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SMOKE-WATER IMPROVES SHOOT GROWTH AND INDIGO ACCUMULATION IN SHOOTS OF INDIGO LINIFOLIA SEEDLINGS

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ABSTRACT

There is scarcity on researches about the effect of smoke on secondary metabolite production in plants, although smoke treatments have successfully been used for promoting the germination of many seeds, This study highlights the effects of smoke-water on shoot growth and accumulation of indigo in shoots of *Indigo linifolia* plant. Results showed that seedlings treated with smoke-water at a dilution of 1:1000 achieved the highest fresh and dry shoot mass, which was significantly (PÂ0.05) different from the control and other smoke-water dilutions tested.

A higher concentration of smoke-water (1:500) significantly increased (PÂ0.05) the indigo concentration in shoots compared with untreated seedlings. The effect of 1:1000 and 1:2000 dilutions gave non-significant increases in the concentration of indigo. These findings suggest the possible use of smoke-water for promoting the growth and accumulation of secondary metabolites in plants.

Keywords: Chinese traditional medicine; *Indigo linifolia*; Smoke-water

INTRODUCTION

It is well documented that plant-derived smoke promotes seed germination of many wild and cultivated plants, irrespective of fire sensitivity. This has generated wide interest in seed technology (Light and Van Staden, 2004) and more recently studies to determine the active compounds present in smoke and their modes of action (Light et *al*, 2009). Smoke treatments also significantly improve post-germinative growth (seedling vigor). Baxter et

al (1994) reported that the seedlings of *Themeda triandra* from smoke-treated seeds grew more vigorously without any abnormalities than untreated seeds. A similar effect was observed for Erica and Asteraceae species (Brown, 1993). Likewise, Sparg et *al* (2006) showed that the seedling vigor of a commercial maize (Zea mays) variety could be enhanced by smoke-water soaking. Furthermore, a number of studies have reported a positive influence of smoke treatments on seedling growth and yield of several crop plants, including celery okra (Kulkarni et *al*, 2007), tomato (Kulkarni et *al*, 2008), and onion (Kulkarni et *al*, 2010). Hence, smoke treatments have great potential for use in horticulture and agriculture (Light and Van Staden, 2004).

Smoke has been mainly assessed for its ability to improve germination of seeds and growth of plants. However, the effect of smoke in promoting the accumulation of secondary metabolites in plants has not been studied. The ever increasing demand for medicinal plants has increased practices in cultivating medicinal plants. Indigo has been reported as one of the major bioactive components in the plant, which is used as the marker for quality control due to its unique pharmaceutical activities. Since the application of smoke may assist in establishing healthy and vigorous seedlings for cultivation of a number of medicinal plants (Sparg et al., 2005), the aim of this study was to determine the possible use of smoke-water in stimulating the accumulation of indigo in shoots of *I. linifolia* seedlings.

METHODS AND MATERIALS

The experimental studies were conducted using seeds obtained from Pusa Agriculture University farm, Bihar. Seeds were surface decontaminated with 0.1% mercuric chloride for 2 min and rinsed with distilled water and then sown in plastic containers containing a twice-autoclaved soil mixture of garden soil and river sand (1:1, v/v). Containers were watered until they reached field capacity and then incubated at 25 °C.

After emergence of the third leaf, uniform seedlings were washed with distilled water and transplanted into pots with sterilized quartz sand as a substrate. Half-strength Hoagland's solution (HS) was used as a liquid growth medium. To determine whether smoke-water can improve the shoot growth and the concentration of indigo in the shoots, the following treatments were used: HS only (control), HS containing smoke-water at dilutions of 1:500, 1:1000, and 1:2000 (100 mL per pot). Each treatment consisted of 10 replicates. The substrate was remoistened with 50 mL HS once a week during the course of the trial. Seedlings were incubated in a greenhouse at 25/22 °C (day/ night) under a 14 h photoperiod (280 ¼mol m^{-2} s^{-1}) at a relative humidity of 65%.

Smoke-water was prepared by continuously bubbling smoke from *Passerina vulgaris* and *T. triandra* plant material through 500 mL water for 45 min (Van Staden et al., 2004). After 30 days, growth parameters were recorded and quantitative analysis of indigo was done. Dry mass of shoots was determined after drying at 60 °C for 72 h until a constant mass was reached. Quantitative analysis of indigo in shoots was performed according to the reported procedure of Fan et al. (2008). Briefly, the dried shoots of *I. linifolia* (500 mg) were ground into a powder (20 mesh) and extracted with chloroform (100 mL) in a Soxhlet apparatus for 8 h at 80 °C.

Statistical analysis was conducted using MINITAB statistical package. Data was subjected to one-way analysis of variance (ANOVA) and Fishers pair wise comparisons at a 5% level of significance.

RESULTS AND DISCUSSIONS

Treating *I. linifolia* seedlings with smoke-water increased shoot growth. Treatment with a 1:1000 dilution of smoke-water resulted in the greatest increase in shoot mass, which was significantly higher (PÂÙ0.05) from the control. Seedlings treated with smoke-water dilutions of 1:500 and 1:2000 showed an increase in shoot mass in comparison to the control, but these results were not statistically significant (Fig. 1). Results for the shoot dry mass showed similar trends to that of the fresh mass (Fig. 1).

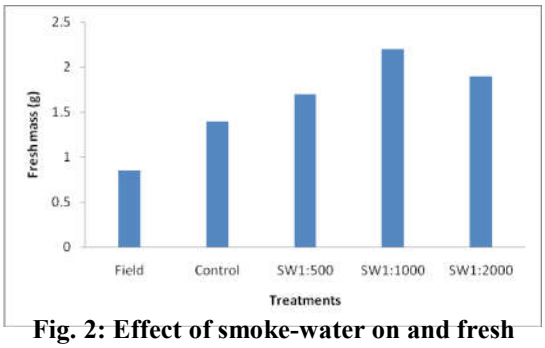

Fig. 2: Effect of smoke-water on and fresh mass of shoots of Indigo linifolia seedlings.

The indigo content of the shoots was improved with smoke water application (Fig. 3). A higher concentration of smoke water (1:500) significantly (PÂÙ0.05) promoted the indigo concentrations in shoot compared with untreated seedlings and smoke-water at dilutions of 1:1000 and 1:2000. Recently it was shown that smoke-water contains inhibitors of growth (Light et *al*, 2010; Thomas and Van Staden, 1998). It is therefore conceivable that these compounds reduced growth and that the increased stress stimulated greater secondary product production involved.

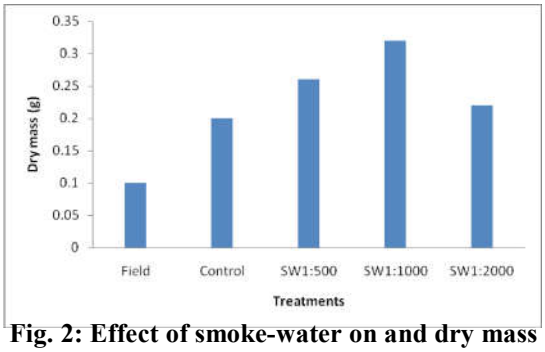

Fig. 2: Effect of smoke-water on and dry mass of shoots of Indigo linifolia seedlings.

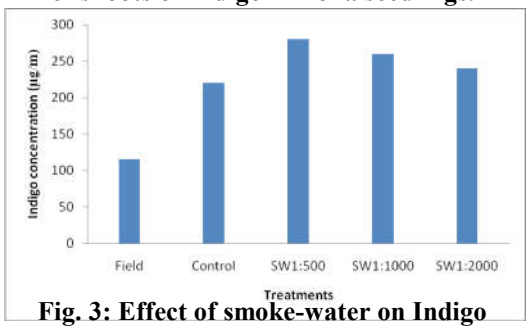

Fig. 3: Effect of smoke-water on Indigo linifolia concentration in plants.

These present findings suggest the possibility of using smoke-water for promoting both the growth and accumulation of secondary metabolites in medicinal plants. It remains to be established whether the results are indirectly due to stress creation or if the actives in smoke have direct effects on the biochemical pathways.

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A REVIEW OF HIMALAYAN BIODIVERSITY WITH REFERENCE TO UTTARAKHAND

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ABSTRACT

Biodiversity means the variety and variability in living beings. India is one of the mega biodiversity centers in the world. Among the 18 hotspots India is considered to possess 2 hotspots extending from Western Ghats on one side and the Eastern Himalayas on the other side. Uttarakhand comprises of about 64.81% forest area of total geographical region. There occur about 21 forests types in Himalayas, which itself is an indicator of the presence of vast biodiversity. According to a survey a total of 280 plants species utilized in 316 formulation by herbal drug companies. Due to over-exploitation and lack of cultivation practices bout 210 species of medicinally valuable plants of Himalayas Region are in Red Data Book.

Biodiversity

Diversity is a concept about range of variation or differences among entities. Biodiversity is the variety and differences among living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are a part. This includes genetic, species and ecosystem diversity. Thus, in essence, biodiversity represents all life. It is the most significant national asset and constitutes an enduring source for supporting the continued existences of human societies. The diversity of plants can be analysed from three viewpoints, phytotaxonomy, ecophysiology and chemotaxonomical diversity.

India is one of the mega biodiversity centers in the world. Among the 18 hotspots of Biodiversity worldwide two are located in the Western Ghats and Eastern Himalayas in India. The Charak Samihata and Susruta Samita describe Himalayas as the richest sources of Biodiversity.

Status of Uttarakhand

It comprises of 13 districts. The total geographical area of Uttarakhand is 53483 Sq.km. out of which 34661.52 Sq.km. is the area under forest, which means about 64.81% of total geographical area is under forest cover. Main species found in these forests are Chir Pine, Deodar,