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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF MAIZE (*ZEA MAYS* L.) TO PLANT DERIVED SMOKE SOLUTION

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Abstract

Plant-derived smoke is known as plant growth regulator by affecting plant growth and developmental processes. Current study highlights the priming effects of smoke solutions on physiological and biochemical attributes of maize. Maize seeds were presoaked in distilled water (as control), concentrated and 500 times diluted smoke solution of *Cymbopogon jawarncusa* (*C. jawarncusa*) plant for 0, 6, 12 and 18h. Significant increase ($p<0.05$) in seed germination, seedling length and biomass with increased presoaking hours was observed. Inhibitory effects of concentrated smoke solutions were higher with the increase in presoaking hours. The content of chlorophyll (*a* and *b*) pigments, total carotenoids and total soluble proteins were increased in seedling treated with smoke dilution (1:500). These results suggested that plant derived smoke solutions have positive effects on plant physiological and biochemical growth parameters and diluted smoke solution has potential application in agriculture since it is economical and environment friendly.

Key words: *Cymbopogon jawarncusa,* Photosynthetic pigments, Smoke solution, Seedling growth.

Introduction

Smoke derived from plant materials has been reported for positive effects on plant's flowering, (Keeley, 1993), somatic embryogenesis (Senaratana *et al.,* 1999), seeds germination (Todorovic *et al.,* 2005, Aslam *et al.,* 2014), seedling growth (van Staden *et al.,* 2006, Moreira *et al.,* 2010; Aslam *et al.,* 2014), pollen performance and elongation of pollen tube (Papenfus *et al.,* 2015). These effects of smoke solution are attributed to the presence of active compounds i.e. butenolide and few more like cyanohydrins (Flematti *et al.,* 2004; Flematti *et al.,* 2011). Smoke derived compounds are heat stable, water soluble and effective at low concentrations (Flematti *et al.,* 2004; van Staden *et al.,* 2004). Smoke solution affects plant growth as it modulates endogenous plant growth hormones (van Staden *et al.,* 2000) and initiating cell cycle activities which assist early emergence of the radicle (Jain & van Staden, 2006). Smoke solution not only affects seed germination but also enhancing the length and mass of the plant's seedling (Sparg *et al.,* 2005).

Maize (*Zea mays* L.) belongs to family Poaceae. It is a profitable, stable and dependable agricultural crop in Pakistan (Ahmed *et al.,* 2003). However, the yield of maize in Pakistan is very low comparing to the world (MINFAL, 2009). Little work is documented on post-germination effects of smoke solution on plant growth. In this paper, we evaluated post germination effects of smoke solution on physiological and biochemical growth parameters of maize plant.

Materials and Methods

Collection of plant material: Leaves of *Cymbopogon jawarncusa* L. were collected from Kohat University of Science and Technology, Kohat, KP, Pakistan. Plant material was surface sterilized with distilled water to remove the dust particles and was semi-dried in shade.

Preparations of plant-derived smoke solutions: Aqueous smoke solution of *C. jawarncusa* L. was prepared by a method as described by De Lange & Boucher (1990) and modified by Baxter & van Staden (1994) and Dixon *et al.* (1995). Semi dried plant material weighing 333 g was heated in a furnace and smoke was passed through 1L of distilled water to gain concentrated smoke solution (pure smoke solution) (Tieu *et al.,* 1999), which was filtered through sterilized filter paper and kept at $4^{o}C$. Concentrated smoke solution was diluted further to 1:500 (500 times).

Seed Source: Maize (*Zea mays* L., Varity Azam) seeds were obtained from Agriculture Research Station Sara-e-Naurang Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. Seeds were surface sterilized in ethanol solution (70%) for 5 minutes (Oyebanji *et al.*, 2009) and rinsed three times with distilled water.

Germination protocol: To find out the effect of smoke solution on physiological and biochemical parameters of maize, seed were presoaked for 0, 6, 12 and 18 h in respective treatments i.e. distilled water/control (Cont), concentrated (Conc.) and 1:500 smoke dilution before germination. Seed imbibition rate was investigated by weighing the seeds (50 seeds) before presoaking and after presoaking in distilled water/control (Cont), concentrated (Conc.) and 1:500 smoke dilution. The difference between these two stages is the increase in imbibition. Seeds were germinated in 9 cm Petri plates lined with 2 layers of filter paper (Whatman No. 41). Direct sowing (without presoaking) seeds were moistened with 5 ml of respective solution distilled water/control, concentrated and 1:500 smoke solution) while the presoaked (6, 12 and 18h) seed were supplied 5ml distilled water when required. In each experiment, 3 replicas were used with 10 seeds per replica. All the experiments were conducted at 28±3°C for 8 days. The data for germination was taken after each 12h. After 8 days of the sowing, seedling length and fresh weight were measured. Different pigments (chlorophyll *a*, *b* and total carotenoids) and total soluble proteins were also determined.

Determination of photosynthetic pigments: Dried plant sample (25mg) was taken in a test tube followed by addition of Magnesium Oxide (MgO) (25 mg). Sample was mixed by shaker (2h) after adding Methanol (5ml). The pigments extract was centrifuged at 25°C for 5 minutes at 4000 rpm (Eppendrop Centrifuge 5427 R). Three wave lengths (666 nm, 653 nm and 470 nm) were used to find out absorbance readings of supernatants against a solvent blank in a UV-2600 spectrophotometer. Different pigments (Chlorophyll "*a*", "*b*" and total carotenoids) were measured using the formula (Lichtenthaler & Wellburn, 1983).

Chlorophyll a = 15.65 A666 – 7.340 A653
Chlorophyll b = 27.05 A653 – 11.21 A666
Total Carotenoids = (1000 A470 – 2.860 Chla – 129.2 Chl b)/ 245

Total soluble proteins determination: Total soluble proteins were determined using the protocol of Bradford (1976). Fresh plant materials (100mg) were homogenized in a 1 ml phosphate buffer with pH 7 using electrical homogenizer. This sample/crude extract was centrifuged at 4000 rpm for 15 minutes. After centrifugation (2 ml distilled water, 20 µl sample extract and 0.5 ml Bradford reagent) was taken in cuvette to check absorbance. Double beam spectrophotometer (UV-2600) was used to calculate absorbance at 595 nm wavelength of light. Bovine serum albumin was used as standard and the below formula was used to measured total proteins in maize seedlings.

Total soluble protein (mg g-1) = C x V/VT x W
C = Absorbance value
V = Volume of phosphate buffer
VT = Volume of sample extract
W = Plant weight

Statistical analysis of data: The results were analyzed by One Way Analysis of Variance (ANOVA). Three replicas were used for all treatments and their means were separated by least significant difference (LSD) test at 5% level of significance.

Results

Effect of smoke solution on imbibition of maize seeds: To study imbibition rate of maize seeds, 50 seeds were presoaked for different time intervals (0, 6, 12 and 18h) in distilled water/control, concentrated and 1:500 smoke dilution (Table 1). Seeds weights were measured before and after imbibition. Results indicated that diluted smoke solution (1:500) significantly increased imbibition of maize seeds as compared to concentrated smoke and control (Table 1).

Table 1. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on imbibition of maize seeds. Maize seeds (50 seeds) were imbibed for 0, 6, 12 and 18h in distilled water (Control), concentrated and diluted smoke (1:500) solution.

Imbibition time (hours)	Control (distilled water)			Concentrated smoke solution			Smoke dilution (1:500)		
	Seed weight before priming (g)	Seed weight after priming (g)	Increase in weight (%)	Seed weight before priming (g)	Seed weight after priming (g)	Increase in weight (%)	Seed weight before priming (g)	Seed weight after priming (g)	Increase in weight (%)
0	9.5	----	------	10.7	----------	--------	10.3	-------	-----
6	9.5	12.3	29.4%	10.7	13.1	22.42%	10.3	14.9	44.66%
12	9.5	12.9	35.7%	10.7	13.6	27.11%	10.3	16.0	55.29%
18	9.5	13.6	43.5%	10.7	14.3	33.66%	10.3	17.0	65%

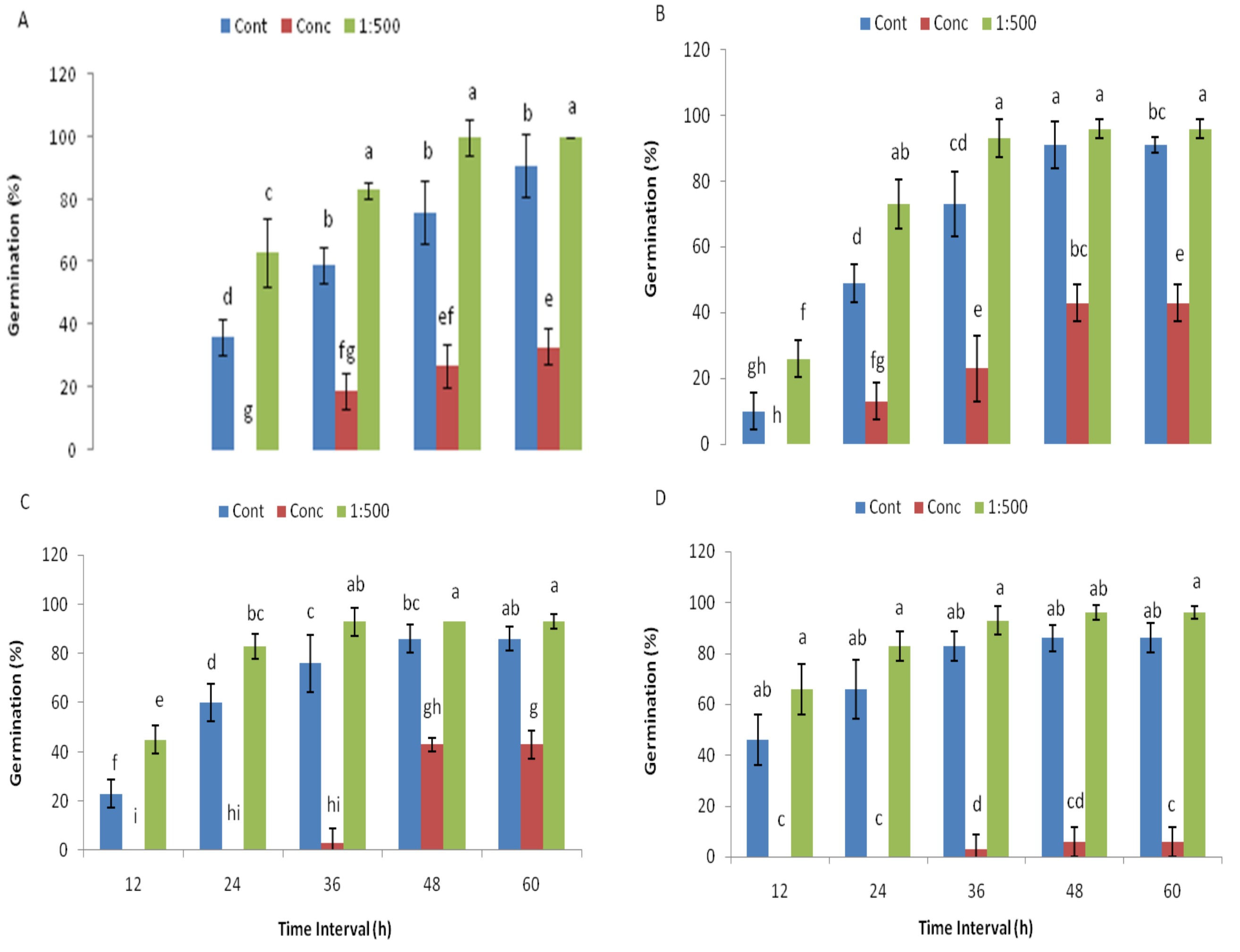

Fig. 1. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on seed germination of maize. Maize seeds were presoaked for 0 (A), 6 (B), 12 (C) and 18 h (D) in distilled water/control (Cont), concentrated (Conc) and dilution smoke (1:500) solution. Vertical error bar (I) indicates standard deviation. Alphabets represent significant difference between treatments with control as per least significance difference.

Effect of smoke solution on seed germination of maize: Seeds which were directly sown (Without Presoaking i.e., W.P) showed no germination after 12h, whereas, smoke dilution (1:500) significantly increased germination (%) after 24, 36 and 48h as compared to control (Fig. 1A). Concentrated smoke solution significantly decreased germination percentage of maize. Concentrated smoke solution delayed the process of germination till 36h of sowing (Fig. 1A).

Germination was observed in control and 1:500 smoke solution dilution after 12h in seeds presoaked for 6h, however, seeds presoaked with smoke dilution had significantly increased germination percentage (Fig. 1B). It was observed that smoke dilution significantly improved seed germination after 12, 24 and 36h. Seed presoaked for 6h in concentrated smoke solution have faster germination as compared to directly sown seeds.

After 12h seed presoaked in control, concentrated and smoke dilution, it was observed that seeds germinated early as compared to without and 6h presoaked seeds. Smoke dilution significantly increased seed germination of maize after 12, 24 and 36h. Seed germination was slow in concentrated smoke presoaked seeds and germination started after 36h (Fig. 1C).

Interesting results were observed after 18h seeds presoaking in control, concentrated smoke solution and 1:500 smoke solution. It was found that seed germination started (15%) even the completion of 18h seeds presoaking in 1:500 smoke dilution. Results showed that smoke dilution significantly increased seed germination (Fig. 1D). It was observed that the germination process was very fast as compared to 6 and 12h of seed presoaking in control and smoke dilution (1:500). Concentrated smoke solution significantly decreased germination of maize and it was slow then 6 and 12h presoaked seeds.

Effect of smoke solution on seedling length of maize: Plant-derived smoke solution also having post germination effects on maize seeds. Smoke dilution (1:500) significantly increased shoot length and its positive effects increases with the increases of presoaking hours (0, 6 and 12h) except 18h presoaking (Fig. 2A and Fig. 3A, B, C and D). Concentrated smoke solution having negative effects on shoot length and it increases with the increase of presoaking hours.

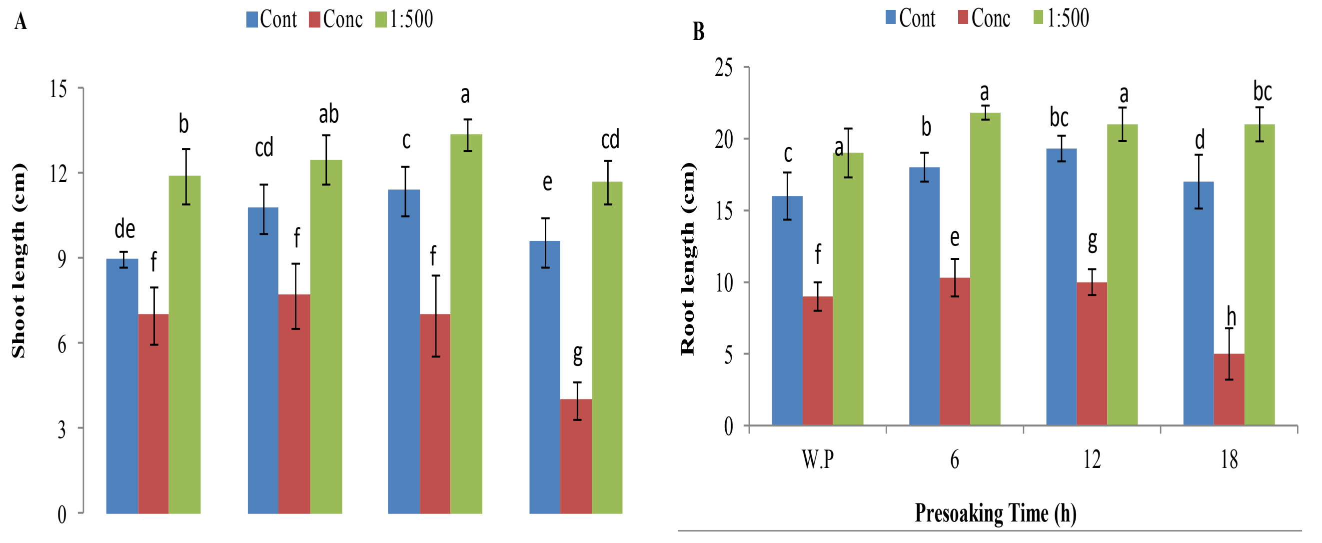

Fig. 2. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on (A) shoot length and (B) root length of maize. Maize seeds were presoaked in distilled water/control (Cont.), concentrated (Conc.) and diluted smoke (1:500) solution for 0, 6, 12 and 18h. Shoot and root length were measured after 8 days. Vertical error bar (I) indicates standard deviation. Alphabets represent significant difference between treatments with control as per least significance difference.

Fig. 3. Images representing maize seedlings of 0 (A), 6 (B), 12 (C) and 18h (D) presoaked seeds in distilled water/control (Cont.), concentrated (Conc.) and diluted (1;500) plant derived smoke solution after 8 days of sowing.

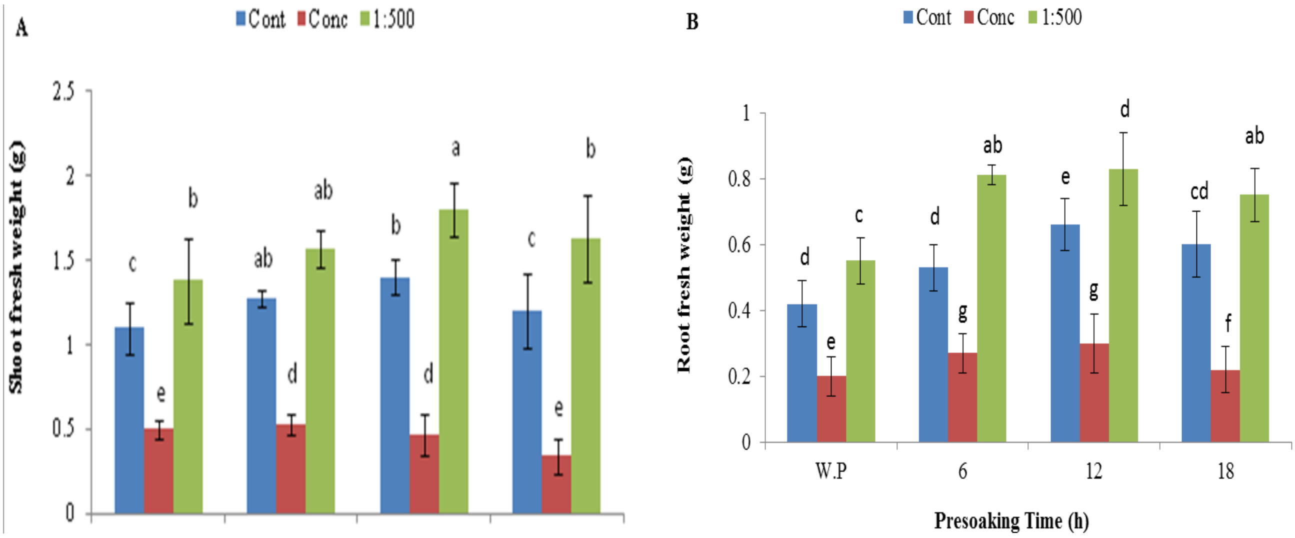

Fig. 4. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on shoot (A) and root (B) fresh weight of maize. Maize seeds were presoaked in distilled water/control (Cont), concentrated (Conc.) and diluted smoke (1:500) solution for 0, 6, 12 and 18h and incubated for germination at 25^{o}C. Shoot and root fresh weights were measured after 8 days. Vertical error bar (I) indicates standard deviation. Alphabets represent significant difference between treatments with control as per least significance difference.

Similar effects were observed on root length of maize. Smoke dilution (1:500) increased root length significantly after 0, 6 and 12h of presoaking while concentrated smoke solution shown negative effects on root length with the increase of presoaking hours (Fig. 2B and Fig. 3A, B, C and D).

Effect of smoke solution on seedling fresh weight of maize: Presoaking of seeds has positive effects on fresh weight of maize. It was found that smoke dilution (1:500) increased shoot fresh weight of seeds presoaked for 0, 6, 12 and 18h. Concentrated smoke solution decreased shoot fresh weight as compared to control (Fig. 4A). Similar effects were also observed for root fresh weight of maize. Smoke dilution has positive effects on fresh weight of root and it increases with the increase of presoaking hours (0, 6, 12 and 18h) (Fig. 4B). Concentrated smoke reduced fresh root weight as compared to smoke dilution (1:500) and control (Fig. 4B).

Effect of smoke solution on pigmentations of maize: Smoke treatment (1:500) have positive effect on amount of Chlorophyll (*a* and *b*) and carotenoids after 0, 6, 12 and 18h of seed presoaking in control and smoke dilution. Smoke dilution (1:500) increased the amount of pigments in maize shoot after 0, 6, 12 and 18h of seed presoaking while concentrated smoke solution have inhibitory effects on pigments (Fig. 5A, B and C).

Effect of smoke solution on total soluble proteins of maize: Total soluble proteins in seedling increased as the presoaking hours of seeds increases (0, 6 and 12h). Smoke solution (1:500) has positive effect on total soluble seedling proteins as compare to control (Fig. 6A and B). It is also observed that shoot have more total soluble proteins as compare to roots treated with concentrated smoke solution while smoke dilution (1:500) has opposite affects.

Discussions

It was observed that maize seeds imbibed more water when treated with smoke dilution than distilled water (control) (Tab. 1), which might have resulted in early and higher seed germination. This study proved that smoke dilution (1:500) has promotry effects on seed germination of maize (Fig. 1A, B, C and D). Seeds priming with plant derived smoke might have reduced germination time by the possibility of having hormones like compounds which help in the early seeds germination. Quicker germination may possibly be due to increased water uptake (Tab. 1) in seeds which in turn accelerated the germination related enzymes. These findings are parallel to Sparg *et al.* (2006) who reported that smoke dilution enhanced germination process while concentrated smoke solution have inhibitory effects on germination of seeds. The present results also showed that concentrated smoke solution have negative effects on seed germination of maize. This inhibitory effect of smoke solution is possibly due to the presence of another butenolide compound (3, 4, 5-trimethylfuran-2(5H)-one) isolated recently (Light *et al.,* 2010) and significantly reduces the promotry effects of related compound present in smoke solution. Furthermore, the positive effects on germination of maize seeds may be due to activating related genes. These stimulatory effects of smoke solution are well known on seed germination for last two decades (Jamil *et al.,* 2014; Aslam *et al.,* 2015). Similar results were also reported by Dayamba *et al.* (2008), that smoke solution obtained from plants and the isolated active compounds from smoke solution have positive effect on seed germination and shorten the overall time of seed germination. Studies revealed that smoke solution have positive effects on germination by stimulating GA (Schwachtje & Baldwin, 2004) which is germination promoter (Kucera *et al.,* 2005).

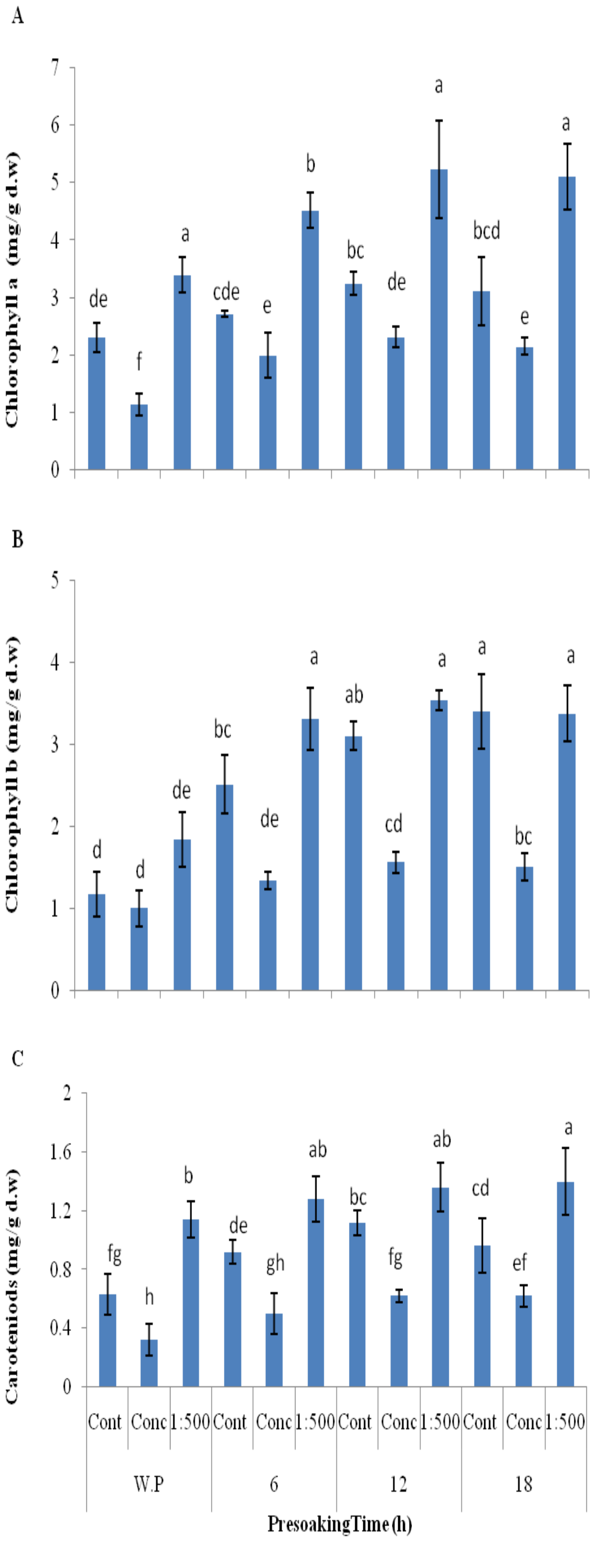

Fig. 5. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on Chlorophyll a (A), Chlorophyll b (B) and Carotenoids (C). Maize seeds were presoaked for 0, 6, 12 and 18h in distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution. Pigmentations were calculated after 8 days. Vertical error bar (I) indicates standard deviation. Alphabets represent significant difference between treatments with control as per least significance difference.

Enhanced seedling length and biomass: Seedling growth is an important growth indicator of plant health because strong roots help in anchoring while green and developed shoots are the main source of food production**.** Plant derived smoke had promotry effects on seedling of maize, okra, bean and tomato (van Staden *et al.,* 2006), okra (Kulkarni *et al.,* 2007). Our results indicated that smoke dilution (1:500) increased seedling length as compare to control (Fig. 2A, B, C and D and Fig. 3A, B, C and D). Smoke solution has the potential to affect various aspects of plant growth like seed germination, seedling vigor, seedling biomass (Kulkarni *et al.,* 2006; Sparg *et al.,* 2005; Jain & van Staden, 2007) and flowering (Keeley, 1993). Our data is also supported by Soo's *et al.* (2009), who reported that pretreatment of seeds with smoke solution have vigorous and lengthy seedling then the non-treated seeds.

Smoke treated maize seedling produced more roots as compare to control treated seedlings (van Staden *et al.,* 2006). It is also reported that the smoke solution behave like cytokinin and auxin which help in cell division and improved seedling growth (Jain *et al.,* 2008). Our results showed that smoke dilution has positive effects on seedling growth of maize (Fig. 2A and B). It is suggested that the smoke solution behave like plant growth hormones (Ma *et al.,* 2006) and affects plant growth parameters by interaction with other plant growth hormones (van Staden *et al.,* 2000).

Smoke solution and smoke derived compounds markedly increased fresh mass of tomato, okra and maize seedlings (van Staden *et al.,* 2006). It helped the plant to produce more leaves which increases seedling mass (Kulkarni *et al.,* 2007). Our results are also accordance to the previous studies (Jain & van Staden 2006; Aslam *et al.,* 2015) that smoke dilution significantly increased seedling mass. Our results elucidated that fresh seedling mass increases with the increase of presoaking hours in the smoke dilution (Fig. 4A and B) while concentrated smoke solution have negative effect on seedling mass. These results are similar to previous study which reported that smoke dilutions increased fresh mass of rice (Jamil *et al.,* 2014). As smoke priming helps the seeds to germinate early so it will have better seedling and more seedlings mass. Smoke water application also increased root and shoot growth in different South African plant species (Sparg *et al.,* 2005). We might conclude that smoke solution play key role in increasing seedling mass by producing vigorous seedlings. Similar results were obtained by Amin *et al.* (2007) when onion seedling were treated with IBA and get more fresh weight then control. Therefore, it is observed smoke solution and its derived compounds have similar mode of action. It is also reported that smoke solution can be a used for improving the rice seedlings which alternately have more seedling mass (Kulkarni *et al.,* 2006).

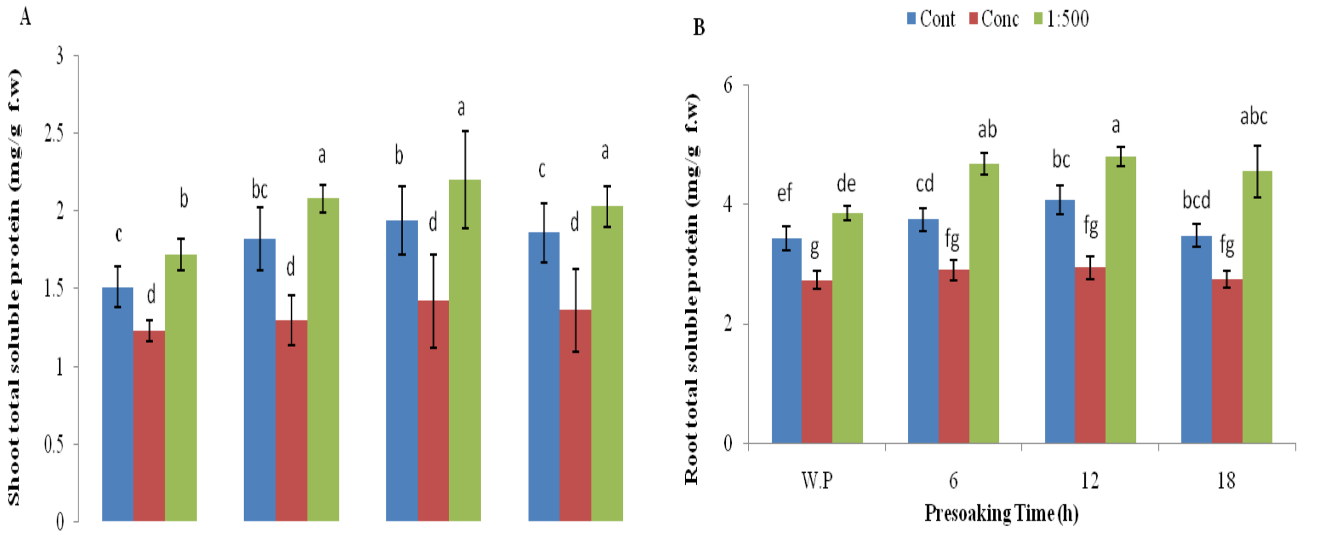

Fig. 6. Effect of distilled water/control (Cont.), Concentrated (Conc.) and diluted smoke (1:500) solution on maize shoot (A) and root (B) total soluble proteins after 8 days. Maize seeds were presoaked for 0, 6, 12 and 18h in distilled water/control (Cont.), concentrated (Conc.) and diluted smoke (1:500) solution. Vertical error bar (I) indicates standard deviation. Alphabets represent significant difference between treatments with control as per least significance difference.

Improved pigments contents and total soluble proteins: Photosynthetic pigments are important for whole plant growth due to regulators of photosynthesis (Parida & Das, 2005). Photosynthetic pigments (especially chlorophylls a, b and carotenoids) are important factors of photosynthetic processes and are very important for plants growth and development (Bollivar, 2006). Our results indicated that smoke dilution has promotry effects on chlorophyll a, b and carotenoids (Fig. 5A, B and C). Kulkarni *et al.* (2006) reported that smoke water had similar effects as plant growth hormones while growth hormones like cytokinin (Riefler *et al.,* 2006) are playing important role in plant development. Gibberellins application also increased protein content, chlorophyll content and produced the highest yield (Khandaker *et al.,* 2015) and it is now common fact that smoke solution has GA like nature and maybe it is the main cause of more chlorophyll content in the smoke treated shoot. Similarly auxin has also positive effects on amount of chlorophyll (Czerpak *et al.,* 1994; Czerpak & Bajguz, 1997). Our results indicated that concentrated smoke solution has negative effects on plant pigments (Fig. 5A, B and C). It is assumed that concentrated smoke has some role in reducing the catalytic activities of chlorophyll and carotene synthesizing enzymes or negative effects on pigmentation due to the higher concentration of inhibitory compound in it. Now it is common fact that smoke solution has promotry effect on shoot growth and those plants having better shoot growth has maximum amount of chlorophyll.

Proteins are important primary metabolites that play a key role in plant life. Smoke-solution application significantly increased nitrogen (N) level in plants (Chumpookam *et al.,* 2012) and nitrogen is the basic part of amino acids which are building blocks of proteins. Smoke dilution (1:500) significantly increased total soluble proteins as compare to control after 6, 12 and 18 h of presoaking (Fig. 6A and B). The increased in the amount of protein might be due to the growth nutrients (Nitrogen) involved in the synthesis of proteins (Jamil *et al.,* 2014). It may be due to the active growth regulator like compounds in smoke dilution while inhibitory compounds in the concentrated form of smoke (Jamil *et al.,* 2014). It is also reported that Gibberellin played an important role in biosynthetic activity of proteins (Wen *et al.,* 2010). Plant-derived smoke extracts can increases endogenous GA levels (Chiwocha *et al.,* 2009) which increases protein content biosynthesis in plants (Khan *et al.,* 2002).

Conclusion

This study highlighted the plant growth promoting potential of plant derived smoke determined through physiological and biochemical parameters of maize. Furthermore, maize seeds have more imbibition rate when presoaked in smoke solution as compared to control. Smoke solution helped maize seeds to germinate earlier and faster, increased seedling length, biomass and amount of pigments with total soluble proteins.

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References

Ahmed, S., A. Shahzad, M. Naeem and M.Y Ashraf. 2003. Determination of Efficacy of cypermethrin, regent and carbofuran against Chilo partellus Swin. and biochemical changes following application of these insecticides in maize plants. *Int. J. Agric. Biol.,* 5: 30-35.

Amin, A.A., M.E. Rashad and H.M.H. El-Abagy. 2007. Physiological effect of indole-3- butyric acid and salicylic acid on growth, yield and chemical constituents of onion plants. *J. Appl. Sci. Res*., 3: 1554-1563.

Anonymous. 2009. Agric. Statistics of Pakistan. Ministry of Food, Agric. and Livestock, Econ. Wing, Islamabad, Pakistan.

Aslam, M. M., A. Akhter, M. Jamil, A. Khatoon, I. Malook and S. Rehman. 2014. Effect of Plant-derived smoke solution on root of *Ipomoea marguerite* cuttings under Cobalt Stress. *J. Bio-Mol. Sci.,* 2: 6-11.

Aslam, M.M., M Jamil, A. Khatoon, S.E. El-Hendawy, N.A. Al-Suhaibani, S.S. Shakirullah, I. Malook and S. Rehman. 2015. Does weeds-derived smoke improve plant growth of wheat. *J. Bio-Mol. Sci.,* 3: 86-96.

Baxter, B.J. M and J. Van Staden. 1994. Plant-derived smoke: an effective seed pre-treatment. *Plant Growth Regul.,* 14: 279-282.

Bollivar, D.W. 2006. Recent advances in chlorophyll biosynthesis. *Photosynth Res*., 90: 173-194.

Bradford, B.B. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal biochem.,* 72: 248-254.

Chiwocha, S.D.S., K.W. Dixon, G.R. Flematti, E.L. Ghisalberti, D.J. Merritt, D.C. Nelson, J.M. Riseborough, S.M. Smith and J.C. Stevens. 2009. Karrikins: A new family of plant growth regulators in smoke. *Plant Sci.,* 177: 252-256.

Chumpookam, J., H.L. Lin, C.C. Shiesh and K.L. Ku. 2012. Effect of smoke-water on seed germination and resistance to *Rhizoctonia Solani* inciting papaya damping-off. *Hort. Sci.,* 37: 13-29.

Czerpak, R. and A. Bajguz. 1997. Stimulatory effect of auxins and cytokinins on carotenes, with differential effects on xanthophylls in the green alga Chlorella pyrenoidosa Chick. *Acta. Soc. Bot. Pol.,* 66: 41-46.

Czerpak, R., A. Bajguz, B. Białecka, L.E. Wierzchołowska and M.M. Wolan. 1994. Effect of auxin precursors and chemical analogues on the growth and chemical composition in Chlorella pyrenoidosa Chick. *Acta. Soc. Bot. Pol.,* 63: 279-286.

Dayamba, S.D., M. Tigabu, L. Sawadogo and P.C. Oden. 2008. Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. *Forest Ecol Manage*., 256: 462-470.

De Lange, J.H. and C. Boucher. 1990. Auto ecological studies on *Audinia capitata* (Bruniaceaae), plant-derived smoke as a germination cue. *S. Afr. J. Bot*., 56: 188-202.

Dixon, K.W., S. Roche and J.S. Pate. 1995. The promotive effect of smoke derived from burnt vegetation on seed germination of western Australian Plants. *Oecologia.,* 101: 185-192.

Flematti, G.R., D.J. Merritt, M.J. Piggott, R.D. Trengove and S.M. Smith. 2011. Burning vegetation produces cyanohydrins that liberate cyanide and promote seed germination. *Nat. Commun*., 2: 360.

Flematti, G.R., E.L. Ghisalberti, K.W. Dixon and R.D. Trengove. 2004. Molecular weight of a germination enhancing compound in smoke. *Plant Soil,* 263: 1-4.

Jain, N. and J. Van Staden. 2006. A plant-derived smoke improves early growth of tomato seedlings. *Plant Growth Regul.,* 50: 139-148.

Jain, N. and J. Van Staden. 2007. The potential of the smoke-derived compound 3-methyl-2H-furo [2,3-c]pyran-2-one as a priming agent for tomato seeds. *Seed Sci Res.,* 17: 175-181.

Jain, N., G.D. Ascough and J. Van Staden. 2008. A smoke-derived butenolide alleviates HgCl2 and ZnCl2 inhibition of water uptake during germination and subsequent growth of tomato—possible involvement of aquaporins. *J. Plant Physiol*., 165: 1422-1427.

Jamil, M., M. Kanwal, M.M. Aslam, K.S. Shakir, I. Malook, J. Tu and S. Rehman. 2014. Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. *Aust. J. Crop. Sci.,* 8(2): 159-170.

Keeley, J.E. 1993. Smoke-induced flowering in the fire lily *Cyranthus ventricosus*. *S. Afr. J. Bot.,* 59(6): 638.

Khan, A., M. Ansari, R. Mir and U. Sami. 2002. Effect of phytohormones on growth and yield of Indian mustard. *Ind. J. Plant. Physiol.,* 7: 75-78.

Khandaker, M.M., A. Majrashi and A.N. Boyce. 2015. The influence of gibberellic acid on the chlorophyll fluorescence, protein content and PAL activity of wax apple (*Syzygium samarangense* var. jambu madu) fruits. *Aust. J. Crop. Sci.,* 9: 1221-1227.

Kucera, B., M.A. Cohn and G. Leubner-Metzger. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.,* 15: 281-307.

Kulkarni, M.G., S.G. Sparg and J. Van Staden. 2006. Dark conditioning, cold stratification and a smoke-derived compound enhance the germination of *Eucomis autumnalis* subsp. autumnalis seeds. *S. Afri. J. Bot*., 72: 157-162.

Kulkarni, M.G., S.G. Sparg and J. Van Staden. 2007. Germination and post germination response of *Acacia* seeds to smoke-water and butenolide, a smoke derived compound. *J. Arid Environ.,* 69: 177-187.

Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determination of total caroteonids and chlorophyll a and b of leaves and different solvents. *Biol. Soc. Trans.,* 11: 591.

Light, M.E., B.V. Burger, D. Staerk, L. Kohout and J. Van Staden. 2010. Butenolides from plant-derived smoke: natural plant growth regulators with antagonistic actions on seed germination. *J. Nat. Prod.,* 73: 267-269.

Ma, E.B., K. Dixon and G. Flematti. 2006. Comparative enhancement of germination and vigor in seed and somatic embryos by the smoke chemical 3-methyl-2H-furo[2,3-c]pyran-2-one in *Tetraphyllum* (Restionaceae). *In Vitro Cell Dev Biol Plant.,* 42: 306-308.

Ministry of Food, Agriculture & Livestock (MINFAL). 2009. Agric. Statistics of Pakistan. Ministry of Food, Agric. and Livestock, Econ. Wing, Islamabad, Pakistan.

Moreira, B., J. Tormo, E. Estrelles and J.G. Pausas. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Ann. Bot.,* 105: 627-35.

Oyebanji, O.B., O. Nweke, N.B. Odebunmi, M.S. Galadima, U.N. Idris, A.S. Nnodi, Afolabi and G.H. Ogbadu. 2009. Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. *Afr. J. Biotechnol*., 8: 5395-5399.

Papenfus, H.B., M.G. Kulkarni, M.P. Jeffrey, F. Finnie and J. Van Staden. 2015. Smoke-isolated tri-methyl-butenolide inhibits seed fermination of different weed species by reducing amylase activity. *Weed Sci.,* 63: 312-320.

Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.,* 60: 324-349.

Riefler, M., O. Novak, M. Strnad and T. Schmu. 2006. Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *The Plant Cell.,* 18: 40-54.

Schwachtje, J. and I.T. Baldwin. 2004. Smoke exposure alters endogenous gibberellins and abscisic acid pools and gibberellins sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuata*. *Seed Sci. Res.,* 14: 51-60.

Senaratna, T., K. Dixon, E. Bunn and D. Touchell. 1999. Smoke-saturated water promotes somatic embryogenesis in geranium. *Plant Growth Regul.,* 28: 95-99.

Soos, V., A. Juhasz, M.E. Light, J. Van Staden and E. Balazs. 2009. Smoke-water-induced changes of expression pattern in Grand Rapids lettuce achenes. *Seed Sci. Res.,* 19: 37-49.

Sparg, S.G., M.G. Kulkarni and J. Van Staden. 2006. Aerosol smoke and smoke-water stimulation of seedling vigour of commercial maize cultivar. *Crop Sci.,* 46: 1336-1340.

Sparg, S.G., M.G. Kulkarni, M.E. Light and J. Van Staden. 2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Bio-resource Technol.,* 96: 1323-1330.

Tieu, A., K.A. Dixon, K. Sivasithamparam and J.A. Plummer. 1999. Germination of four species of native Western Australian plant using plant-derived smoke. *Aust. J. Bot*., 47: 207-219.

Todorovic, S., Z. Giba, S. Zivkovic, D. Grubisic and R. Konjevic. 2005. Stimulation of empress tree seed germination by liquid smoke. *Plant Growth Regul.,* 47: 141-148.

van Staden, J., A.K. Jager, M.E. Light and B.V. Burger. 2004. Isolation of the major germination cue from ¨ plant-derived smoke. *S. Afr. J. Bot*., 70: 654-659.

van Staden, J., N.A.C. Brown, A.K. Jäger and T.A. Johnson. 2000. Smoke as a germination cue. *Plant Species Biol.,* 15: 167-178.

van Staden, J., S.G. Sparg, M.G. Kulkarni and M.E. Light. 2006. Post-germination effects of the smoke-derived compound 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Res.,* 98: 98-105.

Wen, F., Z. Zhang, T. Bai, Q. Xu and Y. Pan. 2010. Proteomics reveals the effects of gibberellic acid (GA3) on salt-stressed rice (*Oryza sativa* L.) shoots. *Plant Sci.,* 178: 170-175.

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