Plant derived smoke - the magical seed sprouter: A view from traditional to recent advancements

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ABSTRACT: Fire, an important ecological factor was considered to play a vital role in stimulating seed germination in traditional farming before later discoveries. Some scientists were interested in heating effect while other in ethylene compound released during burning to be responsible for stimulating germination. Plant derived smoke is an emerging field of great interest for many scientists due to its striking effects on germination, seedling growth, leaf area, photosynthetic capacity and flowering. It can be directly applied to seeds or plants in aerosol or aqueous form by smoldering of plant material in distilled water; however the aqueous form is more effective than aerosol treatment. It is recently reported that plant extracted smoke enhanced ubiquitination of proteins and can also be used as priming solution for better performance of plants. With the identification of new chemical compounds has explored the field and it is reported that butenolide (3-methyl-2*H*-furo[2,3-c]pyran-2-one) present in smoke was most effective than known plant hormone such as gibberellic acid (GA). Recently priming with smoke water elevated the negative effects of salinity in rice plant. It is also observed that smoke containing compounds upregulated genes encoding expansins and a specific aquaporin gene. In the light of current reports it is suggested that plant derived smoke is a best growth promoter and it could be used as a bioenhancer and biofertilizers in future. This magical box can mimic in future the need of phytohormones in various fields of plant sciences.

Keywords: Fire; Smoke; germination; post germination; butenolide; gene expression;

1. INTRODUCTION

Fire is an important ecological factor and plays important role in the rehabilitation of many ecosystems. It is of course an integral part of many ecosystems and when such areas are protected from fire their local ecology becomes severely disturbed. Burning of habitats is also an important source of promoting germination events [1],[2]. Plants of fire prone habitats usually germinated just after fire. There was an idea that fire is necessary for the maintenance of these ecosystem but there was no concept that how the passage of fire stimulate germination of certain species.

Before later discoveries fire was considered to play a role in stimulating seed germination [3]. Farmers used fire and smoke traditionally in grain drying practices and it was thought that these methods improve seedling vigor [4]. In South Africa some farmers use a special method for storing maize; they place maize cobs over a fireplace, thus subjecting the seeds to smoke and heat [5]. The effect of this method was investigated on seed quality of two traditionally maize landraces and the results showed the improved germination rate and final germination in comparison with untreated seeds [6]. Fire stimulated germination of seed has been reported for a variety of fynbos species [7]. The heating effect of fire [8], high-temperature desiccation of the seed coat [9] and stimulation of germination by ethylene and ammonia contained in smoke [10] was considered to be important for seeds. Exposing seeds to temperature of 70°C -150°C for 1 to 2 hour has promoted seed germination of over 40 species. [11], similarly other reports reveal that dry heat may fracture the seed coat of hard-seeded species [12] but Van de Venter and

Esterhuizen. [10] were the first to describe that though high temperature play a role but is not the only necessary cue for germination associated with fire. Possibly the gases released (smoke) during a burn might also contribute to the germination cue and uptill now been ignored [3]. This was proved by performing different experiments on *Emmenanthe penduliflora* (Hydrophylaceae) which is an annual largely restricted to post-fire sites, and its germination is not cued by heat [13] but chemically by charred wood [14] or smoke [15].

Several reviews regarding smoke effect on plants have been published so for but none of them is covering all the aspects of smoke and its effects. This review is an effort to understand the whole story of smoke from traditional use (from where the idea has been developed) to recent advancements regarding efficacy of aqueous and aerosol smoke and new findings in this field including reversion of salinity stress by smoke solution.

2. Plant responses towards smoke

The matter disclosed in 1990's and it was found that one of the most important inducer of germination in post-fire environment is smoke itself [16],[17],[18]. It was found that smoke acted as a cue for breaking seeds of dormancy [19], [20] germination percentage [21], [22], [23], [24] seedling growth [25],[26],[27],[28] flowering [29]) plant biomass [30] of different plant families (Table 1).

The germination response to smoke can be described as an adaptation towards fire-prone habitats [11]. The aqueous extracts of plant-derived smoke induces germination of species of both fire-prone and fire- free habitat and irrespective of seed size viz both large- and small-seeded species comes under the category of positively responding taxa. In celery (*Apium graveolens* L.) seed dormancy was broken by a combination of plant-derived smoke, benzyl adenine (BA) and gibberellins (GA^{3} + GA^{7}) in the dark at 18 °C-26 °C temperature. Smoke may thus act as cytokinin by enhancing gibberellins activity in the celery seed system [20]. Plant communities, like South African fynbos or the Chaparral of California are so adapted to fire that many species can only reproduce when they are subjected to heat or chemical compound of smoke. In addition various scientists have shown the stimulation of germination of numerous species from a range of fire-prone environments worldwide including Australian kwongan [23], Californian chaparral [31], Western Cape fynbos [32] and the Mediterranean basin [33].

The promoting effect does not depend on the material burnt [3], [22] reported that aqueous smoke extracts prepared from burning tissue paper promoted germination of light-sensitive lettuce seeds incubated in the dark. It was also reported that the promotery effect of smoke is independent of seed size, shape and plant life form, whether an annual, perennial, herbaceous, seeder (fire sensitive) or re-sprouter (fire tolerant) [34]. The effectiveness of smoke extracts in breaking seed dormancy does not depends on the pH of the extract [3]. It suggests that positive/stimulatory effect of plant derived smoke is irrespective of habitat, seed size, plant life form, source of smoke and pH of extract.

3. Smoke may substitute light requirement

Smoke may substitute the light requirement for germination [3]. Treatment with a 2000 times diluted smoke solution overcome the light requirement of *Albuca pachychlamys* seeds, resulting in 80% germination compared to the 58% germination observed with the control [24]. In case of lettuce [35] and celery [20] smoke application allowed the seeds to germinate in the dark although these seeds germinate well in light. On the other hand seeds of *Syncarpha vestita* do not germinate in the light but do so when incubated in the dark [16], however in the presence of smoke extracts these (negatively photoblastic) seeds germinate in the light. In *Erica sessiliflora* seeds were dormant when dispersed and require light for germination, but when treated with smoke, the seeds germinated [21]. Thus it is concluded that smoke is the best substitute for the light requirement during germination in both positive and negative photoplastic seeds.

4. Combined effects of heat and smoke

Reports suggested that the combination of cues is also very important. A study by Thomas et al. [36] suggested that heat interact with smoke in promoting germination. The combination of both (smoke and heat) appears to be synergistic [37], [38]. Sometimes the combination of cues reduces while sometime it enhances germination. In chaparral species, the combination of heat shock and combustion products produced a decrease in germination of 12 smoke-stimulated species [31], however Brown et al. [21] reported that along with other distinct cues produced by heat, smoke is a chemical message which independently of the heat can stimulate seed germination of many fynbos species. It is therefore important to investigate the combination of cues (heat & smoke) as it can affect somehow its independent efficacy.

5. Dual Nature of smoke

Reports from earlier studies reveal that plant extracted smoke have dual regulatory function with promoting seed germination on one hand, while on the other hand inhibit it if used in high concentration [39], they also reported that promotion of seed germination depends on the initial uptake of the active component, imbibing seeds before treating with plant derived smoke or smoke solution will show less germination as compared to seeds treated with smoke or smoke solution directly. Another interesting aspect of plant derived smoke is that the promotery effect of smoke is irreversible [40], while the inhibitory effect of smoke is reversible and can be reversed by washing/applying distilled water [16]. Similarly Daws et al. [25] stated that beside stimulation of germination in a number of species smoke also had negative impacts on other species, for example smoke also had a significant negative effect on germination of two species i.e. *Avena fatua* and *Bromus sterilis* . These contrasting effects of smoke on germination and its inhibitory impact at high concentrations reveal that along with the main germination stimulant there are a range of germination inhibitors, whose response is species dependent [35]. In other words we can say that plant derived smoke can both promote or inhibit seed germination depending upon the dilution used and that high concentration inhibits seed germination.

6. Post-germination effects of Smoke

The stimulatory effect of smoke may continue after seed germination for some plants and may increase the biomass of the plant e.g. native grass seeds that were treated with smoke extracts showed better growth than control and produced significantly greater biomass after four months [30]. Similarly Sparg et al. [24] stated that although smoke treatment may not necessarily have an effect at the germination stage, it may play a role at the post-germination stage and suggested that in previous studies where many species have not responded to smoke treatments, may show some response at their seedling stage, viz improved seedling vigor. Therefore it is necessary to extend germination studies till seedling growth; when smoke is used as a germination cue.

Seeds of *Albuca pachychlamys,* and *Tulbaghia violoace* when exposed to aerosol smoke exhibited higher seedling survival percentages than non-smoked seeds, while no significant effect was observed for *Merwilla natalensis* seedlings [24]. Similarly germination of *Themeda triandra* (*T. triandra*) seeds in the presence of smoke or pretreatment with smoke had no adverse effect on seedling growth; in contrast water pretreatment of fresh seeds significantly reduced seedling height and caused a reduction in tiller number and that smoke treated seeds showed rapid germination rate and promotes seedling ability to survive in post-fire environment [41]. Seedling of *T. triandra* as a result of smoke treated seeds showed no morphological changes but grew more vigoursly. The faster growth may be due to increased root growth [3]. A similar effect was reported for Erica species and species of family Asteraceae [16]. Smoke treatments have the potential to improve not only the percent germination but also the seedling vigour of commercially bred maize seeds [5]. Calder et al. [42] reported that 20 minutes of smoke exposure resulted in a more than 50% reduction in photosynthetic capacity in five of the six species examined and this reduction in photosynthetic rate by smoke may be due to reduction in stomatal conductance. Guehl and Aussenac. [43] Suggested that production of smoke can lead to high vapor pressure that can enhance stomatal closure. In a recent study Jamil et al. [44] reported that seeds primed with different smoke dilution increased Ca^{+} and K^{+} while decreased Na^{+} concentration compared to hydroprimed seeds; they also suggested that plant derived smoke increase chlorophyll and total carotene contents under sodium chloride stress condition. They used *Bauhinia variegata* for smoke production and suggested that plant derived smoke may have some regulatory compounds that can reduce the negative effect of NaCl hence increase productivity.

7. Plant derived smoke modifies the expression pattern of genes

As discussed earlier plant derived smoke can change the ionic makeup of the cell helping in homeostasis. These changes will lead to the production of certain proteins that might have regulatory function as discussed by Jamil et al. [44] resulting in down or upregulation of certain genes. To understand the mode of action of plant derived smoke a detailed study was conducted by Soos et al. [45] using maize plant, it was hypothesized that stress-related genes may play a major role in smoke action and seedling vigor. The results showed that smoke water treatment considerably affected the transcription profile of young seedling just entering the early post-germination stage; suggesting that plant response to smoke in the early post-germination phase, may be caused by reactions that are similar to those occurring during abiotic and biotic stress conditions and thus some stress-related genes are up-regulated (by smoke). The common genes that were up-regulated by smoke water have been described previously as stress-related genes, some of which have been well characterized in this regard [46].

8. Other applications

Beside impact on germination and post-germination plant derived smoke also have some other applications. Investigations revealed that application of dry smoke may give seed protection against microbial attack [47]. The use of smoke as an agent to promote seed germination has a wide application including increasing the efficiency of propagation of threatened or commercially important wild species [16]. The use of smoke or aqueous smoke extracts has not been limited to seed germination, promotery effects have also been reported for processes such as flowering [29], rooting [27] and somatic embryogenesis [48]. Beside these smoke may alter endogenous levels of hormones in seeds and may even impact tissue sensitivity to endogenous hormones [49]. Treatment of Grand Rapid lettuce seeds with different dilutions of an aqueous smoke extract produces a response curve similar to that of phytohormones [41]. Similarly previous reports reveal the ability of smoke to substitute for phytochrome requirement during germination of negatively photoblastic *Syncarpha vestita* seeds [3] though before this discovery research reports have shown that it is difficult to germinate bear-grass (*Syncarpha vestita*) seeds under control conditions using hot water, chilling, chlorine bleach, or acid [50] and positively photoblastic *Lactuca sativa* seeds [35]. Keeping in view the above discussion, the role of plant derived smoke cannot be underestimated in any kind of study and needs proper screening for getting optimum conditions to use.

8.1. Smoke and soil structure, environment and micro organisms

It has been a common practice to apply the burned plant materials together with charcoal residues to soil. Carbonized materials are formally authorized for use as soil amendment material in Japan [51]. Butenolide (a compound from smoke; will be discussed later) certainly holds potential for field-scale use in, for example, weed control and re-vegetation of degraded areas [52], [53],[53] found that application of butenolide, at a rate equivalent to 2 g/ha, resulted in improved yield. The chemical constituent of plant derived smoke might have an effect on communities of soil dwelling organisms i.e. bacteria and fungi etc. Merritt et al. [54] hypothesized that soil microbes may also release butenolide during the decomposition of organic matter. Burning of organic material introduce many chemicals in atmosphere as volatiles that eventually settle on soil surfaces [55]. It also has been reported that smoke of rice straw, wheat straw and tobacco leaf had a great antifungal effect against soil fungi [56]. Therefore use of smoke in poorly managed soil or in areas where soil erosion is a problem will be a good practice and seems to be affected one. Furthermore smoke can be used as antimicrobial agent in certain cases.

9. Active compound(s) in smoke

Following the initial reports on smoke-stimulated seed germination in the early 1990s, various researchers world-wide attempted to isolate different chemical(s) present in plant-derived smoke responsible for the promotion of germination. Baldwin et al. [40] identified 71 compounds in active fractions of smoke by GC-MS and atomic absorption (AA) spectrometry, and tested a total 233 compounds using seeds of *Nicotiana attenuate*, however none of these compounds promoted germination. The difficulty in isolating the active compound(s) from aqueous smoke extracts was partly due to the large number of compounds present in the smoke extract, possibly up to several thousand [57], and partly due to the very low concentration of the active compound(s) relative to the other components present in smoke [58]. Afterward, [59] identified seven compounds present in both *Passerina vulgaris* and *T. triandra* smoke extracts. Four of the compounds were tested in the Grand Rapids lettuce seed bioassay at concentrations from 10^{-4} to 10^{-15} M. however, none were found to be active. Jager et al.[34] investigated a smoke extract from *T. triandra* and a liquid food-flavouring concentrate, using Grand Rapids lettuce seeds to detect germination activity. There was some chromatographic evidence indicating that there may be more than one active component in smoke that stimulates seed germination [40].

Van Staden et al. [60] concluded that similar types of compounds are present in smoke derived from different plant material. It was hypothesized that smoke contains various gases, such as ethylene, that may stimulate flowering in *Cyrtanthus ventricosus* and in other fire-stimulated geophytes [61], [62]. Keeley. [29] however suggested that ethylene is not the active component of smoke, as ethylene-treated bulbs failed to flower. Studies using light-sensitive lettuce seed, which is very responsive to smoke extracts, have indicated that neither of these compounds can be considered responsible for the promoting effect of smoke on lettuce seed germination [34]. Furthermore seeds of *T. triandra* and fynbos species tested, give no response to ethylene [19], [17].

A comparison of the active smoke extracts from *Passeriana vulgaris* and *T. triandra* reveal the presence of 7 compounds in both extracts [59]. Plant derived aqueous smoke and extracts prepared by heating agar and cellulose, contained compounds that stimulated the germination of lettuce seeds [34]. Keeley and Fotheringham. [15] reported nitrogen oxides present in smoke responsible for stimulation of germination. Adrianz et al. [63] identified 1, 8-cineole as an active germination enhancer.

The chemical composition of smoke varies with temperature but reports indicates that compounds produced between 160 °C to 200°C (320 to 392 °F) are more active; at high temperatures the stimulatory chemicals lost through volatilization; thus a slow smoldering fire will be effective [34].

9.1Discovery of butenolide

The identity of a compound present in plant and cellulose-derived smoke was reported after continued efforts by both South African and Australian research groups as butenolide, 3-methyl-2*H* furo [2,3-c pyran-2-one [64], [65]; that promotes germination of a variety of smoke responsive species. The compound has become commonly referred as "butenolide" in several studies, although this name refers to the class of compound [58], and recently has been referred to as "karrikinolide" (KAR_1) [66]. The compound was tested for its toxic effect [67]. The results showed that the compound is neither toxic nor genotoxic at the levels tested (1×10^{-4} to 3×10^{-10} M). Although butenolide group of compounds seems to be effective ingredient present in smoke so for but still there are chances of identification of new compounds.

10. Synthesis of Butenolide

After the identification of the active butenolide from smoke, several researchers successfully synthesized active butenolide. Australian researchers who originally reported the active compound, reported the synthesis of 3-methyl-2*H*-furo [2,3-c]pyran-2-one from pyromeconic acid [68]. Goddard-Borger et al. [69] described the synthesis of the compound, and several analogous compounds from d-xylose. Similarly Light et al. [70] prepared butenolide during heating reaction between d-xylose and glycine. Butenolide structurally related to 3-methyl-2*H*-furo [2,3-c]pyran-2-one in smoke are produced by a range of micro-organisms. For example, *Fusarium* sp. produces a 'butenolide' that functions as a mycotoxin with a mode of action resulting from an impact on the intracellular redox [71]. As oxidative stress has been proposed to have a role in germination [72] thus butenolide may also play a role in this area/field. The active compound(s) is soluble in water, maintains stability when stored, stable at high temperatures (its melting point is 118 °C to 119 °C) and is active at very low concentrations (1ppm to 100ppt) [64]), [65].

11. Response of plants towards Butenolide

Plant species responding positively to butenolide include those from fire-prone environments [64], [54], arable weeds [25], [53], hemi-parasitic and holo-parasitic weeds [73], several Australian *Solanum* species [66] and crop plants including lettuce, tomato, okra, bean, maize and rice [26], [65], [28]. The butenolide also stimulates germination of *Sisymbrium orientale* L. (Brassicaceae), *Hordeum leporinum* (Poaceae) and *Echium plantagineum* L. (Boraginaceae) [53]. Unlike smoke solutions butenolide is not inhibitory at high concentration [64], [65]. For example, in a study of 18 arable weed species, it was found that in terms of percentage germination, four species responded positively to smoke whereas eight responded positively to butenolide [25]. In addition, a further two species were negatively affected by smoke treatment, whereas no species were negatively affected by butenolide [58]. A negative effect of smoke on germination has been previously reported [25], [41], [39] and this means that smoke having a positive effect on a narrower range of species than butenolide, and that smoke also contains compounds which may negatively affect germination [39]. Thus it is much better to use butenolide instead of smoke to overcome negative effects of smoke and to get better yield.

12. Butenolide regulates germination at molecular level

Different reports revealed the possible role of butenolide in regulation of germination at molecular level. A study by Jain et al. [74] using differential display during tomato seed germination, reported the up-regulation of genes encoding expansins in the presence of butenolide. Expansin genes are highly conserved genes and mostly have been reported to be involved in cell expansion during tissue growth [75]. In the recent past three analogous compounds have been identified from smoke solution [76], this family of butenolide molecules have been designated as Karrikins [77] The parent molecule is KAR_1 is a potent stimulant that enhances germination (as early discussed) in a range of species at subnano-molar concentrations [64], [53]. In field trials, KAR_1 is effective at less than 5 g/ha compared with 10 ton/ha smoke water and thus may have practical value in agriculture, conservation, and restoration [53]. Karrikins have no distinct structural similarity to known plant hormones, although the "A" ring of the KAR_1 molecule is analogous to the "D" ring of strigolactones [64].

To test whether Karrikins and strigolactones share a similar mechanism of action, the germination response of primary dormant Landsberg *erecta* ecotype (L*er*) seeds was compared with four karrikins and the synthetic strigolactone GR-24 [76]. KAR_2 was the most active analog and clearly enhanced germination at concentrations as low as 10 nM, while KAR_1 and KAR_3 were slightly less effective [76], although KAR_4 stimulates germination of *Lactuca sativa* and *Solanum orbiculatum* [78], KAR_4 was either completely inactive or inhibitory at similar concentrations in *Arabidopsis*. Since its discovery, a widespread capacity for KAR_1 germination response among angiosperms has been demonstrated [64], [64], [53], [28], [65]. Thus Karrikins may be considered as novel class of plant growth regulators with broad impact [76]

Recently Soos et al. [79]) reported that smoke treatment enhanced ubiquitination of proteins and activated genes related to protein-degrading genes in maize kernels while treatment with KAR_1 upregulated a specific aquaporin gene. Therefore it is important to extend smoke related study to molecular level to understand its mode of action.

13. Post-germination effects of Butenolide

Beside germination, butenolide also has positive effect on different plant parts (Table 2). It was found that both $HgCl_2$ and $ZnCl_2$ reduced water uptake during germination and seedling growth in tomato thus impact negatively on germination and seedling growth; however in the presence of butenolide (10^{-7} M) the negative effects on water uptake and growth were largely overcome [80]. This raised the possibility of butenolide treatments being used for plant establishment in contaminated soils. Similarly Van Staden et al. [28] reported that in tomato, the root lengths of seedling treated with butenolide were 10 times longer as compared to the roots of control seedlings. In both okra and bean, the root lengths were increased, on average, up to three-fold, suggesting that the smoke compound may stimulate either cell elongation or division [28].

The data reported by Daws et al. [25] tells us that butenolide may have wide applicability as a germination and seedling growth stimulant in species irrespective of their habitat. He also stated that butenolide does not affect non-dormant seeds e.g. for non-dormant seeds of tomato, butenolide had no effect on germination percentage but significantly increase seedling growth [26]. This suggests that like smoke solution butenolide also has its promoting effect on post germination processes.

14. Other applications of Butenolide

Butenolide had a wide range of applications. Though it is derived from smoke, it has been proved more effective than smoke [25], [64], [65]. Beside other effects, butenolide also affects fruit set. Kulkarni et al. [81] investigated the effect of smoke-water and butenolide (10^{-9} M) on growth and final yield of tomato under greenhouse conditions. Both smoke-water and butenolide-treated plants produced fruits earlier and in greater number than control treatment. Furthermore in *vitro* experiments incorporation of butenolide into the culture medium reduce the period of embryo development and improved the frequency of root formation in *Baloskion tetraphyllum* compared to a control treatment [82]. Promotion of lateral root formation is one of the processes controlled by Auxins [71] and also by butenolide revealed by Kulkarni et al. [83], that smoke water and butenolide act in a similar manner just like plant hormones. If this statement is true, butenolide and smoke solution can be used instead of plant hormones in various experiments as well as in field use to avoid the costly use of plant hormones.

15. Stability of Smoke solution

The stability of smoke solution was also a question to consider because the production of smoke solution is not an easy practice. Scientists tried to find out that for how long smoke treatment can sustain its effectiveness and it was confirmed that smoke is thermo stable and can sustain its effectiveness for a long time. Baxter and Van Staden. [41] Showed that seeds of *Themeda triandra* imbibed in aqueous smoke solution for 12 hour and dried retained the promoting effect after 21 days storage. Seeds of species of Asteraceae and Restionacea treated with smoke extract and then dried have retained the dormancy breaking effect for at least 1 year. Smoke treated seeds of some wild flower species retained their promoting effect after a year dry storage [84]. Similarly seeds which were treated with 1:100 concentration of smoke extract for 1 hour after two weeks showed a similar germination response to seeds which were allowed to germinate directly following an equivalent treatment [39].

16. Period of smoke treatment

The period of smoke treatment is also very important. Exposure of the lettuce seeds to 1000X smoke solutions for periods of 1 hour or less show almost similar germination to that of water control; the percentage germination show a marked increase above water control when seeds exposed to smoke solution for 2 hours or more, with a maximum 90% germination for the 6 hour treatment [39]. Experiments performed by Keeley. [85] found that the length of exposure to smoke was very important in some species, a three minute difference in exposure resulted in the death of some seeds. Similarly Pennacchio et al. [86] reported that aerosol smoke significantly inhibits germination when seeds were exposed to prolonged periods of aerosol smoke. Smoke may be applied to seeds immediately before sowing, or the seeds may be pretreated and stored until conditions are favorable for sowing [49]. Both smoke and aqueous smoke-water is active in this respect. It is evident from the study that period of smoke treatment depends upon the nature of the smoke, in case of aerosol smoke long period is dangerous while in case of aqueous smoke the efficacy of smoke solution increases with increase in treatment period but upto certain time.

17. Comparison between butenolide and Gibberellin

Daws et al. [25] reported a significant relationship between the germination response to butenolide and gibberelic acid 3 (GA_3), the results showed that butenolide was the most effective and did not result in the elongated internodes (abnormal condition) that were resulted by application of gibberellins. Thus butenolide is of greater value than GA_3 for germination testing on diverse species. The action of GA was compared to that of butenoloide for three species *Angianthus tomentosus*, *Myriocephalus guerinae* and *Podolepis canescens*; and both compounds were found to stimulate germination [87]. This study provides evidence that butenolide can act in a similar fashion as gibberellic acid in promoting seed germination of light-sensitive seeds. There are clear similarities in the response of seeds to butenolide/smoke and GA_3; and also in the chemical structures of the two compounds [58]. Consequently, butenolide has great applications in agriculture, horticulture and re-vegetation of degraded landscapes.

18. Smoke reverses the negative effect of salinity

The dramatic role of plant derived smoke in enhancement of plant growth attributes diverted the scientists to test smoke water for the reversion of different stress conditions. a recent study by Ijaz et al. 2014 [88] experimented the possible role of *Buhania varigata* and *Cymbopogon jwarancusa* derived smoke water on rice plant under 50, 100 and 150 mM salt stress condition. They reported that with increasing salinity, the germination percentage, seedling growth, K^+, Ca^+, cell membrane stability, total nitrogen and protein contents were compromised while Na^+ concentration was increased. However seeds pre-soaked with 5000X *Buhania* and 500X *Cymbopogon* derived smoke water significantly reduced the negative effects of salinity on all the growth attributes studied.

This discussion indicates that keeping in mind the positive role of plant derived smoke in the land management and rehabilitation of ecosystems smoke water can be used against both biotic and abiotic stress conditions.

19. Conclusions

The above discussion reveals that plant derived smoke has a wide range of applications, with more effective on germination of all kinds of seeds in general and that of fire prone habitats in particular. This efficacy is independent of any taxonomic position some members of the family may respond positively while other members of the same family will respond negatively or will show no response. Although the pattern of smoke action is not well understood but it is confirmed that smoke is more effective in diluted form than concentrated one and can have different effects on different parts of the same plant. The comparision with GA confirmed that butenolide an active compound from smoke performs better than GA, some published and unpublished data confirmed that pretreatment with smoke solution can enhance the germination and seedling growth compared to hydroprimed seeds. Recent investigations revealed the positive role of smoke water in the elevation of salt stress in rice plant; this practice can be used for other plants against both biotic and abiotic stress conditions by studying in detail the mode of action and the effect in metabolic pathways. As well as the confirmation of smoke effect on soil structure and soil microorganisms suggests that plant derived smoke can be used in soil management and biofertilizers in future.

Acknowledgment:

Thanks to Barani agriculture research center Jarma Kohat and Pir sabak research agriculture farm Nowshehra for providing seeds for different experiments.

Table 1: Response of different plants families towards smoke

S/ No	Family name	Response	**Representative plants	Reference
1	Bruniaceae	Positive	*Audouinia capitata*	De Lang & Boucher, 1990 [17]
2	Asteraceae	Positive	*Syncarpha vestita & other sp*	Brown et al., 1993 [89]
3	Ericaceae	Positive	*Erica sessiliflora*	Brown et al., 2003b [21]
4	Restionaceae	Positive	*Staberoha cernua*	Brown et al., 1993 [89]
5	Proteaceae	Positive	2 species	Brown et al., 1993 [89]
6	Crassulaceae	Positive	*Crassula capensis*	Brown et al., 2003b [21]
7	Geraniaceae	Positive	*Pelargonium* sp.	Brown et al., 2003b [21]
8	Mesembryanthemaceae	Positive	*Erepsia anceps*	Brown et al., 2003b; Pierce et al., 1995 [21], [90]
9	Poaceae	Positive	*Themeda triandra*	Le Maitre & Midgley, 1992 [7]
10	Portulacaceae	Positive		Le Maitre & Midgley, 1992 [7]
11	Scrophulariaceae	Positive	*Nemesia versicolor, N. lucida, Selago sp.*	Le Maitre & Midgley, 1992 [7]
12	Haemodoraceae	Positive	*Anigozanthos manglesii*	Dixon et al., 1995 [34]
13	Epacridaceae	Positive	*Lysinema ciliatum*	Dixon et al., 1995 [34]
14	Amaryllidaceae †	No Responce	*Cyrtanthus* sp	Brown et al., 2003b [21]
15	Hyacinthaceae*	Positive	*Albuca pachychlamys*	Sparg et al., 2005
16	Iridaceae	No Responce	*Bobartia gladiata* *Geissorhiza sp.*	Brown et al., 2003b [21]
17	Haemodoraceae	No Responce	*Wachendorfia paniculata*	Brown et al., 2003b [21]

18	Rutaceae +	Positive	*Geleznowia*	Dixon et al., 1995 [34]
19	Dilleniaceae +	Positive	*Hibbertia*	Dixon et al., 1995 [34]
20	Proteacea +	Positive	*Stirlingia*	Dixon et al., 1995 [34]
21	Myrtaceae +	Positive	*Verticordia*	Dixon et al., 1995 [34]
22	Cupressaceae +	Positive	*Actinostrobus*	Dixon et al., 1995 [34]
23	Thymelaeaceae +	Positive	*Pimellia*	Dixon et al., 1995 [34]
24	Lilliaceae	Positive	*Xerophyllum tenax*	Shebitz et al. (2009) [91]
25	Cistaceae	Positive	*Cistus albidus*	Reyes and Trabaud, 2009 [92]
26	Fabaceae	Positive	*Spartium junceum* L.	Reyes and Trabaud, 2009 [92]
27	Apiaceae	Positive	*Apium graveolens* L.	Thomas and Van Staden, 1995 [20]
28	Thymelaeaceae	Positive	*Themeda triandra*	Taylor & Van Staden, 1998 [93]

*: The family was described previously as non-responding to smoke by Brown et al., 2003a but described by Sparg et al., 2005 as positively responding to smoke.

**. Representative plant means that plant that was subjected for study.

+: Germination of these families was improved by smoke previously recorded as very difficult or impossible to germinate using usual methods

†: the family was reported not responding to smoke by Brown et al., 2003b but Keely (1993) reported that smoke enhance flowering in *Cyrtanthus ventricosus*

Table 2: Response of different plants families towards Butenolide

S/ No	Family name	Response	Representative plants	Reference
1	Fabaceae	Positive	*Acacia, Phaseolus*	Kulkarni et al., 2007a; Van Staden et al., 2006 [94], [28]
2	Poaceae	Positive	*Eragrostis tef, Oryza sativa*	Ghebrehiwot et al., 2008; Kulkarni et al., 2006 [95] [83]
4	Asteraceae	Positive	*Arctotheca calendula*	Stevens et al., 2007 [53]

5	Brassicaceae	Positive	*Brassica tournefortii*	Stevens et al., 2007 [53]
6	Solanaceae	Positive	*Lycopersicon esculentum*	Van Staden et al., 2006; Merritt et al., 2005; Jain et al., 2006 [94], [87], [26]
7	Malvaceae	Positive	*Abelmoschus esculentus*	
8	Apiaceae	Positive	*Dacus carota*	
9	Dioscoreaceae	Positive	*Dioscorea dregeana*	Kulkarni et al., 2007b [96]
10	Restionaceae	Positive	*Baloskion tetraphyllum*	Ma et al., 2006 [82]

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