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# Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition

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**Abstract**

Plant growth and productivity are adversely affected by various forms of abiotic stresses all over the world. Soil salinity affects various physiological and biochemical processes which results in reduced biomass production. From the last two decades, Plant-derived smoke solutions have been used as growth promoters, therefore we designed some experiments to investigate the effect of priming with different dilutions (1:100, 1:500 and 1:1000) of two plant-derived smoke solutions (*Bauhinia* and *Cymbopogon*) on physiological and biochemical aspects of indica rice variety (NIAB-IR-9) under salt (NaCl) stressed conditions. The seeds were primed both in water and smoke solutions for 24 hrs. Ten day experiment was conducted for germination and seedling vigor, while the plants were subjected for 30 days to sand culture salinized with four salt levels (0, 50, 100 and 150mM NaCl) to assess biochemical parameters. Significant increase occurred in germination percentage (23.3 %) and seedling vigor of smoke primed seeds as compared to non-smoke primed seeds at high salt concentration. Smoke primed seeds showed a significant increase in fresh and dry weight as well. Plants raised from seeds primed with different smoke dilutions had increased $K^+$, $Ca^{2+}$ and decreased $Na^+$ content compared to plants raised from hydro primed seeds under saline conditions. Similarly plant raised from primed seeds had higher chlorophyll a and b and carotenoids contents as compared to non-smoke treated plants. Concentration of total nitrogen and protein contents also raised in plants treated with smoke solutions as the level of salt increased. *Bauhinia* (1:500) was the most promontory dilution that significantly alleviated salt stress both at the physiological and biochemical level. It can be concluded that plant-derived smoke solution has some growth regulatory compounds that can increase productivity by reducing the negative effects of salt (NaCl) stress in rice plants.

**Keywords:** biochemical aspect, growth, salinity, seed priming.

## Introduction

Smoke has the potential to be used in horticulture and agriculture industries for the production of more vigorous crops (Light and Van Staden, 2004). A number of cues associated with the fire itself, have been identified as stimulant for germination (Paul et al., 2007). Smoke from burning plant materials induces a remarkable increase seed germination in over 1200 species from 80 genera (Dixon et al., 2009). Smoke and smoke solutions formed from the burning of plant material enhance germination in a large range of species differing plant growth form, reproductive strategy and seed size (Brown and Botha, 2004). Smoke solution is formed by burning a mixture of some plants and forcing the deriving smoke to bubble into a bottle of water for some time (Sidzabda et al., 2010). After the discovery by De Lange and Boucher (1990), various studies have revealed the potential of smoke in many aspects of plant biology such as seed germination, seedling growth (Tigabu et al., 2007), plant flowering (Keeley, 1993) and somatic embryogenesis (Senaratna et al., 1999). Smoke affects plant growth because it interact, in some way with endogenous plant growth regulators (Van Staden et al., 2000) and may be involved in the early beginning of the cell cycle activities, thus accelerating radical emergence in germinating seeds (Jain and Van Staden, 2006). It is observed from the previous studies that aqueous smoke solutions show hormone like responses in different species and interact with auxins, gibberellins, cytokinins, ABA and ethylene in various kinds of seeds (Daws et al., 2007). The effect of smoke and smoke solutions extends beyond the post germination events resulting in the activation and improvement of seedling vigour (Sparg et al., 2005). Butenolide in smoke water has been shown of have positive effects on seedling growth (Sparg *et al.*, 2005), *Eragrostis tef* (Ghebrehiwot et al., 2008), woody Acacia species (Kulkarni et al., 2007) and rice (Kulkarni et al., 2006). Improved seedling vigour included increased leaf production, shoot length and root mass. According to the previous studies, an active butenolide compound in smoke plays a regulatory role in plant growth and development. As all these physiological effects are in part controlled by plant growth regulators (PGR), indications are that, the smoke extracts interact in the same way with endogenous PGRs (Van Staden et al., 2000). There are many studies on salinization and different priming agent which alleviate the adverse affect of Stalinization, effect of smoke solution priming is a novel work and its effect on physiological and biochemical aspect on rice are limited. The present study was therefore conducted to find out the protective effect of smoke solution priming on rice against salinity by investigating growth, ion uptake, photosynthetic pigments, total nitrogen and protein contents.

## Results

### *Effect of plant-derived smoke solutions on seed germination*

Significant differences were observed in germination of both smoke and non-smoke primed seeds at different salinity levels throughout the germination test (Fig. 1 and 2). Decrease in germination was recorded with an increase in salinity from 0 to 150 mM, however smoke primed seeds showed overall high germination percentage than hydro primed seeds under both normal and saline conditions. Germination started within twenty four hours in control and smoke primed seeds of both the varieties. Maximum germination percentage for non-saline seeds (control) was 93.3 %, which decreased to 60 % at 150 mM salinity stress. All the seeds of control and 50 mM salt stress showed maximum germination within 72 hours. While the seeds treated with 100 mM and 150 mM salt stress showed maximum germination after 72 hours (Fig. 1 and 2). Among smoke dilutions, maximum germination was noted for seeds primed with Bauhinia (1:500) under both normal (100%) and saline conditions (96.7, 90 and 83.3%) at 50, 100 and 150mM salt stress compared to control (hydro primed) treatments (Fig. 2).

### *Effect of plant-derived smoke solutions on seedling growth*

In both hydro primed and diluted smoke solution primed treatments, gradual reduction was observed in root and shoot length from control to 100 mM salt (NaCl) stress, while a significant reduction was observed at 150 mM, which was significantly alleviated by smoke primed (saline treatments) plantlets (Fig. 3 and 4). Maximum value was recorded for *Bauhinia* (1:500) where length of shoot was 6.76cm that reduced to 6.4, 4.57 and 3.6cm at different salinity levels than other dilutions (Fig. 4). Seeds primed with Bauhinia (1:500) smoke water showed maximum root length at 50mM salt stress i.e 7.63cm, while significant reduction i.e 5.3 and 4.58cm at 100 and 150mM salt stress was observed as compared to control and *Cymbopogon* dilutions (Fig. 3). There was a remarkable decrease in non-smoke primed seedlings of root and shoot fresh and dry weights at different salinity levels. With the increase in NaCl concentration root and shoot weight decreased (Fig. 3 and 4). Rice seedlings (hydro and smoke primed) grown at lower levels of NaCl (0mM) reached relatively higher fresh and dry weights and did not imply toxicity symptoms, while significant reduction at higher levels (150mM) indicated the symptoms of salt toxicity as growth depression. Maximum shoot fresh weight (0.307g) and root fresh weight (0.17g) were observed in *Cymbopogon* (1:1000) dilution that reduced to 0.161g and 0.03g at 150mM salt stress as compared to control (0.265g and 0.13g) (Fig. 3 and 4) .

### *Effect of plant-derived smoke solution on ions contents ($Na^+$, $K^+$ and $Ca^{2+}$)*

Significant differences were observed in $Na^+$, $K^+$ and $Ca^{2+}$ contents of plants raised from both smoke and non smoke primed seeds at different salinity levels. Decrease in $K^+$ and $Ca^{2+}$ while increase in $Na^+$ content was recorded with increasing salinity from 0 to 150mM (Fig. 5.). However plants raised from seeds primed with different smoke dilutions had increased $K^+$, $Ca^{2+}$ and decreased $Na^+$ content compared to plants raised from hydro primed seeds under saline conditions. High $K^+$, $Ca^{2+}$ and low $Na^+$ content in plants raised from hydro primed were found in control, which were 33.5, 19.7 and 22 µg $g^{-1}$ respectively but at 150mM salinity, these values were reduced to 20.35, 11.6 and 63.4 µg $g^{-1}$ respectively (Fig. 5). Among smoke dilutions, high $K^+$, $Ca^{2+}$ and low $Na^+$ were found in plants grown from seeds primed with *Bauhinia* (1:500) under saline conditions compared to plants grown from seeds primed with other smoke dilutions (Fig. 5). High concentration of $K^+$, $Ca^{2+}$ while low concentration of $Na^+$ ion in plants raised from *Bauhinia* (1:500) primed seeds under 50mM salt stress were 53.4, 25.6 and 23.2 µg $g^{-1}$ respectively, but at 150mM salinity, they were changed to 42.6, 21.7 and 28.4 µg $g^{-1}$ respectively (Fig. 5).

### *Effect of plant-derived smoke water on chlorophyll and carotene contents*

Significant differences were observed in both chlorophyll "a" and chlorophyll "b" under both normal and salt stressed conditions. Smoke primed plants showed increased chlorophyll content than non smoke primed seedlings (Fig. 6). Smoke priming increased chlorophyll "*a*" and "*b*" under saline conditions, while under normal conditions it increased chlorophyll "*b*" but decreased chlorophyll "*a*" compared to hydro priming. High chlorophyll "a" and "b" content (3.53, 2.25, 1.92 and 1.70 µg $g^{-1}$) and (11.86, 7.98, 7.34 and 6.52 µg $g^{-}$) were found in case of *Bauhinia* (1:500) compared to other dilutions under both normal and saline conditions at different salinity levels i.e 50, 100 and 150mM. Cymbopogon (1:500) had maximum value (8.37µg $g^{-1}$) of Chlorophyll 'b' at 150 mM salt (Fig. 6). By increasing the NaCl concentration, carotene content was decreased in plants raised from both hydro primed and smoke primed seeds, however, the decrease was lower in case of smoke primed than hydro primed seedlings (Fig. 7). High carotene contents in plants due to hydro priming were found in control (0mM) which were 520.75 µg $g^{-1}$ while these were reduced to 270.84 µg $g^{-1}$ under 150mM salt stressed conditions. Priming with Bauhinia (1:500) dilution showed high carotene content than priming with other dilutions under both normal and saline conditions (Fig. 7).

### *Effect of plant-derived smoke water on total nitrogen and protein contents*

Both nitrogen and protein contents in rice were decreased with increasing salinity from 0 to 50mM, however, plants grown from seeds primed with smoke solutions showed lower decrease than plants grown from non smoke or hydro primed seeds (Fig. 8 and 9). All smoke dilutions showed increase both in nitrogen and protein content under normal as well as saline conditions. Among smoke dilutions, Bauhinia (1:500) dilution showed high nitrogen and protein content which were 371.19 and 2323.62 µg $g^{-1}$ respectively, which were changed to 280.14 and 1753.68 µg $g^{-1}$ at 50mM salt stress. When salinity increased to 150mM, nitrogen and protein contents of plants reduced to 210.11 and 1315.26 as compared to control (98.05 and 613.79 µg $g^{-1}$) (Fig. 8 and 9).

## Discussion

The most vital stage in seedling establishment is usually considered as seed germination which consequently determines the successful crop production. It is a complex phenomenon involving many physiological and biochemical changes.

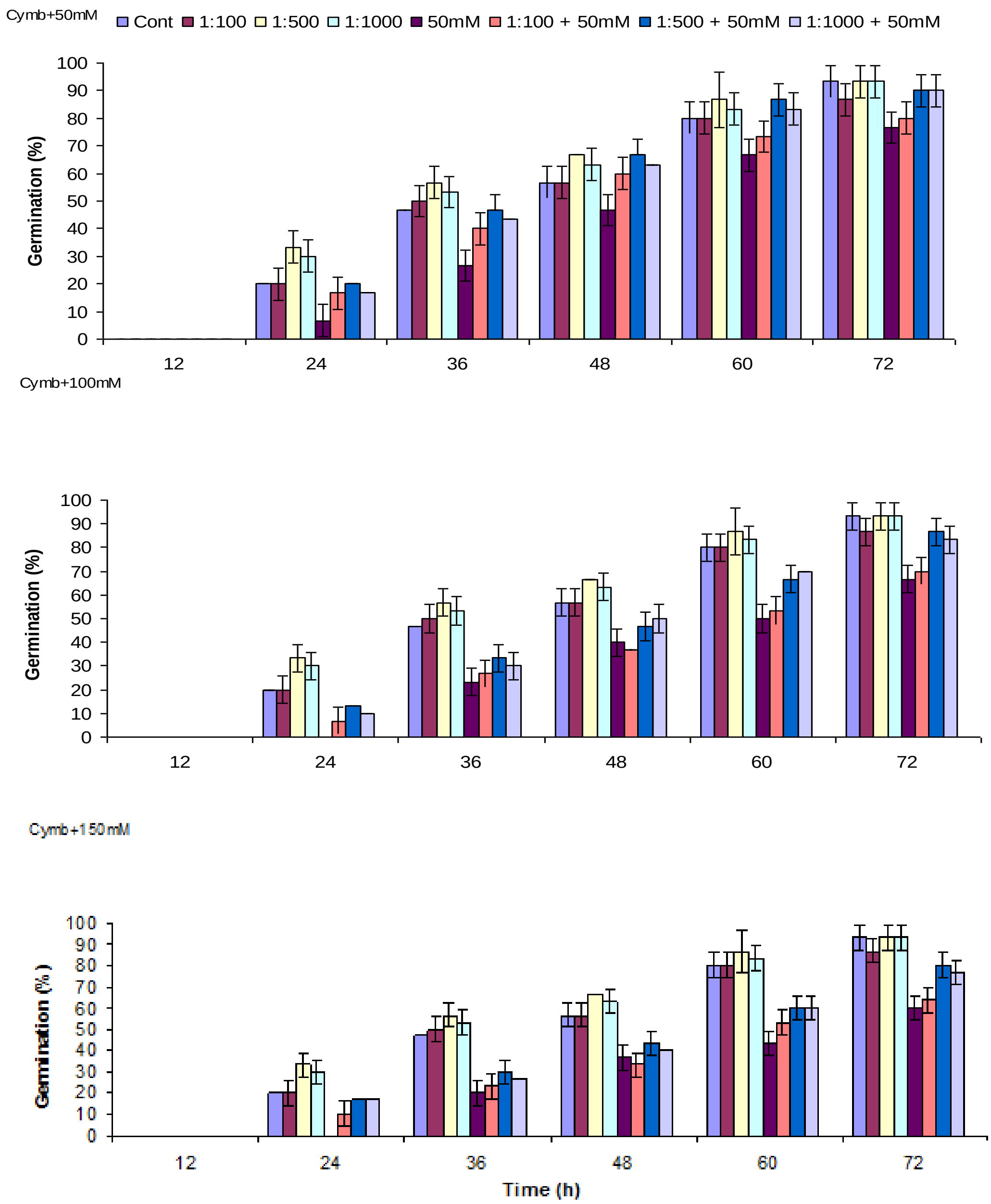


**Fig 1.** Effect of plant-derived smoke solutions (1:100, 1:500 and 1:1000 dilutions) of *C. jawarancusa* on germination % of smoke and non-smoke primed rice (NIAB-IR-9) seeds under both normal (0mM) and saline (50, 100 and 150mM) conditions after 72 hours of treatment. Vertical error bar (I) indicates LSD at ($P < 0.05$).

Salinity induces numerous disorders in seeds during germination. The responses of plants at these stages are mainly important for understanding the mechanisms of salt resistance or sensitivity in plants and their survival. Decrease in germination was recorded with increase in salinity from 0 to 150 mM, however smoke primed seeds enhanced germination percentage and rate than hydro primed seeds under both normal and saline conditions (Fig. 1 and 2). Under normal conditions, salt stress adversely affected germination percentage and rate may be due to the high accumulation of both $Na^+$ and $Cl^-$ in tissues which is likely to have contributed to growth reduction. It might be due to osmotic and ionic stress resulting from high $Na^+$ concentration in soil. All smoke dilutions were effective in promoting germination percentage and rate but the most effective dilution was Bauhinia (1:500 dilutions) (Fig. 1 and 2). Lower dilutions of smoke have shown better germination than higher dilutions. It might be due to the presence of important chemicals which become diluted at higher dilutions and do not show positive effects on germination. These results are in accordance with previous studies of Sparg et al. (2006) who observed that a concentration of 1:500 smoke solution increased seed germination while at higher concentrations, smoke reduced seed germination. Smoke priming reduced germination time due to the possibility of possessing hormone like chemicals helping in breaking the physical dormancy of seeds and alleviating salt stress in plants. Smoke priming may be involved in reducing osmotic stress created by salt and increasing water absorbance in seeds. According to Nelson et al. (2009) the active germination stimulant in smoke is karrikinolide. These results are also in line with those of Brown and van Staden (1997); smoke-solutions improved the germination of seeds. The present study is also in accordance to Dayamba et al. (2008), who reported that plant derived smoke and the active compounds in smoke, shortens the overall

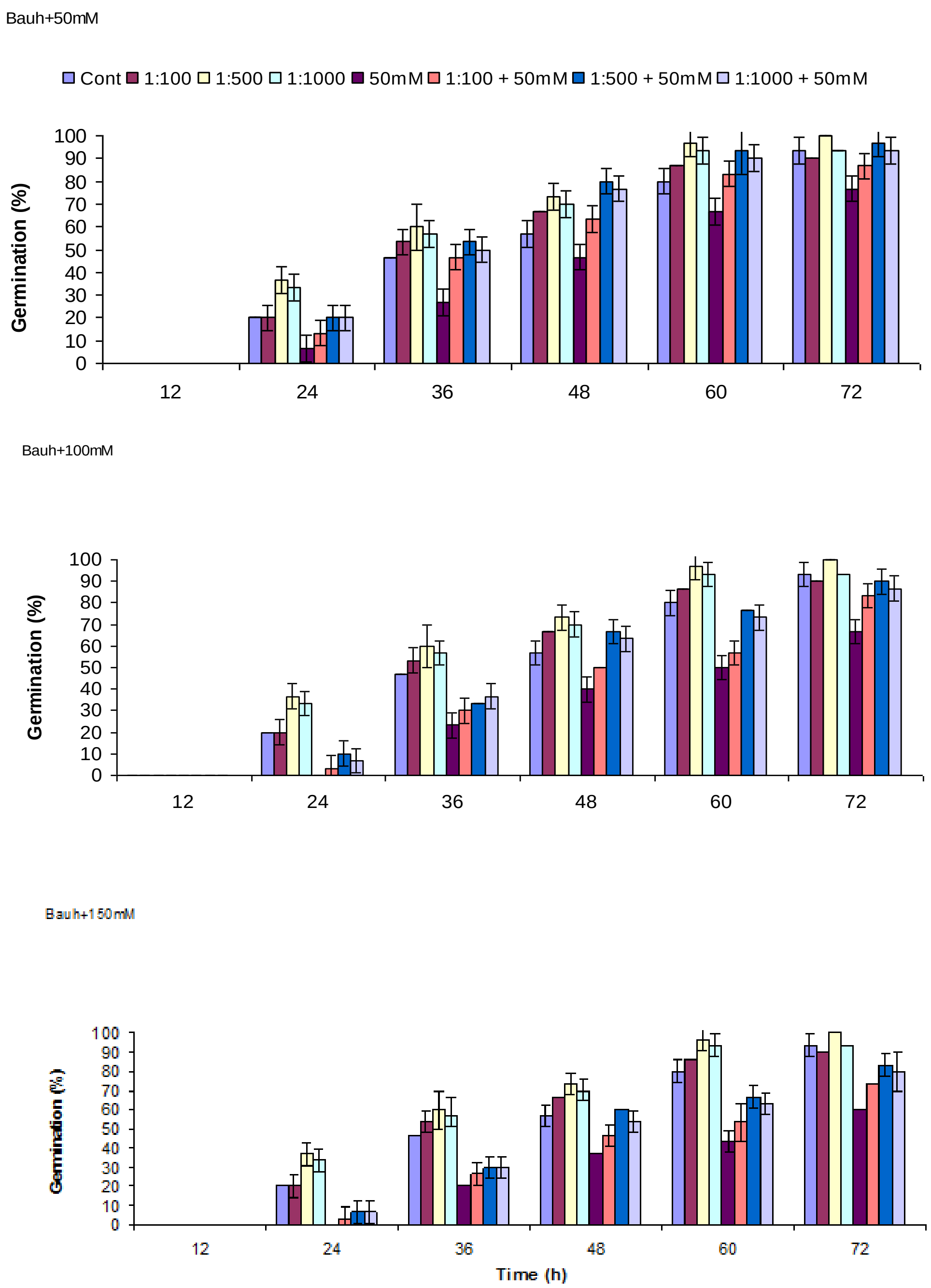


**Fig 2.** Effect of plant-derived smoke solutions (1:100, 1:500 and 1:1000 dilutions) of *B. variegata* on germination % of smoke and non-smoke primed rice (NIAB -IR-9) seeds under both normal (0mM) and saline (50, 100 and 150mM) conditions after 72 hours of treatment. Vertical error bar (I) indicates LSD at ($P < 0.05$).

germination time of seeds. The shoot and root lengths are the most important parameters for salt stress because roots are in direct contact with soil that absorb water from the soil while shoot supplies it to other plant parts (Jamil and Rha, 2004). Rice seedlings (hydro and smoke primed) grown at the low levels of NaCl (0mM) reached relatively higher fresh and dry weights and did not imply toxicity symptoms, while significant reduction at higher levels (150mM) indicated the symptoms of salt toxicity as growth depression. Salinity reduces the growth of plant through osmotic effects, reduces the ability of water uptake and results in reduced growth. Results are also in accordance to studies of Naseer et al. (2001) who observed that germination percentage, shoot and root fresh and dry weights decreased in barley varieties with an increase in salinity. Amirjani (2011) has also reported that salinity limits crop growth, development and agricultural productivity worldwide. Reduction in fresh and dry weight of barley plant by means of salt stress has been reported by Abdel-Hady (2007). Increased duration of salt stress significantly reduces the growth of seedling (Zeng and Shannon, 2000). Under saline conditions, Cymbopogon (1:1000) and Bauhinia (1:500) smoke dilutions showed alleviation of different levels of salt stress (Fig. 3 and 4). Increased seedling growth in smoke primed seeds might be due to the presence of active compounds like growth hormones. It is evident from the studies of Van Staden et al. (2000) that smoke affects all plant processes, in same ways, by interacting


**Fig 3.** Effect of plant-derived smoke solutions (1:100, 1:500 and 1:1000 dilutions) of *B. variegata* on seedling of smoke and non-smoke primed rice (NIAB -IR-9) seeds under both normal (0mM) and saline (50, 100 and 150mM) conditions

with endogenous plant growth regulators. It has been observed from the previous studies that aqueous plant derived smoke solutions show hormone like responses in different species and interact with auxins, cytokinins, gibberellins, ethylene and ABA in various kinds of seeds (Daws et al., 2007). It has been investigated that plant derived smoke is an effective stimulant that enhances germination of seed and improves seedling vigor (Rokich et al., 2002). It has been investigated that effects of smoke and smoke solutions extends beyond the post germination events resulting in the activation and improvement of seedling vigor (Sparg et al., 2005). Salt stress reduced the plant fresh and dry biomass. Reduction in growth might be due to accumulation of $Na^+$ content in shoot and roots. However, smoke priming reduced the accumulation of $Na^+$ and $Cl^-$ and enhanced the $K^+$ and $Ca^{+2}$ (Fig. 5). Daws et al. (2007) reported that seeds treated with smoke-water or butenolide germinate faster and have increased vigor and fresh weight. By increasing the NaCl concentration $Ca^{+2}$ and $K^+$ ion reduced but $Na^+$ ion increased from control to 150mM (Fig. 5). It might be due to ionic stress which interferes with the uptake of nutrients and causes direct toxicity due to the $Na^+$ and $Cl^-$ accumulation in plants. $Na^+$ competes with useful ions like $K^+$ and $Ca^{2+}$, and in turn inhibits their absorption. Yildirim et al. (2011) reported that salt stress not only reduces macro nutrients in plant leaves, especially $Ca^{+2}$ and $K^+$ availability, but also reduces $Ca^{+2}$ and $K^+$ transport and mobility to growing regions of the plants. Greenway and Munns (1980) reported that NaCl, the most pre-dominant form of salt in most saline soils, enhances the $Na^+$ and $Cl^-$ contents, which affect the uptake of other mineral elements. Previously, studying on rice Zhang et al. (2011) demonstrated that the uptake and distribution of K and Ca decrease along the continuum from root to shoot when the concentration of NaCl in culture solution increases. Under saline conditions, all smoke dilutions had reduced negative effects of salts but the most effective dilution was Bauhinia (1:500 diltuion) (Fig. 5). Smoke priming improved the rate of $Ca^{+2}$ and $K^+$ ion channel but reduction occurred in $Na^+$ ion content (Fig. 5). Smoke priming decreased the accumulation of $Na^+$ ion which may enhanced the ions transport and mobility to growing regions of the plants. It is seemed that smoke may have some role in reducing the accumulation of toxic ions, $Na^+$ and $Cl^-$ by increasing the absorption and accumulation of useful ions like $K^+$ and $Ca^{2+}$. Photosynthetic pigments are important regulators of photosynthesis (Parida and Das, 2005). However, these pigments are greatly degraded in plants exposed to salt stress. Likewise, in the present investigation, higher levels of salt stress markedly declined chlorophyll and carotenoids (Fig. 6 and 7). The decrease in chlorophyll content under salt stress is a commonly reported phenomenon in various studies, due to its adverse effects on membrane stability (Ashraf and Bhatti, 2000). Ashraf and Rasul (1988) reported that the reduction in Chlorophyll content under salinity is due to the suppression of enzymes required for chlorophyll synthesis under higher salinity. Many reports show salt induced reduction in photosynthetic pigments in rice plant (Chaum et al., 2007). Singh and Dubey (1995) reported that the decrease in total chlorophyll concentration of susceptible rice cultivars was mainly attributed to the destruction of chlorophyll '*a*'. All smoke solutions alleviated the adverse effects of salinity on chlorophyll and carotene contents but Bauhinia (1:500) dilution, had maintained significantly higher total chlorophyll *a*, *b* and carotene content at all salinity levels. It seemed that smoke has some role in inhibiting suppression of enzymes responsible for synthesis of chlorophyll and carotene contents under salinity but under normal conditions, smoke enhances chlorophyll '*b*' and negatively affects chlorophyll '*a*'. Similar increase in chlorophyll content by priming with other compounds has been reported by El-Tayeb (2005) in barley. By increasing the NaCl concentration significant decrease occurred in nitrogen and protein content while smoke primed seedlings improved the nitrogen and protein contents (Fig. 8 and 9). Salinity has significant reducing effect on shoot and

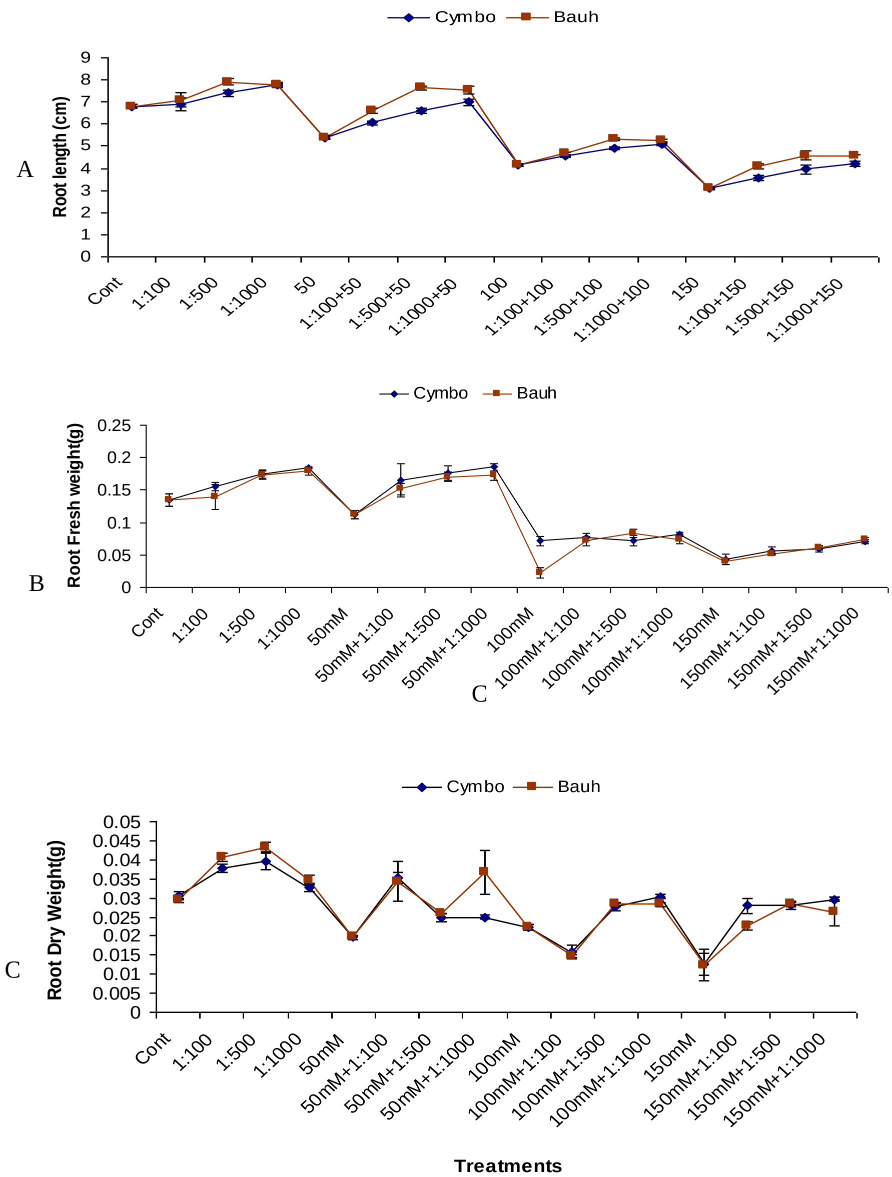


**Fig 4.** Effect of Plant-derived smoke solutions (1:100, 1:500 and 1:1000 dilutions) on root length (A), root fresh weight (B) and root dry weight of smoke (C) (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at (P < 0.05).

root K concentration. Potassium plays important role in protein synthesis (Ashrafuzzaman et al., 2002). So decrease in potassium content due to salt stress may also inhibit protein synthesis and hence total protein content of subsequent salt treated plantlets. Salt stress not only inhibits the absorption of useful ions, but it decreases chlorophyll of rice leaves and protein content under salinity (Singh and Dubey, 1995; Jamil et al., 2012). Like all other parameters, nitrogen and protein contents were significantly increased by priming with smoke Bauhinia (1:500) solutions compared to hydro priming (Fig. 8 and 9). As plant-derived smoke solutions possess essential nutrient elements like Ca, Mg, K, N, Fe, Mn and Cu (Irfan, 2011), it can be said that smoke may be involved in enhanced synthesis of protein by increasing absorption of growth nutrients. The proteins that accumulate under salt stress conditions may provide a storage form of nitrogen that is re utilized in post-stress recovery (Singh et al., 1987). It was concluded that smoke solutions of both plants *Bauhinia variegata* and *Cymbopogon Jwarancusa*, were affective against salt sress but *Bauhinia* (1:500) was the most promontory dilution that significantly alleviated salt stress both at physiological and biochemical level. It can also be concluded that plant-derived smoke solution has some growth regulatory compounds that can increase rice growth by reducing the negative effects of salt (NaCl) stress.

## Material and Methods

### *Seeds collection and sterilization*

Seeds of rice variety (NIAB–IR 9) were procured from the National Agriculture Research Center (NARC) Islamabad. The seeds were stored in brown paper bags at room temperature before being used for experiments. Healthy seeds of both rice varieties were carefully hand-sorted, surface sterilized with 3.5% (v/v) sodium hypochlorite for five minutes and rinsed thrice thoroughly with distilled water, in order to avoid the

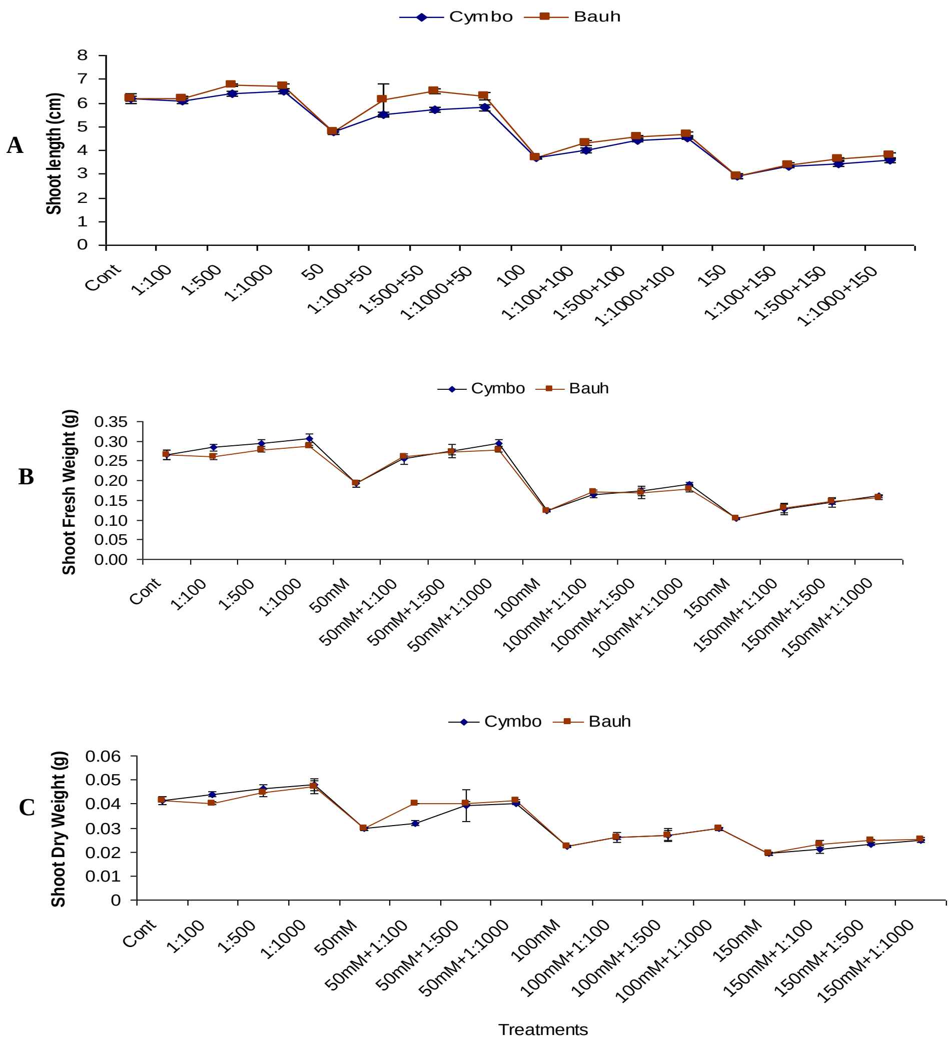


**Fig 5.** Effect of Plant-derived smoke solutions (1:100, 1:500 and 1:1000 dilutions) on shoot length (A), shoot fresh weight (B) and shoot dry weight of smoke (C) (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

chemical effects of the solution, then air dried on sterilized filter paper at room temperature.

### *Collection and preparation of smoke solution*

Trunk (or stalk) and leaves of two plant species, *Bauhinia variegata* and *Cymbopogon Jwarancusa*, were collected from from Kohat District, Pakistan. The collected plant materials were washed with tape water and then distilled water to remove dust and sand particles. They were then shaded dried at room temperature for 10 days for smoke preparation. Aqueous smoke solutions of both plants were prepared by following the method of Tieu *et al*. (1999) with slight modification. Shade dried plant materials having weight 333g were combusted in a furnace. An electrical heater was used for generating heat in order to burn the plant material slowly. The smoke thus produced was passed through the exhaust pipe and collected in a beaker containing one liter of distilled water. The concentrated smoke solutions were further diluted to 1:100, 1:500 and 1:1000.

### *Seed priming*

Seeds of rice variety (NIAB-IR-9) were primed in three kinds of diluted solutions (1:100, 1:500 and 1:1000) of two original smoke solutions (*Bauhinia variegata* and *Cymbopogon jwarancusa*) and in distilled water for 24 hours. After priming, seeds were air dried on filter papers at room temperature.

### *Seed germination and seedling growth*

Selected seeds of NIAB-IR-9 in each treatment were allowed to germinate in sterilized Petri plates containing double layered filter papers (Whatman No.1). Seeds primed in distilled water (hydro primed seeds) and in three kinds of diluted solutions of both smoke solutions were treated with 0 (normal conditions), 50, 100 and 150mM (saline conditions) NaCl solutions. Three replicas with 10 seeds per replicate were used for each treatment. The seeds were germinated in an incubator at 30 $^{o}$C under continuous dark condition. Seed germination was evaluated after every 12 hours up to 96 hrs.

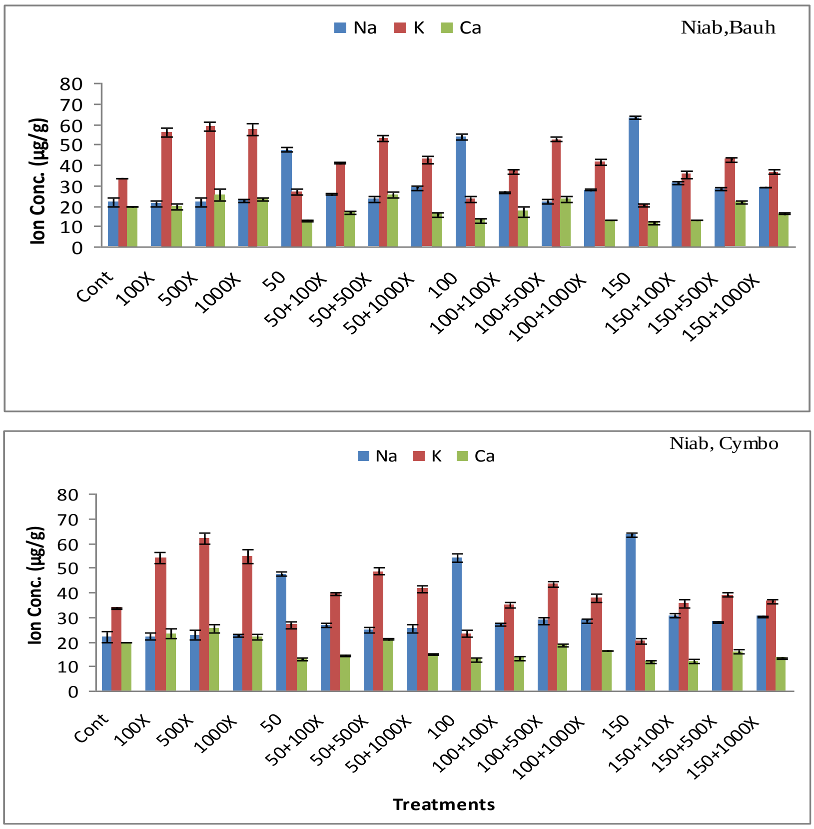


**Fig 6.** Effect of Plant-derived smoke solutions (100X (100 times), 500X and 1000X dilutions) on $Na^{+}$, $K^{+}$ and $Ca^{+2}$ ions contents of smoke (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

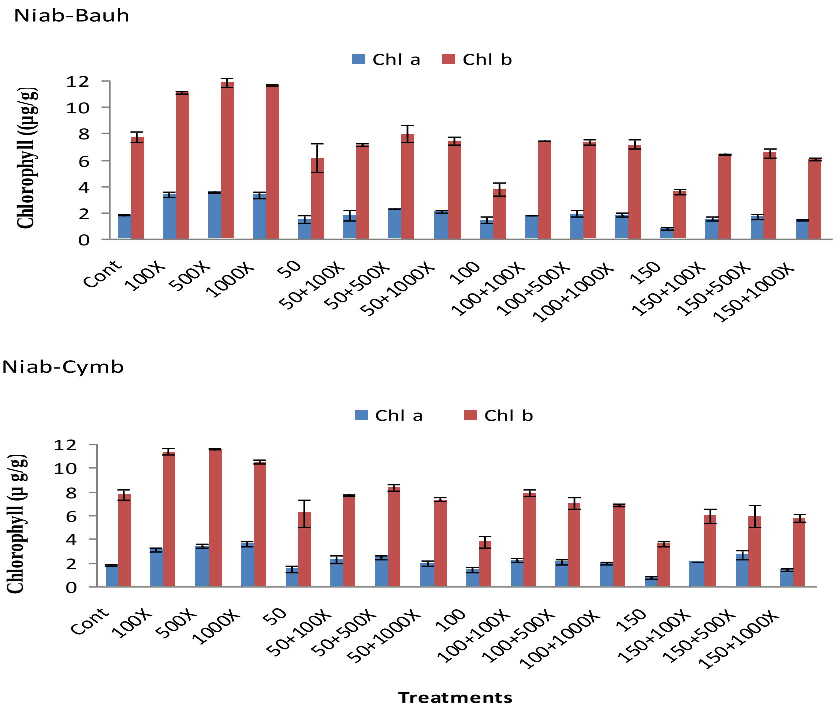


**Fig 7.** Effect of Plant-derived smoke solutions (100X (100 times), 500X and 1000X dilutions) on chlorophyll contents (Chl a and b) of smoke (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

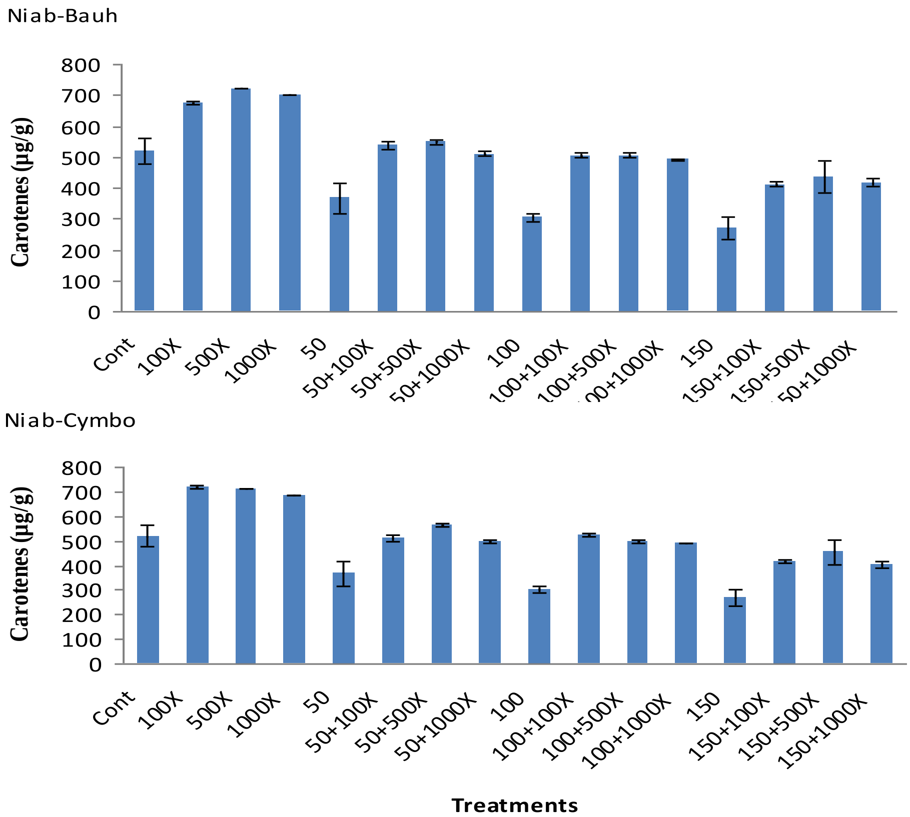


**Fig 8.** Effect of Plant-derived smoke solutions (100X (100 times), 500X and 1000X dilutions) on carotene contents of smoke (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

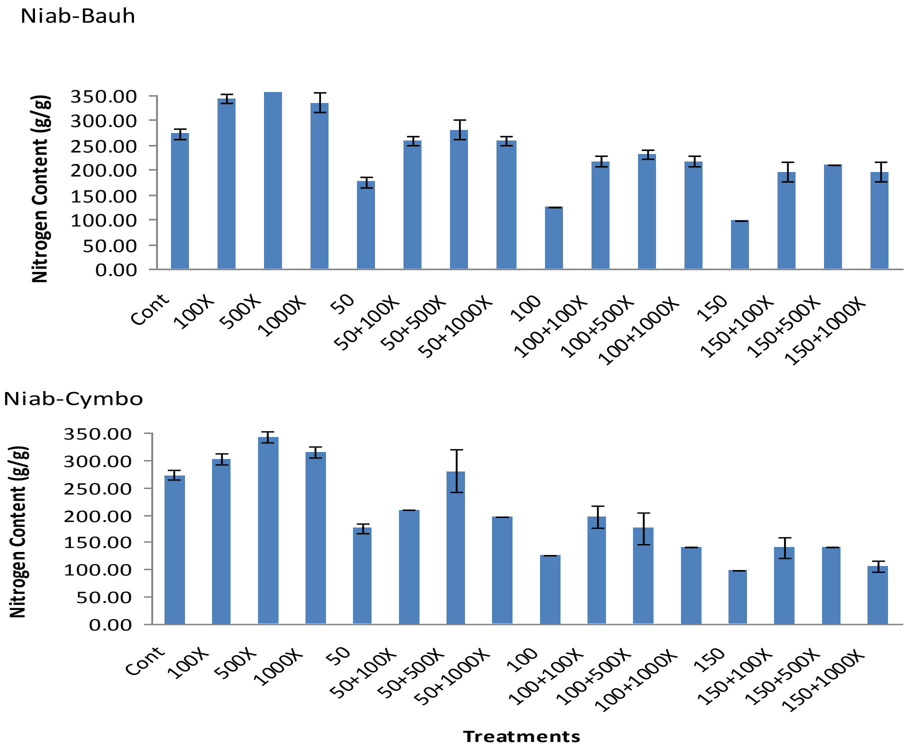


**Fig 9.** Effect of Plant-derived smoke solutions (100X (100 times), 500X and 1000X dilutions) on nitrogen content of smoke (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

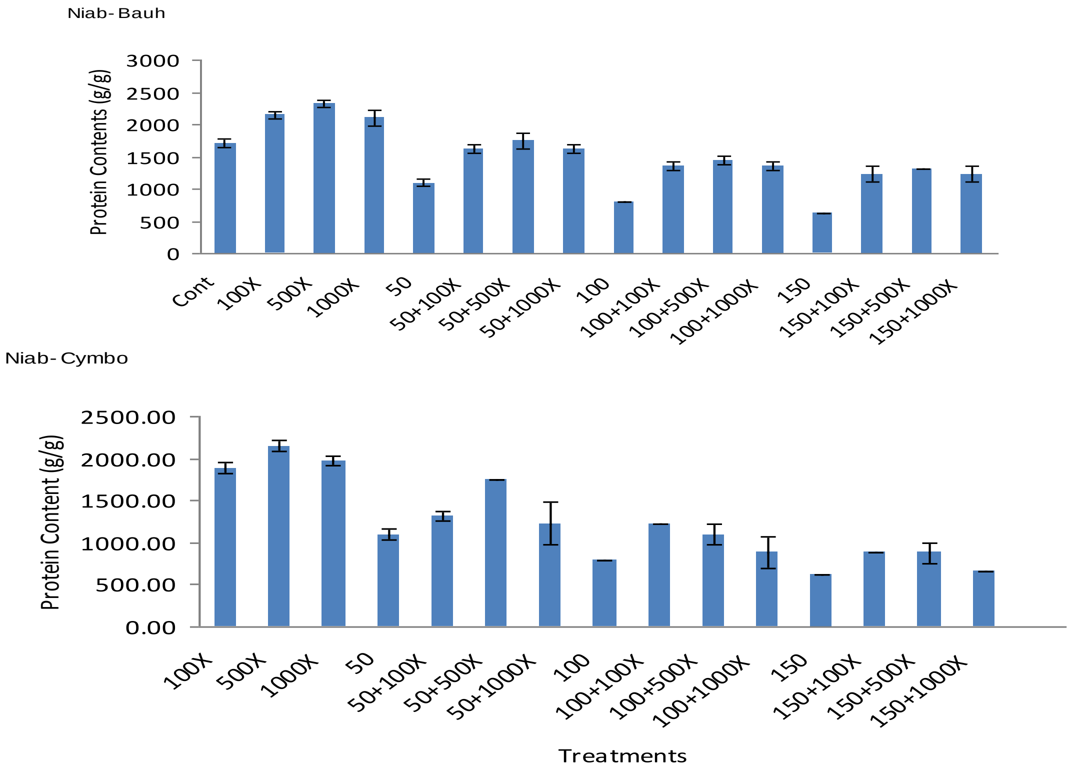


**Fig 10.** Effect of Plant-derived smoke solutions (100X (100 times), 500X and 1000X dilutions) on protein content of smoke (Cymbo & Bauh) and non-smoke primed rice seeds (NIAB-IR-9) under both normal and saline (50, 100 and 150mM) conditions. Vertical error bar (I) indicates LSD at ($P < 0.05$).

Seeds with the emergence of 2mm radicals were considered as germinated (Jamil et al., 2012). After 10 days, root and shoot lengths and fresh weights of both hydro and smoke primed rice seedlings of all the seeds were measured and properly recorded, while dry weight was taken by keeping them in oven for 48 hrs at 80 $^{o}$C.

***Sand culture experiment***

The seeds (hydro and smoke primed) were initially germinated in distilled water in Petri plates with double layered filter papers. Young seedlings with two leaves were then transferred to plastic pots (12 centimeters diameter and with a small hole in the bottom) filled with sand. The seedlings were allowed to be fully grown for three weeks with a Hoagland nutrient solution (Hoagland and Arnon, 1950), irrigated on weekly intervals with continuous maintenance of pH (5.5-5.9). After three weeks of transplanting, salt stress treatments of 0 (control), 50, 100 and 150mM NaCl concentration were imposed to the sterilized fully growing plants for 5 days with a full strength Hoagland solution. The whole experiment was conducted under greenhouse conditions (humidity 65 – 75 percent, air temperature 30– 35$^{o}$C and photoperiod of 16 hours). After stress treatment, the plants were harvested, dried at 80˚C in the incubator and powdered to assess different biochemical tests.

***Ion contents ($Na^{+}$, $K^{+}$ and $Ca^{2+}$)***

Ion contents were determined by following the method of Awan and Salim (1997) with slight modification. Twenty five mg of plant powder was thoroughly digested by a mixture of concentrated sulphuric acid and hydrogen peroxide mixed in 2:1 (v/v) ratio in a small beaker followed by heating for 15 minutes. After digestion, 20 mL distilled water was added to each sample followed by shaking to get filtrate for determination of ions through flame photometer (Jenway PFP7).

***Chlorophyll and carotene contents***

Chlorophyll and carotenoids were determined by using a method given by Lichtenthaler and Welburn (1985). To determine total chlorophyll concentration, 25 mg of plant powder of each treatment was taken in test tube and 25 milligrams of Magnesium oxide was added to it. After that, 5 mL methanol was added to the test tube containing the mixture, which was then homogenized on shaker for 2 hours. After homogenization, turbid pigment extract (methanol extract) was transferred to 5 mL graduated centrifuge tube and centrifuged for 5 minutes at 300 to 500 rpm at room temperature. The absorbance reading against a solvent blank in a UV-VIS spectrophotometer was taken at 666 nm (chlorophyll a), 653 nm (chlorophyll b), 470 nm (carotenoids) wavelengths.

***Nitrogen and protein content***

The nitrogen content of both smoke and non smoke primed plants was determined using the micro-Kjeldahl technique, which was adopted by Theymoli and Sadasivam (1987). Then, protein content was calculated from the following equation.

Total crude protein content = N content × 6.25

**Statistical analysis**

The experiments were designed by randomized complete block design with five and three replications for each experiment, respectively. The data, of both experiments with different treatments, were analyzed by one way analysis of variance (ANOVA). The fisher's least significant difference (LSD) at the 5% level was used to analyze the differences between the means of growth parameters under both saline and non saline conditions.

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