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PLANT DERIVED SMOKE PROMOTES SEED GERMINATION AND ALLEVIATES AUXIN STRESS IN CARROT

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ABSTRACT

The stimulation of seed germination by smoke and aqueous smoke extracts has received much attention in recent years. An application of aqueous smoke solutions stimulates seed germination in a number of plant species. The present study was designed to investigate the reversion of negative effect of auxin by smoke treatments. It was observed that smoke solutions of *Acacia nilotica* (*A. nilotica*) improved seed germination percentage of *Dacus carota* (*D. carota*) at 500 and 1000 times (1000X) dilutions while *Cymbopogon jwarancusa* (*C. jwarancusa*) smoke solution significantly improved germination percentage at 300X and 500X dilutions. However, concentrated smoke solutions of both plants had inhibitory effect on germination percentage of *D. carota*. Smoke solution from *Acacia* plant at 100 times dilution significantly increased root length, while no significant effect was observed for shoot length compared to control. *C. jwarancusa* smoke solutions at 300X and 500X dilutions significantly increased root length while shoot length was increased at 50 times dilution but the difference was not significant. Auxin solutions at dilutions of 15, 30 and 50 ppm inhibit germination of carrot seeds that was alleviated by using both alleviating solutions (auxin 15 ppm + *C. jwarancusa* 1:500 and auxin 15 ppm + acacia 1:100) which improved germination percentage and reversed the inhibition of auxin. In present investigation it has been observed that smoke solution can alleviate the negative effects of auxin.

Keywords: smoke, auxin, germination, seedling vigour, *Daucus carota*, *Acacia nilotica*, *Cymbopogon jwarancusa.*

INTRODUCTION

Smoke is a highly complex chemical mixture with plant active compounds that can provide stimulant to plants in ecosystems that have been affected by fire. The chemical compounds in smoke that stimulated seed germination has been identified (Flematti *et al*., 2004; Van Staden *et al*., 2004), and shown to be thermo stable, water soluble and acting at very low concentration (Baldwin *et al*., 1994; Flematti *et al*., 2004). There are different species which showed specific responses to smoke (Drewes *et al*., 1995). Smoke and smoke solutions from the combustion of plant material stimulated germination in a wide range of species (Brown *et al*., 2003; Brown and Botha 2004). Smoke also significantly improved post-germinative growth in seeds of the *Amaryllidaceae,* even though in these species there was no effect on germination (Brown *et al*., 2003, Sparg *et al*., 2005). Several studies have focused on the ability of smoke to promote germination in a variety of plant species in South Africa, Australia, and North America (Brown. 1993, Tieu *et al*., 2001). Researchers who have screened large numbers of plants often reported that the seed of some species are inhibited by smoke. This phenomenon occurred when seeds were exposed to prolonged periods or high concentrations of smoke and smoke solutions (Brown, 1993; Light *et al*., 2002).

The treatment of Grand Rapids lettuce seeds with different dilutions of an aqueous smoke extract produced a response curve similar to that of phytohormone response curves (Drewes *et al*., 1995).

In the past many studies on Acacia species have focused on physical aspects of fire-related cues for releasing dormancy and seed germination and little attention has been given to the chemical/physiological influence of smoke components at post-germination levels. A few studies have shown that the effects of smoke extend beyond post-germination events resulting in the stimulation of seedling vigour (Baxter and Van Staden., 1994; Sparg *et al*., 2005).

The carrot (*Daucus carota* L. Apiaceae.) originated in Asia, is a very common vegetable grown throughout the world. In Pakistan, it is grown on an area of about 11, 000 hectares annually with a total production of 192, 000 metric tons carrot roots (Anonymous, 1999). The germination of carrot at low temperature was enhanced by priming (Szafirowska *et al*. 1981).

Plant growth regulators are the chemicals which influence the plant growth when applied in very minute quantity. The addition of growth regulators to seed has proved beneficial in stimulating germination under adverse conditions. Cytokinins have been used to overcome thermodormancy in lettuce seed (McCory and Harrington, 1970; Reynolda and Thompson, 1973). Auxin has been shown to be a potent inhibitor of seed germination in several strain of lettuce (Sankhla and Sankhla, 1968). Ethylene and auxins are interrelated in their action (Morgan and Gausman, 1966). Ethylene can negate the inhibition of seed germination caused by both auxin as well as abscisic acid (Sankhla and Sankhla, 1972). Auxin action on elongation of roots was posed by (Chadwick and Burg, 1967). They suggested that the inhibition of root elongation by auxin resulted from auxin - inducted ethylene production.

The aim of the present work was to study the influence of smoke solution derived from two different plant sources on germination and post germination

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behavior of carrot seeds and to alleviate the inhibitory effects of auxin by using alleviating smoke solution (the solutions formed by the mixture of smoke solution and Auxin dilutions).

MATERIAL AND METHODS

Seed source

Seeds of *D. carota* (Carrot) used in these experiments were collected from Tarnab Research Centre Peshawar, KPK, Pakistan.

Preparation of smoke solution

Stem and leaves of two plants *A. nilotica* and *C. jwarancusa* were collected from Kohat region. Plant materials were washed to remove dust particles and semidried under shade for 14 days. Aqueous smoke solutions of plants were obtained by following the method of (Tieu *et al*., 1999) with slight modifications. Plant material was smoldered in a liter of distilled water until the whole plant material converted to ash. The solution was filtered through a sterilized filter paper in a clean sterilized bottle and was stored at 4°C.The smoke solution was further diluted to 50, 100, 300, 500, 1000, 3000, 5000 and 10000 times dilution while auxin solution was diluted upto 10, 15, 30 and 50 ppm.

Alleviating solution

Alleviating solutions were made by the combination of smoke and auxin solution. The dilution of auxin 15 ppm (this dilution was selected because of its moderate inhibitory effect on germination) was made in Cymbopogon smoke dilution (1:500) and Acacia (1:100). Selection of smoke dilutions for auxin was done on the basis of strong vigour among all dilutions.

Experimental procedure

The experimental trial was divided into three phases. In the first phase smoke effect on carrot seed germination and seedling vigor was studied. In the second phase all the auxin dilution were screened for its inhibitory effect. In the third phase effect of alleviating solutions (smoke+auxin) were studied for germination and seedling vigor. For all the experiments healthy and mature seeds of equal size of *D. carota* were selected. All the seeds were surface sterilized in seventy percent ethanol solution for five minutes, then rinsed in distilled water for three times. The experiment was conducted in 9 cm Petri plates with double layer of filter paper. Each treatment was replicated 3 times with 10 seeds in each replica. Each Petri plate was irrigated with 5 ml of distilled water and respective smoke/alleviating solution and remoistened when required. The Petri plates were placed in dark at $\pm 25^{0}$ C and germination counts were observed for every 24h on daily basis for 10 days. Criterion for germination was the emergence of radical 2mm in length. Root, shoot length, and fresh weight of root and shoot were recorded after 10 days and the results were compared with that of control. Fresh material of shoot and root were kept in oven at 80^{0}C for 48h to find dry weight.

Statistical analysis

All the data was analyzed for analysis of variance (ANOVA) using statistix 9 software followed by LSD and $P \leq 0.05$ was considered as a criterion for significance.

RESULTS

It was observed that smoke solutions of *A. nilotica* and *C. jwarancusa* inhibit the germination of carrot seed when used in concentrated form; after 120 hrs the acacia smoke solution at 500 times dilution significantly increased ($P<0.05$) germination percentage as compared to control (Figure-1A). The *C. jwarancusa* smoke solution at 300, 500, 1000 and 5000 times dilution significantly increased ($P<0.05$) germination percentage as compare to control (Figure-2A). The acacia smoke solution at dilution of 1:50 and 1:100 increased root length of carrot while all the other dilutions decreased root length as compared to control (Figure-1C). The *C.jwarancusa* smoke solution at dilution of 1:300 and 1:500 significantly increased while all other dilutions decreased root length of carrot (Figure-2C). Acacia smoke at 50 times dilution promote shoot length but the difference was not significant, rest of the dilutions either decreased or performed similar to control (Figure-1B). *C.jwarancusa* almost at all dilutions performed parallel to control in case of shoot length (Figure-2B).

After 96 hrs the auxin solutions at dilutions of 15 ppm, 30 ppm and 50 ppm significantly decrease in germination percentage (Figure-3A). The auxin solution at dilutions of 30 and 50 ppm showed significant decrease while other dilutions significantly increased root length of carrot as compared to control (Figure-3C). In case of shoot length the auxin solutions at dilution of 10 ppm significantly increased shoot length of carrot while rest of the dilutions showed parallel results as compared to control (Figure-3B).

The acacia smoke (1:100) and both auxin alleviating dilutions (auxin 15 ppm + Cymb 1:500 and auxin 15 ppm + Ac 1:100) highly significantly increased the germination percentage of Carrot as compare to control (Figure-4A). In case of shoot length the, *Cymbopogon* smoke (1:500), acacia smoke (1:100) and both auxin alleviating solution significantly increased shoot length of carrot (Figure-4B). Root length was significantly increased by almost all treatments used (Figure-4C).

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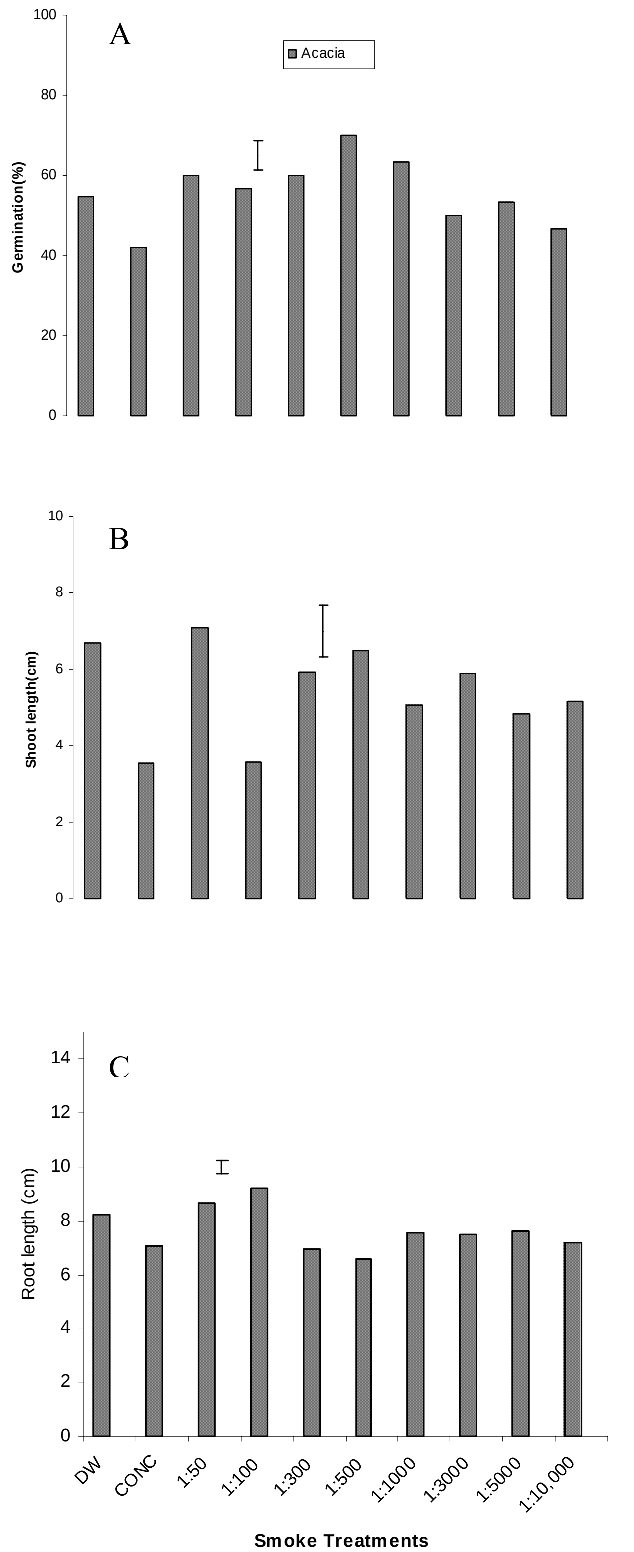

Figure-1. Response of carrot seeds towards *A. nilotica* smoke on (A) Seed germination (B) Shoot length and (C) Root length. All the data points are the mean of three replicates with 20 seeds in each replica. The bar indicates Least Significant Difference (LSD).

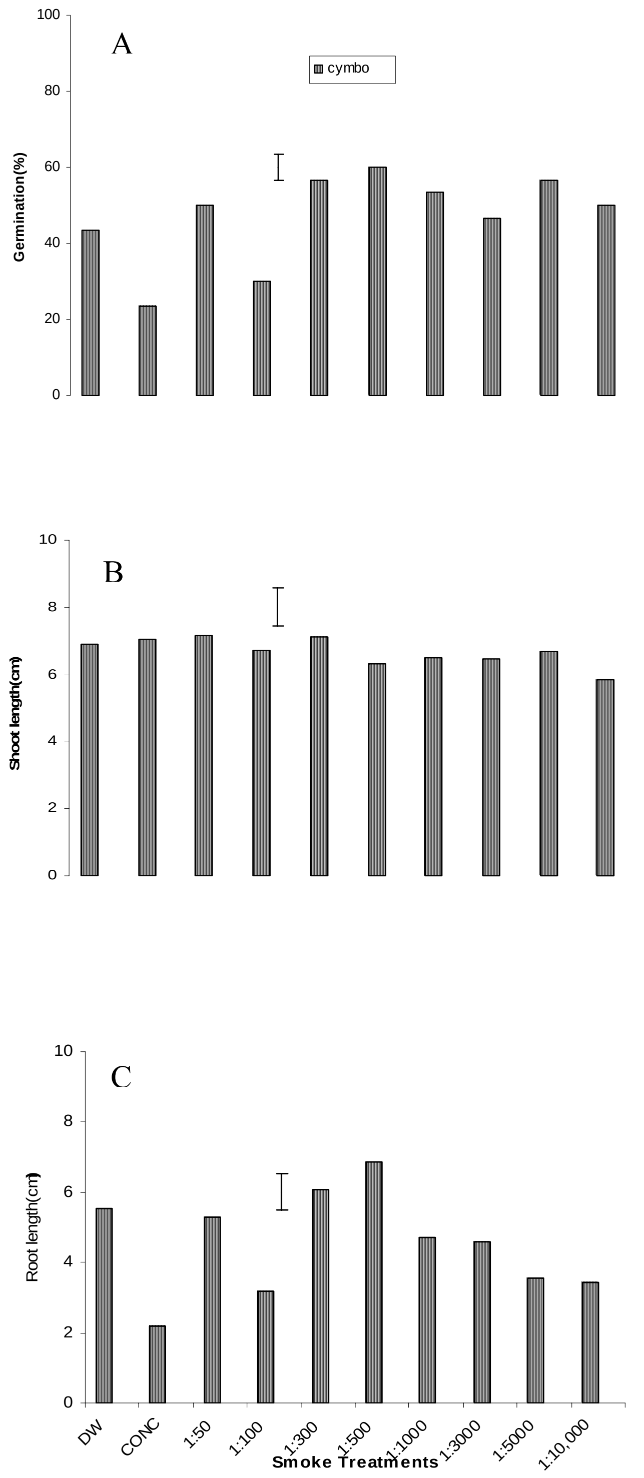

Figure-2. Response of carrot seeds towards *C. jwarancusa* smoke on (A) Seed germination (B) Shoot length and (C) Root length. All the data points are the mean of three replicates with 20 seeds in each replica. The bar indicates Least Significant Difference (LSD).

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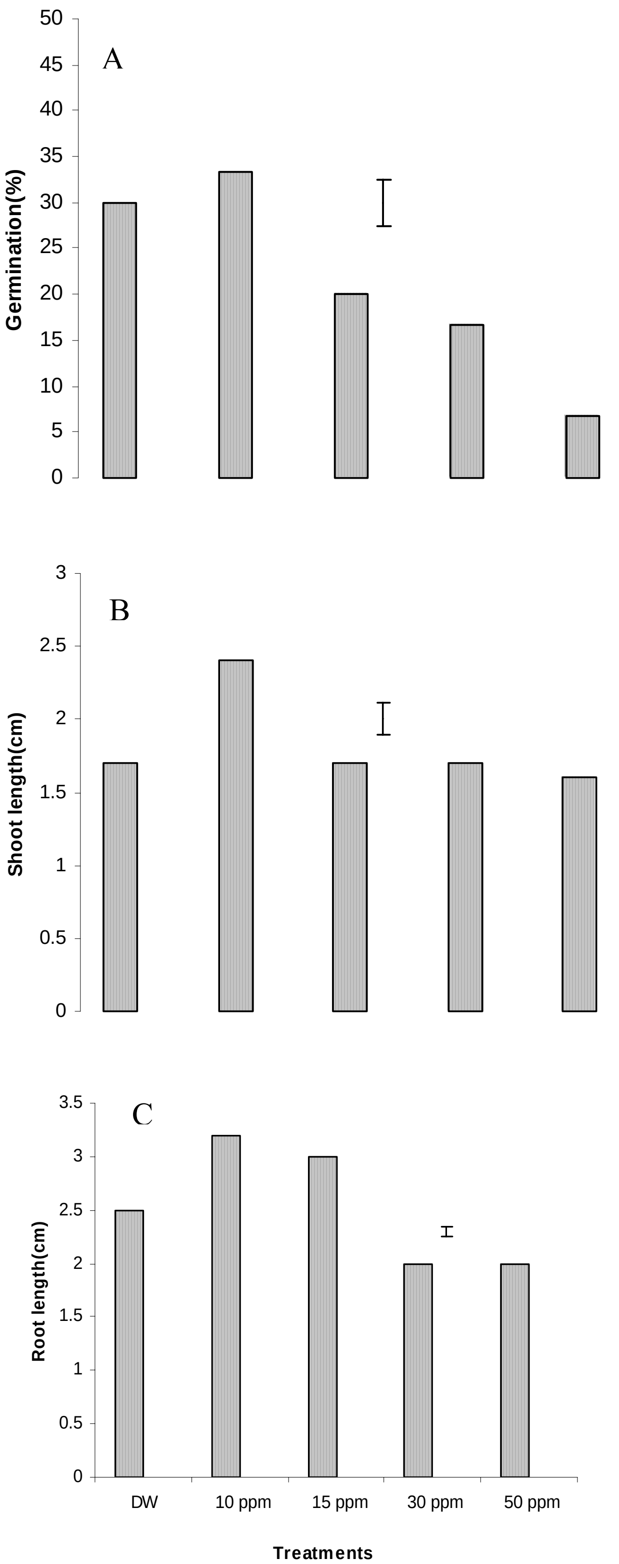

Figure-3. Effect of different dilutions of auxin on (A) carrot seed germination (B) Shoot length and (C) Root length. All the data points are the mean of three replicates with 20 seeds in each replica. The bar indicates Least Significant Difference (LSD). The treatments in ppm are the different dilutions of auxin.

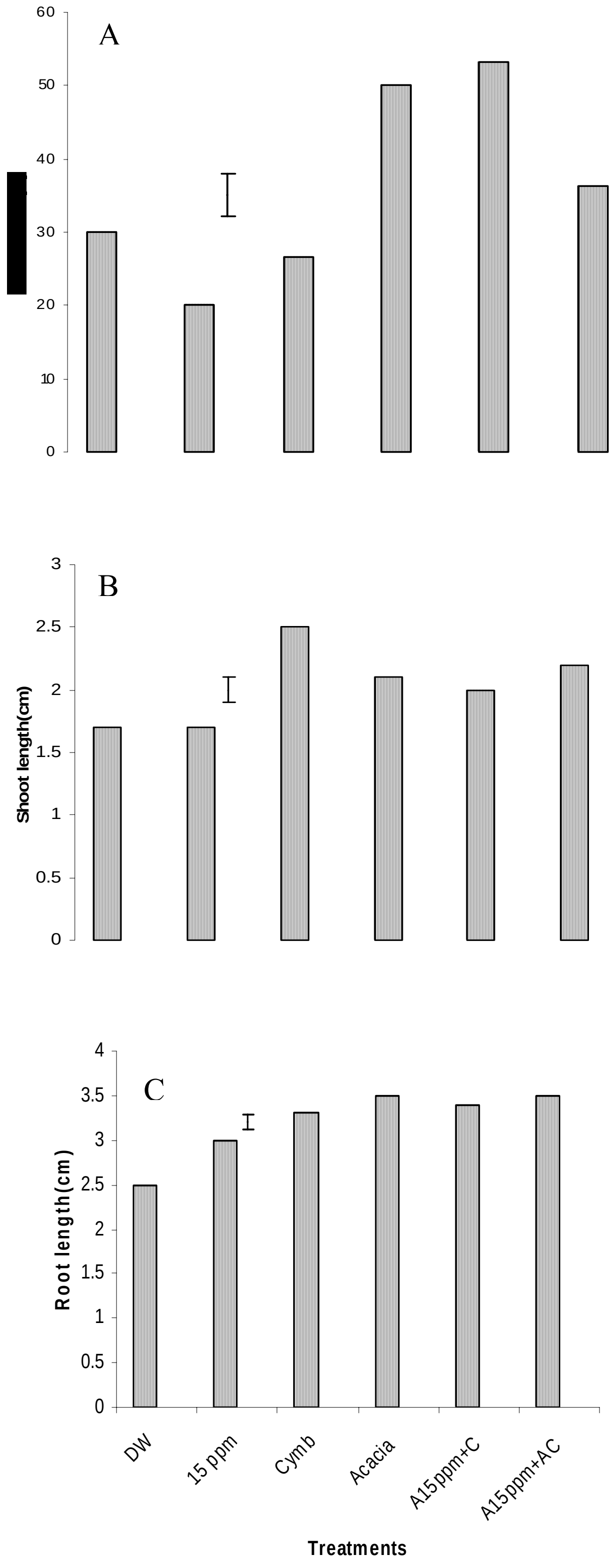

Figure-4. Alleviation of Auxin stress by smoke solution: (A) carrot seed germination (B) Shoot length and (C) Root length. All the data points are the mean of three replicates with 20 seeds in each replica. The bar indicates Least Significant Difference (LSD). Treatments A15ppm+C = auxin 15 ppm+ cymbopogon smoke 1:500 and A15ppm+AC= auxin 15 ppm+ Acacia smoke 1:100.

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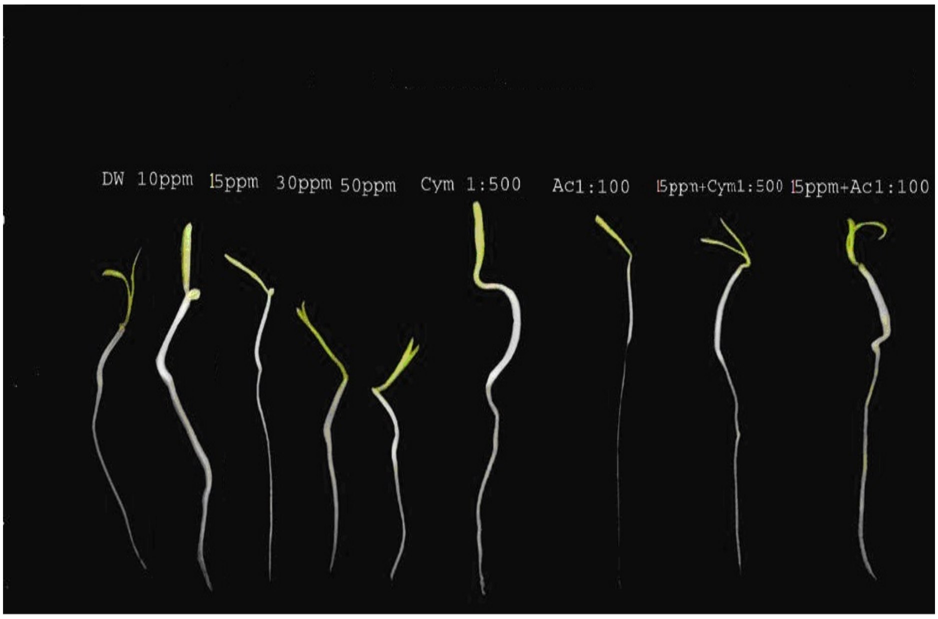

Figure-5. Carrot seedlings showing a response towards different treatment: DW= distilled water control, dilutions in ppm are different dilutions of auxin, Cym 1:500= cymbopogon smoke solution 500 times diluted, Ac 1:100= Acacia smoke 100 times diluted, 15ppm+Cym 1:500 = Alleviating solution of auxin with cymbopogon and 15ppm+Ac 1:100 = Alleviating solution of auxin with acacia smoke solution.

DISCUSSIONS

In this study effect of *A. nilotica* and *C. jwarancuasa* smoke solution was studied on *D. carota* seeds germination and seedling vigour. In addition we focused to study the effect of auxin solutions, auxin alleviating solutions and smoke solutions (Cymbopogon 1:500 and Acacia 1:100) on carrot seed germination and seedling vigour. As stated earlier, smoke promotes seed germination and seedling vigour so it was of a great interest whether smoke can alleviate auxin stress or not? Smoke solutions derived from both plant sources improved seed germination percentage at different dilutions (Figure-1A, 2A). Concentrated smoke solutions of both plants decreased germination after 120 hrs (Figure 1A, 2A); these results are in accordance with that of Van Staden *et al*. (2000) who suggested that plant derived smoke can both stimulate and inhibit seed germination depending on concentrations used. Similarly Brown and Van Staden. (1997) reported that at high concentrations, smoke solutions are known to inhibit seed germination and suggested that more dilute solutions improved seed germination. Early reports by Brown *et al*. (2003) and Sparg *et al*. (2005) reveal that Smoke also significantly improves post-germination growth (seedling vigour) in species of *Amaryllidaceae*. Modi (2002, 2004) reported that smoke-treated kernels of traditional maize landraces produced more vigorous seedlings (heavier and taller) than untreated kernels. Similarly Baxter and van Staden (1994) reported that seedlings of *Themeda triandra* from smoke-treated seeds grew more vigorously without any abnormalities than untreated seeds; similar effect was reported for Erica species (Brown 1993), grasses (Blank and Young 1998) and indigenous medicinal plants (Sparg *et al*., 2005). Our results also showed that shoot and root length of carrot was significantly improved by *acacia* smoke solution at 100 times dilution and Cymbopogon smoke solution at 300 and 500 times dilution as compared to control (Figure-1, 2).

Auxin has been shown to be a potent inhibitor of seed germination (Evenari and Mayer, 1954; Khan and Tolbert, 1966; D. Sankhla and N. Sankhla, 1968). Auxin solutions at dilutions of 15, 30 and 50ppm inhibit the germination percentage of carrot seeds while at 10 ppm, Cymbopogon smoke (500 times dilution) and Acacia smoke (100 times dilution) improved germination percentage as compared to control (Figure 3A; 4A). On the other hand both the auxin alleviating solution (auxin 15 ppm + Cymb 1:500 and auxin 15 ppm + Ac 1:100) improved germination percentage of carrot seed as compared to control. These results show that both smoke promotory dilutions (Cymbopogon 1:500 and Acacia 1:100) alleviate the auxin stress and promote germination percentage as compared to auxin 15 ppm dilution as shown in (Figure 3A; 4A). Chauhan *et al*. (2009) reported that germination percentage decreases when the auxin concentration increased, which shows that higher concentration inhibit germination; they also showed that the germination percentage under auxin treatment at 10 ppm recorded maximum in both the crops Black gram and Horse gram. As suggested by (Khan and Tolbert 1966, D. Sankhla and N. Sankhla, 1968) that kinetin can effectively undo the inhibition of seed germination caused by several naturally occurring growth inhibitors; therefore it is suggested that smoke may also act like kineten in this regard.

Burstrom (1969) suggested that under certain condition auxin stimulated cell elongation in intact roots at low concentrations (10^{-10} M). Lower concentration of

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growth regulators favour the increased enzymatic activity which leads to the favourable environment for the germination as well as the growth of the radicle and plumule (Chauhan *et al*., 2009). Increase of root and shoot length by auxin, smoke and alleviating solutions are in accordance with the above statement (Figure 3 C; 4C).

The experimental results presented in this study were consistent with the hypothesis that plant derived smoke solutions enhance seed germination and seedling vigour. Furthermore, these results supported the hypothesis that seed germination inhibition by auxins can be alleviated by using smoke solution.

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