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RISK EVALUATION OF BRICK KILN SMOKE TO KIDNEY BEAN (*PHASEOLUS VULGARIS*)

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Abstract

In view of global change caused by green house gases, bean plants (*Phaseolus vulgaris* L.) were exposed to brick kiln smoke in close chambers under laboratory conditions. Brick kiln smoke was produced by firing bituminous coal and furnace oil. The duration of smoke exposure was 4, 8 and 12 hours on daily basis which lasted until crop maturity. Additional control plants were grown in ambient air for evaluating the 'brick kiln smoke effect on plants. A significant reduction in mean length of shoot and roots of plants exposed to smoke for 8 and 12 hours compared with the control were observed. The pollution effects were directly proportional to the intensity of the exposure. The average pore areas of stomata from plants treated for 8 and 12 hours were significantly smaller compared with control plants. The continued uptake of the gaseous pollutants through leaf stomata eventually resulted in cellular damage, which was manifested through effects on growth and the foliar characteristics. Microscopic studies of stomata revealed that a longer duration of smoke exposure resulted in deformation and destruction of stomatal shape. A critical look on the experimental data revealed that the epidermis on adaxial surface was more affected as compared with one present on abaxial surface.

Introduction

Regulation, handling and bioremediation of hazardous materials require an assessment of hazard or risk to some living species other than human being, or assessment of risk to entire ecosystem. Assessment endpoints are values of the ecosystem that are to be protected and are identified early in the analysis. Such endpoints may include life cycle stages of a species, reproductive patterns or growth patterns. Ecosystem risk assessment is at its dawn and this area of environmental sciences still requires extensive work in the industrialized nations of the world for the sustainability of global ecosystem.

Air pollution is most dangerous among all type of pollutions because man and even plants need fresh air for their normal metabolic pathways. Honjyo *et al*., (1980) in a study found that the trees in the polluted area show vital decay. Rao (1985) argued that pollutants and their combinations cast toxic effects on plants. Data on air pollution in Pakistan is insufficient (Anon., 1999). Although ambient air standards (Pakistan Environment Protection Ordinance, 1983) and Law on air pollution exist in Pakistan which demands curtailing the emission of toxic gases into atmosphere but strict legislation implementation is required to enforce these laws to save natural vegetation and habitats.

In Pakistan one of the most common sources of air pollution is old-fashioned brick kilns, which are spread, in the whole country and release poisonous smoke in the environment. According to a careful estimate there are many thousands of brick kilns working at present throughout the country. Old rubber tyres, low-quality coal, wood and used oil is used in these kilns as fuel. Consumption of these fuels, combined with inefficient combustion process produces a large quantity of hazardous gaseous that is injurious to the health of the community living in the surroundings the kilns as well as the workers of the kiln (Anon., 2004). In view of ever-increasing air pollution problems during the past few years, and Pakistan being an agricultural country, it is highly desired to take serious notice of this alarming situation and is an urgent need to carry out such studies in Pakistan.

Materials and Methods

Plant materials and soil characterization: The seeds of *Phaseolus vulgaris* were procured from Pulses Programme, National Agricultural Research Centre Islamabad, Pakistan. Bean seeds (*Phaseolus vulgaris* L.) were sown in agricultural farm soil and maintained in a greenhouse for 4-5 d, until the cotyledons were fully expanded. Agricultural farm soil was taken from a ranch in college campus in Islamabad. The samples were sieved through a 2 mm sieve and air-dried for 3 d. NH_4NO_3 and KH_2PO_4 were applied as basal fertilizers @ 0.43 and 0.33 g kg^{-1}, respectively (Wu *et al*., 2004). After soil amendment, following parameters were determined: pH value (solid distilled water 1:2.5, w/v); total organic matter (450 and 600^{o}C, after heating for 6 h in a muffle furnace); total nitrogen content; total phosphorus and water-soluble P; water-soluble N; water-soluble K; total Pb, Zn, Cu and Cd contents (mixed acid digestion with concentrated HNO_3, HCl, and HF = 3:1:1, v/v); and water-soluble metal contents (solid distilled water 1:2.5, w/v) (Bao, 2000).

Seedlings were then transplanted into a steam sterilized agricultural farm soil and grown in containers under greenhouse conditions for another 10-11 d at a temperature ranging between 17 and 23°C and RH between 55 and 85%, with a 14 h photoperiod. Uniform plants were selected and treated with brick kiln smoke in a treatment room when the primary leaves were fully expanded (about 15 d after sowing).

Smoke treatment: The experiment was a fully randomized block design, with three replicates. In each block there was one treatment "chamber" and one control "chamber". The exposure to brick kiln smoke was performed in a treatment chamber. A small room was used to produce brick kiln smoke and thus was designated as treatment chamber. Brick kiln smoke was produced by firing bituminous coal and furnace oil. Three blocks of treatment were established i.e., the duration of smoke exposure was 4, 8 and 12 hours on daily basis. This smoke exposure lasted until crop maturity. Additional control plants were grown in ambient air for evaluating the 'brick kiln smoke effect' on plants.

Growth measurements: Five control and five treated shoots were sampled at random from each of the *Phaseolus vulgaris* L. Each of the sampled shoots was removed carefully using a pair of scissors and was transferred to a large polythene bag which was duly labeled and further characterized. The fresh weight of shoots (gm), total length of shoots (cm), number of leaves and number of nodes were studied.

Epidermal imprints: One normal and one damaged leaf were selected from control and treated *Phaseolus vulgaris* L., and the epidermal imprints of the adaxial and abaxial surfaces were obtained by the help of a cellulose acetate paper and acetone. These epidermal imprints were mounted on glass slide and observed under microscope.

a. Number of stomata: Using a compound microscope number of stomata on adaxial and abaxial epidermal layers was noted from the relevant epidermal imprint in a randomly drawn line transect. Three transects were studied per slide and their mean values and standard errors were calculated.

b. Measurements of stomatal area: These were taken with the help of a micrometric eyepiece of a compound microscope. 15 measurements were taken per slide in 3 randomly drawn transects.

Statistical analyses: Experiment was conducted by analysis of variance (ANOVA) and the data were analyzed through SPSS v.11.5. Means were separated through Least Significant Difference (LSD) test at ($p<0.05$).

Results

Response of *Phaseolus vulgaris* to brick kiln smoke: The observations on the physical response of plants after the smoke treatments have been presented in Figs. 1-5. The results revealed that *Phaseolus vulgaris* plants exposed to smoke for four hours wilted immediately and few dark yellowish spots appeared on their leaves caused by smoke. Similarly, the plants exposed to smoke for 8 hours also wilted and dark yellowish spots also appeared but the number and affected area of these spots were slightly more than plants exposed to smoke for four hours. Wilting was also observed in plants exposed to smoke for areas of dark yellowish spots were quite larger than plants exposed for less duration. However, the plants recovered from wilting after two days, though the spots were permanent and in some cases the dark yellowish patches fell down producing holes on the leaf surface.

Effect of smoke on growth: The growth responses of *Phaseolus vulgaris* plants exposed to smoke for various durations have been presented in Fig. 1-3. A careful look at Fig. 1 reveals that there was no significant ($p<0.05$) difference between the mean values of the maximum length of shoot and root of the plants exposed to smoke for four hours compared with control. However, there was a significant ($p<0.05$) reduction in mean length of shoot and roots of plants exposed to smoke for 8 and 12 hours compared with the control. Thus smoke exposure for duration longer than 8 hours caused a significant reduction in the length of both root and shoots of *Phaseolus vulgaris*. In Fig. 2 a comparison of mean number of nodes, leaves and leaf area of *Phaseolus vulgaris* exposed to various smoke concentrations has been presented. A careful look at Fig. 2 a reveals that there is a slight difference between the number nodes of plant exposed to smoke for 4 hours and those of control plants. However the mean number of nodes in plants exposed to smoke for 8 and12 hours was reduced significantly ($p<0.05$).Similarly, mean number of leaves and leaf area in plants treated for 4 hours was reduced compared with control plants. In plants treated for longer duration (exposed for 8 and 12 hours), similar trend was noted i.e., the pollution effects were directly proportional to the intensity of the exposure. Comparative account of shoot fresh weights, dry weight and root dry weights have been shown in Fig. 3. Though dry weight of plants exposed to 4 hours was less than those of control plants, it was evident that there were no significant differences between mean shoot fresh weights and dry weights of plants treated for 4 hours over control. However, there was a significant reduction ($p<0.05$) in these parameters in plants exposed to smoke for longer duration. The differences were significant between plants exposed to 8 and 12 hours to smoky fire.

Effect of smoke on stomata: Figure 4 shows the effects of brick kiln smoke on stomatal pore length, width and pore area from adaxial and abaxial surfaces of leaves of treated and control *Phaseolus vulgaris* plans. The data presented in Fig. 4 clearly indicates that the difference in means of pore areas of stomata from epidermis on abaxial surface of *Phaseolus vulgaris* for control plants and the ones treated for four hours were statistically non significant ($p<0.05$). On the contrary, the average pore areas of stomata from plants treated for 8 and 12 hours were significantly smaller compared with control plants. Figure 4 shows the average values for stomatal size and area from leaf epidermis on adaxial surfaces of treated and control plants. A trend similar to epidermis on abaxial surface was also noted i.e. the effects of pollutant gases on leaf structure were directly proportional to exposure period. A critical look on the experimental data revealed that the epidermis on adaxial surface was more affected as compared with one present on abaxial surface.

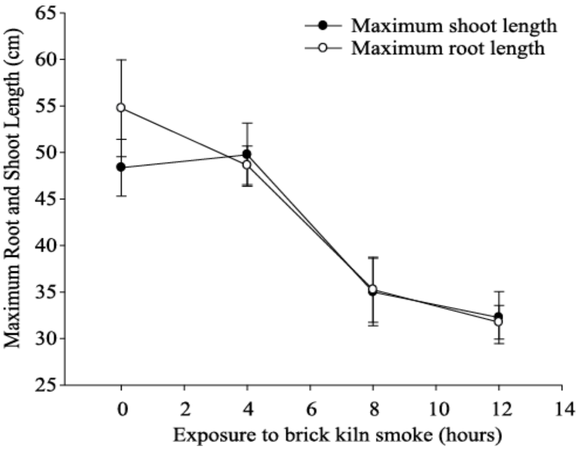

Fig. 1. Effect of brick kiln smoke on growth of *Phaseolus vulgaris*.

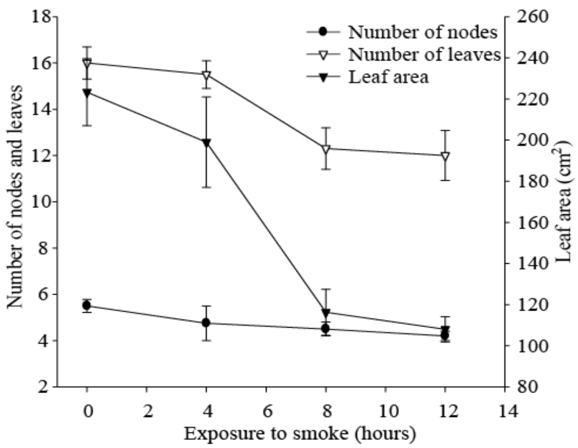

Fig. 2. Effect of brick kiln smoke exposure on number of nodes, leaves and lead area in *Phaseolus vulgaris*.

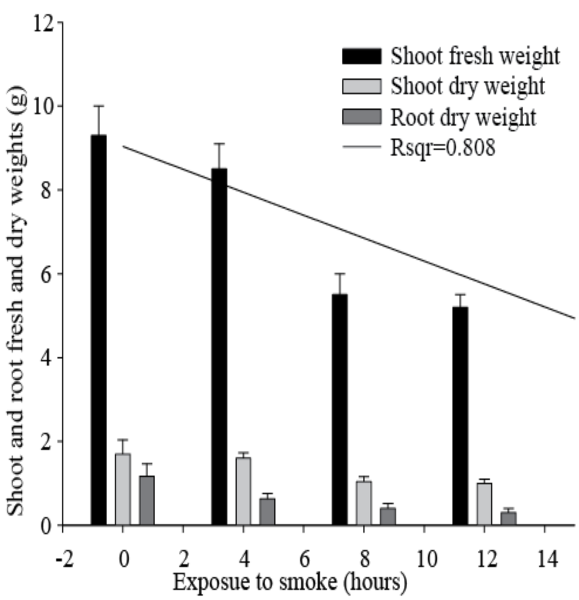

Fig. 3. Effect of brick kiln smoke exposure on shoot, root, fresh and dry weight of *Phaseolus vulgaris*.

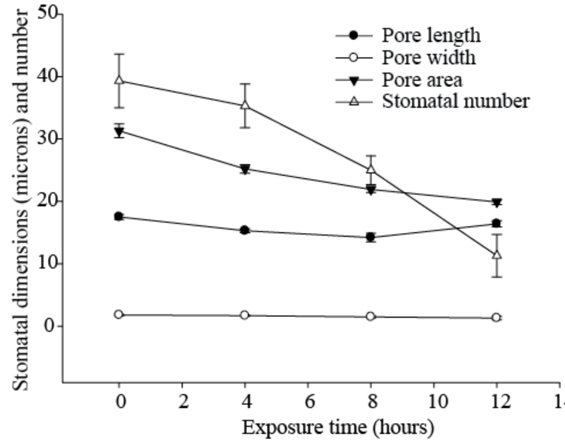

Fig. 4. Effect of brick kiln smoke exposure on stomatal features of adaxial surface of leaves of *Phaseolus vulgaris*.

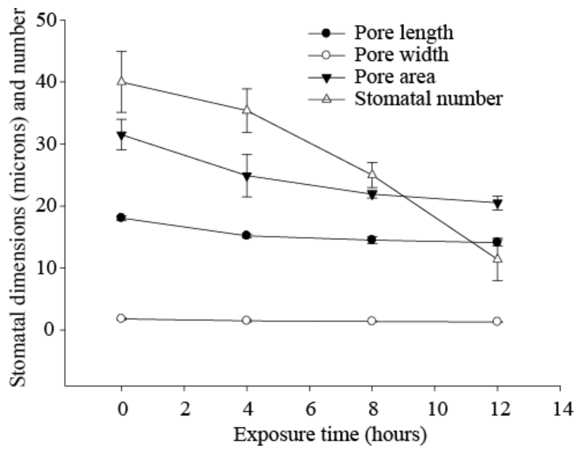

Fig. 5. Effect of brick kiln smoke exposure on stomatal features of adaxial surface of leaves of *Phaseolus vulgaris*.

Figure 5 shows the number of stomata on lower epidermis of leaves of *Phaseolus vulgaris* under various treatments. There was gradual reduction in stomatal number with the increase in time duration of smoke exposure. Such a decrease in stomatal number was marked in plants exposed to smoke for 8 and 12 hours. Likewise the number of stomata in epidermis on adaxial surface of leaves of *Phaseolus vulgaris* under the influence of brick kiln smoke showed a significant reduction. The difference was not significant between control and plants exposed to smoke for four hours. The reduction in stomatal number was greater in the epidermis on adaxial surface. Microscopic studies of stomata revealed that a longer duration of smoke exposure resulted in deformation and destruction of stomatal shape.

Discussion

Present investigation on the evaluation of impact of brick kiln smoke on kidney beans has clearly demonstrated that the growth of the plants was affected seriously when the plants were exposed to brick kiln smoke. The intensity of the harmful effects was dependent on the time duration for which the plants were exposed to smoke. The production of particulate matter during burning of fuel in brick kiln resulted in its accumulation on surface of leaves that may have clogged the stomatal openings causing hindrance in gaseous exchange. Moreover, the entry of toxic gases into leaves may have resulted in the production of toxicity symptoms in leaves. The appearance of toxicity symptoms and changes in growth serve as basis to evaluate the damage caused by brick kiln smoke. The reduction in shoot and root dry and fresh weights is an important indication of harmful effects of brick kiln smoke.

The results of the measurements performed show a clear influence of brick kiln air pollution upon the morphology of bean plants. The air pollution levels currently reached over large areas of the industrialized world may interfere with biochemical and physiological mechanisms of crop plants to an extent which ultimately leads to yield losses (Heck *et al*., 1988). Continuous intake of gaseous pollutants or deposition of particulate matter can also result in cellular damage which is manifested by visible changes in leaf morphology. Changes in growth pattern serve as the basis for ascertaining injury limits.

The smoke from brick kiln contains dust, a byproduct of brick production that may cause hazardous effects upon the nearby vegetation. After settling down on leaves and shoots the dust can clog stomatal openings to interfere with the normal gaseous exchange and thus exerting considerable bearing on photosynthesis (Creed *et al*., 1973). Hence air pollutants can be viewed as additional stress agents that can limit tree growth and productivity in concert with other stresses (Winner, 1994).

During the microscopic studies of the epidermal imprints of both surfaces of the normal and smoke effected leaves of kidney beans, it was found that number of stomata were significantly reduced in the smoke affected leaves of *Phaseolus vulgaris* (Figs.. 4 and 5). Some of the epidermal cells including stomata were badly injured and lost their shape and identity caused by the deleterious effects of brick kiln smoke. Chaudhari and Rao (1984) also mentioned these sorts of findings in their research work on the leaf epidermis of *Lycopersicon* sp**.**

Industrial effluents have adverse effects on the growth of plants (Farooqi *et al*., 2009; Kabir *et al*., 2008; Yousaf *et al*., 2013). Elevated levels of the trace gases SO_2, NOx, CO_2 and O_3 resulting from industrial emissions have well-documented physiological effects on plants (Keller, 1984; Ceulemans & Mousseau, 1994; Matyssek *et al.,* 1995). With the exception of CO_2 these trace gases are toxic to plants and cause reductions in their growth (Matyssek *et al.,* 1995). These gases enter leaves almost exclusively through the stomata and impact the internal physiology through effects on mesophyll tissue. The resultant reductions in photosynthetic processes in leaves are the primary mechanism of direct effects of these pollutants on growth and productivity. The reduction in number and photosynthetic efficiency of leaves may actually affect the carbon allocation to the whole plant resulting in reduced growth and yield as investigated here (Wahid *et al.*, 2001; Wahid *et al*., 2006a, b).

The brick kiln smoke exposure for varied durations evidently resulted in visible injury to the *Phaseolus vulgaris* when grown in laboratory conditions. The continued uptake of the gaseous pollutants through leaf stomata eventually resulted in cellular damage, which was manifested through effects on growth and the foliar characteristics. Microscopic studies of stomata revealed that a longer duration of smoke exposure resulted in deformation and destruction of stomatal shape.

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