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Research article

Effect of wood ash application on the morphological, physiological and biochemical parameters of *Brassica napus* L.

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ABSTRACT

The present study was conducted to determine the effect of wood ash application on different parameters of *Brassica napus* L. including seed germination, seedling growth, fresh and dry biomass, water content in seedlings, photosynthetic pigments, soluble sugars, total protein and cell viability. In addition, the effect of wood ash on soil microflora and accumulation of trace elements in seedlings were determined. The seeds of *B. napus* were grown at different doses of wood ash (0, 1, 10, 25, 50 and 100 g (wood ash)/kg (soil)) and the effect on various parameters was determined. Wood ash significantly inhibited seed germination at doses above 25 g/kg and there was no germination at 100 g/kg of wood ash. At lower concentrations of wood ash, most of the growth parameters of seedlings were stimulated, but at higher concentrations of wood ash most of the studied parameters were adversely affected. Wood ash was found to be very detrimental to *B. napus* when applied above 25 g/kg. Wood ash application resulted in an increased bioaccumulation of trace elements in seedlings of *B. napus*. Almost all trace elements were significantly higher in seedlings grown in wood ash above 10 g/kg as compared to the control. An increase in total microbial count was observed with wood ash treatment which was statistically significant at 1 and 10 g/kg of wood ash. It is concluded that at very high concentration, wood ash can be detrimental to plants; however, its application at lower application rate can be recommended.

1. Introduction

For a healthy growth and development, plants require a variety of different nutrients. These nutrients can be supplied by inorganic or organic or a combination of both inorganic and organic sources. In modern agriculture, the inorganic sources mostly include synthetic fertilizers. The role of fertilizers in increasing plant growth and yield have been reported fairly well (Niehues et al., 2004; Stewart et al., 2005; Mohamed et al. 2008). Due to the drastic increase in prices of synthetic fertilizers, the search for alternative sources of nutrients became increasingly important.

Wood ash, a product of incomplete combustion of wood containing organic and inorganic compounds, is considered as an important source of minerals. It can be utilized as a source of plant nutrition to save fertilizers and to realize nutrient cycling in agriculture (Bhattacharya and Chattopadhyay, 2002). Wood ash contains a mixture of oxides, hydroxides, carbonates and silicates but usually has very little nitrogen as it gets volatized during combustion process (Ozolincius et al., 2005). Wood ash has high solubility and availability of macronutrients for plants following the order as potassium > magnesium > calcium > phosphate (Eriksson, 1998). These nutrients play vital physiological roles in the formation of chlorophyll, nucleotides, phosphatides, and alkaloids as well as in the synthesis of many enzymes, hormones and vitamins (Mohamed et al., 2008).

Wood ash is getting wide spread acceptance as alternative of chemical fertilizers. Its application is similar to lime application, i.e. both materials cause liming effect, but wood ash has an additional advantage of supplying additional nutrients. However, both wood ash and lime are strongly alkaline and could damage crops if over applied. Wood ash application to crops may have both positive and negative effects. The positive effects can be attributed to higher

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availability of P, K and B in wood ash, while the negative effects can be due to the decreased supply of N (Bramryd and Fransman, 1995). Application of wood ash has been reported to increase soil fertility by increasing respiration and other activities of soil microorganisms (Fritze et al., 1993; Martikainen et al., 1994). Wood ash application has been observed to have significant positive effects on the growth and yield of some crops, especially maize (Roger and Sharland, 1997; Nottidge et al., 2005; Mbah and Nkpaji, 2009; Owolabi et al., 2003).

In addition to essential nutrients, wood ash also contains some toxic substances including organic compounds (chlorobenzenes, polyaromatic hydrocarbons and chlorophenols), radioactive elements (^{137}Cs) and toxic elements (As, Co, Cu, Ni, Pb, and Zn) which create certain environmental problems (Perkiomaki, 2004) and can be toxic to plants. Among the toxic elements, Cd has been regarded as the most hazardous metal present in wood ash because of its high toxicity and comparatively high mobility (Levula et al., 2000; Demeyer et al., 2001). At high application rate of wood ash, these toxic substances may disturb microbiological processes and nutrients cycling in soil which will adversely affect plant growth (Lundborg, 1998).

Several studies regarding the application of wood ash to forests exist, but very few studies have been conducted on its application to cereal and vegetable crops. Therefore, the present study was conducted to investigate the effect of wood ash application on an oilseed crop, *Brassica napus* L. *B. napus* is a member of the family Brassicaceae. Both winter and summer annual forms of *B. napus* are cultivated as oilseeds world wide and are considered as the most productive oilseed species under cultivation. It is the third most important source of vegetable oil after soybean and palm oil. It is also commonly used as a hyper-accumulator for phytoextraction of trace elements from soil and water (Ashraf et al., 2011). In the present study, different parameters of *B. napus* like seed germination, root and shoot growth, fresh and dry biomass, photosynthetic pigments, total sugars, total proteins and cell viability were used as end points to evaluate the effect of wood ash. In addition, the effect of wood ash on toxic metal accumulation in seedlings and microbial density in soil were investigated.

2. Materials and methods

2.1. Collection of wood ash and soil

The wood ash for the present study was derived from the combustion of *Zizipus jujuba* wood obtained from Kohat region. The soil used was collected from the Botanical Garden of Kohat University of Science and Technology, Kohat.

2.2. Physicochemical analysis of wood ash and soil

For physicochemical analysis, samples were prepared as per method of Halonen et al. (1983). pH and electrical conductivity (EC) were determined from a soil–water suspension by pH meter and EC meter, respectively. For elemental analysis, the soil samples were dried at 40–60 °C and milled to pass through a 2 mm sieve. Extractable nutrients (Ca, K, Na and Mg) in soil and ash were extracted with 1 M ammonium acetate (pH 4.65) and analyzed by flame atomic absorption spectrophotometer. Phosphorus (P) was determined calorimetrically. Total nitrogen (TN) was measured by the Kjeldahl method as described by Halonen et al. (1983). For analysis of trace metals, samples were prepared according to the method of Saeed et al. (2010, 2011). Two grams of sample was taken in a small beaker and 10 ml of concentrated HNO_3 was added and was kept for 24 h. Sample was heated on a hot plate until the production of red NO_2 fumes ceased. The samples were then cooled down and perchloric acid (2–4 ml) was added. Samples were heated again to evaporate until a small volume was left. Then aqua regia (10 ml) was added and samples were heated again to evaporate to a small volume. The samples were then transferred to 50 ml volumetric flasks and diluted with deionized water. The concentrations of different toxic metals in samples were quantified by Atomic Absorption Spectrometer (Parkin Elmer AAS 700).

2.3. Collection of *B. napus* L. seeds

Seeds of *B. napus* L. (variety Gangon) were obtained from National Agriculture Research Centre (NARC) Islamabad, Pakistan.

2.4. Sterilization of seeds

The seeds were surface sterilized with 80% (v/v) ethanol and then were rinsed with distilled water for two to three times. Sterilized seed were kept at room temperature to make them completely dried.

2.5. Germination and growth conditions

All experiments were conducted in Petri plates of 18 cm diameter. The soil was mixed with wood ash in different ratios to obtain the required doses of wood ash {0 (control), 1, 10, 25, 50, and 100 g wood ash/kg of soil}. Approximately 200 g mixture of soil and ash was filled in each Petri plate. For each treatment, there were three independent replicates. The seeds were incubated in dark for first three days for germination at a temperature of 30 °C. After 3 days of germination, seedlings were shifted to light in growth chamber at a temperature of 30 °C and 60% relative humidity. All parameters (except germination) were recorded after 10 days of seedling growth.

2.6. Germination analysis

The number of germinated seeds in each plate was examined after every 24 h for seven days. A seed with the emergence of radical was considered to be germinated. Germination was calculated as a percentage of total seeds in a Petri plate.

2.7. Determination of root and shoot length

After ten days of growth, the seedlings were separated into root and shoot and their length was measured with a measuring tape. The average length of root and shoot of seedlings was calculated and expressed in centimeter (cm).

Average length of seedlings (cm) = the sum of length of all seedlings in a Petri plate/total number of seedlings in a Petri plate.

2.8. Determination of fresh and dry weight of root and shoot

For determination of the fresh biomass of seedlings, fresh root and shoot were separated and weighed with an electric balance in gram (g). For dry weight, the fresh materials were oven dried for 24 h at 80 °C, and weighed individually with an electronic balance.

2.9. Determination of water content of shoot and root

For determination of the water content of seedlings, dry weight of root and shoot was subtracted from fresh weight of root and shoot as follow: