein (cm)	6.24A	4.89B
Length of leaf (cm)	6.01A	4.83B
Breath of leaf (cm)	0.74A	0.49B
Length of petiole (cm)	0.52A	0.41A
Leaf area (cm^2)	4.13A	2.87B

Existence of same letters in every row showed the no significant difference at 5% level

Table 2: Effect of air pollution on biochemical characteristics of *Callistemon citrinus*

Characteristic	Unpolluted	Polluted
Chl A	4.20A	7.98B
Chl B	1.83A	2.07B
Chl T	5.99A	10.02B
Car	1.57A	3.30B
Soluble sugar	43.00A	51.00B
Proline	0.05A	0.07A

Existence of same letters in every row showed that no significant difference at 5% level

DISCUSSION

The results showed that leaf area, length breadth of blade and length of vein reduced significantly. Totally describe of air pollution is related to morphology of area of leaf visible damage including reduction of leaf area, changes in morphology as compare to unpolluted condition, necrosis and chlorosis (Heath, 1980). Naido and Chricot (2004) showed that by the effect of air pollutant exchange of gases on area of leaf of *Avicenia* marine decreased. Significant reduction in length and area of leaflets and length of petiole of G. officinal of polluted plants was recorded (Jahan and Iqbal, 1992). One way to increase tolerance in contrast with stress is to balance the water content of tissue by decrease the leaf area (Maynard and Orcutt, 1993). It and seems that this species use this way as defense mechanism. Similarly leaf biochemical effects of above mentioned species also showed changes. The content of chlorophyll A,B, total chlorophyll, carotinoid soluble sugar showed increase significantly and concentration of proline also increase. Investigation proved that chlorosis, is the first indicator of Flour effect on plant (Kendrick *et al*., 1956). Hui (2007) showed reduction in photosynthesis because of the PSII function damage, in sensitive species of tobacco. Carotinoids exist in plasma of plant tissues, photosynthetic or non photosynthetic, the function of carotinoids in chloroplasts is as pigments to capture the light. But probably more important role is in protecting the cells and live organisms encounter with damage of free radical oxidative (Fleschin *et al*., 2003). It seems that *C. citrinus* by increase in amount of pigments tolerates in stress of pollution and doesn't suffer damage and metabolic disorder. Other workers showed changes in soluble sugar concentration by the effect of stress in plants. Investigations revealed that the more resistant species plants to the air pollution as compare to sensitive species showed more concentration of soluble sugar (Kameli and Losel, 1993; Ludlow, 1993). On the other side researches showed that Concentrations of total and soluble sugars decreased significantly in the sensitive trees to the air pollution. In damaged *Q. cerris* leaves the decrease in concentrations of sugars was higher in September. The decrease in total sugar content of damaged leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate (Tzvetkova and Kolarov, 1996). This contrast is because of choosing chlorosis and damaged leaves in tzvetkova and kolarov's research however in this research we selected undamaged pant leaves. Furthermore, increase in amount of soluble sugar is a protecting mechanism of leaves it has been shown in Pinto bean in exposure with different concentration ofozone (Dugger and Ting, 1970). So increase in amount of total soluble sugar in leaves of *Callistemon citrinus* could indicate that tolerance of this species. In this study the concentration of proline in *C. citrinus* is increased in polluted area but not significantly (<0.05). Obviously proline has main role in protection in different kinds of stress. Accumulation of proline in plants is a physiological response to osmotic stress (Szekely, 2004). Contribution of proline in salt stress is reported (Melony *et al*., 2001) and concentration of proline in almond seedling in drought stress is also shown as far as the sensitivity to stress is in positive correlation with proline accumulation, no significant changes in amount of proline in leaves of *Callistemon citrinus* probably indicate that the plant tolerate to toxic effect of air pollution to some extent. *Calistemon citrinus* that is analyzed in this study may be good bioindicators. In relation to biochemical effects showed the high resistance to industrial emissions. This tree species could be recommended for afforestation of industrial regions. According to accessible information this research has been done for the first time in this region.

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