**Review Article**

**Plant derived smoke treatment of seeds for enhancement of germination and seedling growth**

**ABSTRACT**

Smoke act as an important evolutionary factor involved in post fire germination cues. The role of smoke in stimulating germination was first highlighted in South Africa in a study on a threatened fynbos species *Audouinia capitata*. Farmers of South Africa uses conventional method of exposing seeds to smoke as it give protection against insects and pathogens. Further this method proved to increase germination and seedling vigor. The potential benefits of smoke in agriculture were achieved by exposing seeds directly to aerosol smoke, treating with smoke water or dynamic compounds extracted from smoke. Smoke water is convenient and shows promising results. Butenolide (3-methyl-2H furo [2,3-c]pyran-2-one) derived from plant-derived smoke is a well-known seed germination agent. Following the initial isolation of KAR1, a whole new family of plant growth regulators, named ‘karrikins’ were isolated. Glyceronitrile or cyanohydrin is another compound isolated from plant derived smoke which stimulated germination in wide variety of plant species. Application of plant-derived smoke had found to stimulate seed germination, dormancy breaking, seedling growth, and mitigating stress in various ecosystem.

***Keywords:*** *Smoke treatment;Karrikins;Cyanohydrins;Germination; Plant derived smoke*

1. **INTRODUCTION**

Fire act as a common disturbance element in many ecosystem and also provides opportunity for recreation of new species as a acquired response of plants to fire. Post fire events involves removing the canopy and leaf litter, which alters the amount and quality of light, adding more nutrients and moisture to the soil, eliminating inhibitory allelochemicals ([Dixon et al., 2009](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9); [Nelson et al., 2012](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9)). Plant-derived smoke produced during fire is known to boost plant growth and development (Kamran et al., 2017) and has a favorable impact on plant species in different ecosystem (van Staden et al., 2007). The germination of some plant species, fully efficient after fire and subsequently rain, is a well-known phenomemon called “pyrophytism”, and these plants are referred to as “fire ephemeral” or “pyro endemic plants” that only after many years of absence, can emerge after a forest fire (Troumbis et al., 2021). Many aspects of fire have been studied for their effects on plant growth and/or seed germination. These include heat, the rapid release of nutrients from burned plant tissue, and compounds found in ash. Fire's stimulating mechanism could be from the physical one of desiccation on seed coat morphology, the physiological one of desiccation on seed embryos, and/or the dormancy-breaking properties of volatile chemicals such as ethylene and ammonia (Keeley & Fotheringham, 2000). The chemical germination cues can be found in smoke which is released by burning vegetation. These compounds function as important environmental signals which either promote or inhibit the germination of many plant species following a fire (Dixon et al., 2009). The significance of smoke compounds in breaking seed dormancy and promoting germination for certain species has been the focus of attention since 1990.

 The discovery of compounds in plant smoke accelerated seed germination was initially reported by De Lange & Boucher, (1990). The significance of smoke in promoting germination was initially brought to light in a South African study focusing on the endangered fynbos species *Audouinia capitata*. Additional research on Australian species, Californian chaparral, and South African fynbos has demonstrated how smoke can generally encourage the germination of numerous species from fire-prone regions. Smoke has also been found to elicit germination of many crop and weed species such as rice (Oryza sativa), wild oats (Avena sativa), and lettuce (Lactuca sativa) (Kulkarni et al., 2006; Light et al., 2009). Thus far, smoke has been found to stimulate the germination of seeds in over 1200 species from 80 genera across various ecosystems (Dixon et al., 2009).

The research by Brown & Van Staden, (1998) confirmed certain water-soluble substances included in smoke from burning plant tissues are crucial for thawing seed dormancy. From burned cellulose and smoke derived from plants, a biologically active butenolide compound (3-methyl-2H furo [2,3-c]pyran-2-one) was isolated (Flematti et al., 2004). As per Merritt et al., (2007), this compound is known as karrikinolide (KAR1). It has the ability to stimulate seed germination in numerous plant species, even at incredibly low concentrations. Smoke water was shown to have effects akin to those of plant growth regulators by Kulkarni et al., (2006). Additionally, Karrikins, a novel family of plant growth regulators discovered in smoke, were reported by Chiwocha et al., (2009) to have the ability of breaking seed dormancy, initiating seed germination, and regulating seedling growth in a variety of taxa. Smoke may have a dual regulatory function during germination, (Light et al., 2010). It may stimulate germination but also inhibit it until there is enough water available. In post-fire settings, this kind of dual signal system which includes both promotory and inhibitory compounds may be crucial. 2, 3, 4-trimethylbut-2-enolide, or 3, 4, 5-trimethylfuran-2(5H)-one, was the inhibitory compound that was isolated. Cyanohydrins is the another compound isolated from plant derived smoke known to release cyanide upon hydrolysis also reported to enhance seed germination and seedling growth by Nelson et al., (2012).

**Bioactive compounds in smoke as a trigger of growth and germination**

Chemically smoke contains many different compounds, and several attempts have been made to identify these active compounds. The two main active substances that have been identified and isolated with potential for use in agriculture are karrikins (KARs) and cyanohydrins. These are long-lasting, heat-stable, and water-soluble substances.

 **Karrikins (KARs):** A biologically active butenolide compound (3-methyl-2-H-furo [2,3-c]pyran-2- one) was isolated from burnt cellulose and plant-derived smoke (Flematti et al., 2004) as a by product of pyrolysis. This compound was termed as karrikinolide (KAR1) (Flematti et al., 2009), is stable, crystalline in nature having melting point of 118–119 °C and are easily soluble in organic solvent while sparingly soluble in water (Antala et al., [2020](https://link.springer.com/article/10.1007/s00344-021-10473-5#ref-CR5)) and even at very low concentrations it is effective in promoting seed germination in many different plant species. Following the initial isolation of KAR1, a whole new family of plant growth regulators, KAR2–KAR6, are collectively known as karrikins, were identified in smoke (Chiwocha et al., 2009), and several related compounds have been synthesized (Flematti et al*.,* 2007). Of all the KARs the most abundant in smoke and active in seed germination is KAR1. KAR1 stimulates potent germination activity of seeds when used in nano molar concentrations (< 1 ppb or 1 nM). It is estimated that between 2 - 5 g of KAR1 is more than sufficient for 1 ha of land application rates that are commercially viable (Dixon et al., 2009).

Karrikins responses are not specific to fire-prone species but have been effective in the germination, vigor, and stress tolerance of many crops (Antala et al., 2019). The study of KARs promoting germination and seedling photomorphogenesis in *Arabidopsis thaliana* opened doors to the further understanding of KAR perception and signaling (Nelson et al., 2009). In a study conducted on *Arabidopsis thaliana*, it was demonstrated that two genes play a signifcant role in KAR responses. The first one is *MORE AXILLARY GROWTH* (*MAX2*), which is responsible for encoding an F-box protein (also responsible for plant hormone SL response). The second gene responsible for KAR response is *KARRIKIN INSENITIVE*. KAR1 is perceived by α/β hydrolase *KARRIKIN INSENSITIVE 2* (*KAI2*), a KAR receptor, further causing a conformational change in *KAI2*. The activated *KAI2* enhances its interaction with *MAX2*, and both altogether lead to a complex degrading *SUPRESSOR OF MAX2* (*SMAX1*) and *SMAX1-LIKE2* (*SMXL2*). From this suppression, transcription factors are revealed, and a KAR response occurs. As KAI2 is essential for the response of KAR1 and has been well explained to bind with KAR1, it is generally called a KAR receptor.Karrikins boost seed germination by up-regulating the Gibberellic acid (GA) production genes, activating ROS-scavenging antioxidants, and mobilizing soluble carbohydrates in seeds (Sunmonu et al., 2016; Banerjee et al., 2019; Shah et al., 2020).

**Fig. 1.** Karrikin signaling pathway

**Smoke**

**KAR**

Karrikin

Interacts

AR acceptor

KAR acceptor

**SMAX1**

**MAX2**

SUPRESSOR OF MAX2

**SMXL2**

MORE AXILLARY GROWTH 2

SMAX1-LIKE2

**Cyanohydrins:** Several smoke-responsive plant species were found to be non-responsive to karrikinolide in studies conducted to isolate active compounds from smoke. It was discovered that smoke, but not karrikinolide or smoke extracts made from burning cellulose, caused the fire ephemeral *Tersonia cyathiflora* (Gyrostemonaceae) to germinate (Downes et al., 2010). A novel nitrogen-containing bioactive compound known as cyanohydrin glyceronitrile was isolated by Flematti et al., (2011) using a bioassay based on kangaroo paw seed germination. Afterwards, it was discovered that glyceronitrile was more widely active against Haemodoraceae species as well as other smoke-responsive species from southern Africa and North America (Flematti et al., 2011). Mandelonitrile, glycolonitrile, and acetone cyanohydrin were among the other cyanohydrins that could induce germination. The activity of cyanohydrins was found to be due to hydrogen cyanide released upon hydrolysis. Cyanide has been widely reported to stimulate seed germination in many plant species. There are a number of reports that have suggested cyanide as a regulator of seed germination and dormancy release, in rice (*Oryza sativa*), apple (*Malus domestica*), *Helianthus tuberosus*, and *Arabidopsis* ([Cohn & Hughes, 1986](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9); [Bogatek et al., 1991](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9); [Bethke et al., 2006](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9)). Treatment with cyanide breaks *Arabidopsis* seed dormancy, and the emission of hydrogen cyanide from many seeds has been detected during the pregermination period. It was hypothesized that cyanide acts by creating nitric oxide *in vivo* ([Bethke et al., 2006](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9)). However, [Oracz et al., (2007)](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9) suggested that cyanide signaling interacts with reactive oxygen species (ROS) and, in a subsequent paper, [Oracz et al., (2009)](https://www.cell.com/molecular-plant/fulltext/S1674-2052%2814%2960878-9) showed that the effect of cyanide on germination of dormant sunflower embryos is associated with a marked increase in hydrogen peroxide and superoxide anion generation in the embryonic axes. This rise is caused by activation of NADPH oxidase as well as inhibition of the activities of catalase and superoxide dismutase. It was thought that ROS was likely to mediate the effect of cyanide on gene expression. Additionally, it was shown that transcription factor ERF1, a part of the ethylene signaling pathway, is activated by both cyanide and ROS. According to the theory (Oracz et al., 2009), ROS functions as a secondary messenger of cyanide and is crucial for sunflower seed germination.

**Application of plant-derived smoke**

**Fig. 2.** Method of smoke application



**Smoke fumigation:** Applying aerosol smoke directly is one of the simplest way of treatment. This technique exposes seeds directly to smoke produced by burning plant matter. Ghebrehiwot et al., (2013) reported on the aerosol treatment of tef [Eragrostis tef (Zucc.) Trotter] seeds, which were placed in sieves and exposed to cooled (~28 ℃) aerosol smoke for 10 min. The sieves were placed inside a chimney (150 cm high) and smoldering smoke of semi-dry *Themeda triandra* Forssk (Poaceae) was generated at the bottom. This method was relatively simple and affordable technique for tef growers. Aerosol smoke can also be used to treat soil or potting media into which seeds could be planted (Abdollahi et al., 2011; Keeley & Fortheringham, 1998). This treatment offers an additional advantage of gradual release of active molecules over smoke water treatment (Dixon et al., 2009).

**Smoke water extract:** The active ingredient in smoke was found to be soluble in water (Nelson et al., 2012), this property was used to prepare smoke solution. The most common technique to produce plant-derived smoke solution is by bubbling the smoke through water so that the metabolites or the biologically active components present in the smoke dissolve well into water. The smoke can be generated in a drum/fask functioning as a combustion chamber and passed through a compressed air or vacuum pump channeled into the water, thus dissolving the smoke and its metabolites in water (Khatoon et al., 2020). In general, any type of plant can be used to make smoke water. A concentrated aqueous smoke extract can be mixed with water in proportions of 1:250, 1:500, 1:1000, 1:1500, and 1:2000 (v/v). Dilutions within this range tend to be quite effective in enhancing germination, although the effectiveness may differ among species (Van Staden et al., 2004).

**Smoke derived compounds:** Burnt cellulose and smoke derived from plants were used to isolate 3-methyl-2H furo [2, 3-c] pyran-2-one, a biologically active butenolide compound (Flemattiet al., 2004; Van Staden et al., 2004). This compound referred to as ‘karrikinolide’ (KAR1) ([Commander et al., 2008](https://www.sciencedirect.com/science/article/pii/S0254629908003098%22%20%5Cl%20%22bib11)). It was discovered that the compound facilitated the germination of specific seeds across a broad range of concentrations, with effective levels as low as 10−9 M for Grand Rapids lettuce seeds, and around 10−7 M for *Conostylis aculeata* and *Stylidium affine* (Flematti et al., 2004). In a similar vein, Van Staden et al. (2004) demonstrated activity in Grand Rapids lettuce seeds from concentrations of 10−4 M down to 10−9 M. [Verschaeve et al., (2006)](https://www.sciencedirect.com/science/article/pii/S0254629908003098%22%20%5Cl%20%22bib72) tested the butenolide compound for possible mutagenic and genotoxic effects using the VITOTOX® test and the Ames assay. The results confirmed that the compound is not toxic nor genotoxic at the levels tested (1 × 10− 4 to 3 × 10− 10 M), which indicating the possibility of wide-scale usage of the compound as both a germination stimulant and in a field setting.

Smoke stimulates germination positively, and it has been shown that high smoke-water concentrations have the opposite effect. The compounds in the smoke-water that are inhibitory, that is, different from the stimulatory karrikins, are most likely responsible for this effect. The inhibitory compound isolated was 3, 4, 5-trimethylfuran-2(5*H*)-one (2, 3, 4- trimethylbut-2-enolide TMB). But compared to karrikins, which encourage germination, a higher concentration of TMB compound is needed for inhibition action.

**Extraction of smoke compounds**

**Isolation of smoke compound:** By employing the lettuce seed bioassay for bioactivity-guided fractionation, smoke components were separated. Twenty litres of smoke-saturated water derived from burned *Passerina vulgaris* Thoday and *Themeda triandra* L. were concentrated under vacuum to 2l. Using dichloromethane, this concentration was extracted completely.1% (w/v)  aqueous  NaOH  was  used  six  times to  wash  the  combined  organic  extracts,  and  then H2O was used three times to get the pH down to neutral. A 5 x 27 cm column loaded with 200g of silica gel 60 (Merck, 0.040-0.063 mm) was used to subject the extract (9.8g) to vaccum liquid chromatography (VLC). A hexane:ethyl acetate gradient was used to elute the extract (hexane proportions: 100%, 85%, 80%, 75%, 70%, 65%, 60%, 50%, 40%, 30%, 20%, 10%, 0% (v/v); 400ml aliquots of each mixture were used. Fractions that eluted in 70:30 and 65:35 hexane:ethyl acetate (v/v) had germination-stimulating effect. After combining these two fractions, they were separated on a 90 x 2.5 cm Sephadex LH-20 column and eluted with 35% EtOH at a rate of 15 milliliters per hour. The range of activity eluted was 560–630 ml. To recover the active ingredient, the aqueous residue was extracted using dichloromethane after the active ingredient was concentrated under vaccum. Repeated HPLC (60 runs) using 30% MeOH as the mobile phase at 2 ml min–1on a C18 reverse phase column (Haisil 300 C18, 5 m, 250 x 10 mm, Higgins Analytical) allowed for further purification. At 20–21 minutes, the active ingredient eluted. The methanol was allowed to evaporate at room temperature and the active fractions combined and extracted with dichloromethane yielding 3.1mg of the target compound (Van Staden et al., 2004).

**GC-MS analysis of active constituent:** Volumes of 1–2 μl of the active HPLC fraction were analyzed using preparative capillary gas chromatography (Carlo Erba 400 series model HRGC), which was equipped with an on-column injector and a 40m x 0.3mm glass capillary column that had a 0.375 μm coating of OV-1701 as the stationary phase. A capillary effluent splitter was constructed to direct 10% of the column effluent to the detector while sending 90% to the collection device. To minimize spatial band broadening expected from injecting samples with highly polar solvents, the column was connected to a 3m retention gap. Fractions were collected manually. Preparative capillary gas chromatography presents challenges since some of the organic material in the effluent may be lost due to aerosol formation. To address this issue, fractions were gathered in either methanol or dichloromethane using small conical sample vials. Another challenge is the potential for cross-contamination of fractions resulting from the condensation of organic substances where hot capillary fractions are collected in a cold solvent. To mitigate this issue, each fraction was collected with a clean glass exit capillary that was securely connected in a ‘press-fit’ manner to the fused silica GC column. All fractions were evaluated for germination activity, and the active fraction underwent GC-MS analysis on both more polar and less polar capillary columns to assess the purity of the isolated active substance. The preparative separations were conducted 20 times, collecting the active fraction each time. Following this, the preparative separations were carried out initially on a glass capillary column measuring 40m x 0.3mm, coated with 2.5μm of the nonpolar phase PS-255, and subsequently on a fused silica column measuring 30m x 0.32mm, coated with 0.25μm Carbowax 20M. Based on analyses performed using GC and GC-EIMS (Carlo Erba QMD1000 quadrupole instrument, Carbowax 20M column), this method yielded the active component in a very pure form. The compound that was isolated exhibited a mass spectrum identical to that of the primary component found in the active material obtained through HPLC. This compound underwent high-resolution mass spectral analysis using a MicroMass Autospec-TOF instrument, where the theoretical value for C8H6O3 was calculated to be 150.0317 and the measured value was found to be 150.0316 (Van Staden et al., 2004).

**Factors affecting seed response to smoke**

**Pretreatments:** Certain plant seeds react to smoke when they are dry, while seeds from other plants require presoaking in water. Traditional seed treatments like mechanical or acid scarification or soil storage can occasionally boost the effectiveness of smoke treatments (Roche and others 1997).

**Type of Plant Material:** The material used for combustion has ranged from sawdust to both fresh and aged grass, and tree species tissue. Since their combustion would encourage the natural smokes produced by wild land fires, using the branches and foliage of native species is typically preferred. Smoke's chemical makeup, might differ amongst plant species and even between different tissue types within a species. However, commercial smoke flavoring products and tissue paper have proven to be effective (Brown & Van Staden, 1997).

**Combustion Temperature:** The chemical makeup of smoke changes with temperature, tests show that the most active compounds are generated between 160 and 200 °C (320 and 392 °F) (Jager et al., 1996). It appears that at higher temperatures, volatilization causes the stimulatory chemicals to be lost. Therefore, a slow, smoldering fire will work best in practice (Brown & Van Staden, 1997).

**Species Response:** Dixon et al., (1995) found that the effect of smoke vary greatly on different species and ecotypes. Growers will need to experiment with different dilutions to get the best results, as highly concentrated solutions of smoke water may prevent the germination of certain species. Researcher from China performed an experiment in which 13 plant species were studied and out of which one plant species named *A. debilis* showed positive response whereas other plant species such as *T. magnifica* and *A. auriculiformis* showed negative effects on seed germination this shows that not every plant species shows positive response (Zhou et al., 2014).

**Table 1.** Positive response of different crop plants towards application of smoke

|  |  |  |
| --- | --- | --- |
| **Family name** | **Representative plants** | **Reference** |
| Poaceae |  *Triticum aestivum* | Iqbal et al., 2016 |
| Amaryllidaceae | *Allium cepa* | Kulkarni et al., 2010 |
| Malvaceae  | *Abelmoschus esculentus* | Van Staden et al., 2005 |
| Apiaceae | *Dacuscarota*  | Akeel et al., 2019 |
| Caricaceae | *Carica papaya* | Chumpookam et al., 2012 |
| Asteraceae | *Lactuca sativa* | Kamran et al., 2017 |
| Cucurbitaceae | *Cucumis sativus* | Elsadek and Yousef, (2019) |
|  Iridaceae  | *Gladiolus spp.* | Elsadek and Yousef, (2019) |
| Poaceae | *Zea mays* | Aslam et al., 2017 |
| Solanaceae | *Lycopersicon esculentum* | Indriati and Saparita, (2020) |
| Brassicaceae | *Raphanus sativus* | Gupta et al., 2024 |

 **Effects of smoke on seed germination:** Light & van Staden, (2004) investigated the smoke and aqueous smoke extracts can potentially be used for a variety of applications. These include uses in horticulture, agriculture, ecological management and rehabilitation of disturbed areas. Baldwin et al., (1994) proposed that smoke interacts chemically with inhibitors in the seed coat, endosperm or embryo to enhance seed germination and that stimulation of germination is due to smoke-specific signal molecule(s), possibly promotive hormones. Plant-derived smoke solution significantly promoted seed germination in Arabidopsis and lettuce by increasing the activity of hydrolytic enzymes, which supports the mobilization of stored food reserves (Nelson et al., [2011](https://link.springer.com/article/10.1007/s00344-023-11221-7#ref-CR93); Khatoon et al., [2020](https://link.springer.com/article/10.1007/s00344-023-11221-7#ref-CR67)) Daws et al., (2007) and Stevens et al., (2007) have shown that smoke extract promotes the germination of weed seed banks both in farming and restoration environment. As per the study done by Iqbal et al., 2016, smoke aerosol treatment of wheat- *Triticum aestivum* seeds of different varities for about 1 hr recorded 3-4% increase in germination compared to control and also recorded significant increase in germination index. Elsadek and Yousef, (2019) reported that seeds soaked in smoke water generated from different plant species for about 24 hr increases the germination and post-germination parameters in cucumber, tomato and marigold. Also,treated seeds under light conditions recorded high α-amylase activity and low abscisic acid which helps to establish relationships between α-amylase activity, ABA content, and germination parameters in the studied crops.

**Effect of smoke on seed dormancy:** Plant-derived smoke compounds were found to break dormancy present in seed of *Arabidopsis thaliana* after fire. Using a transcriptomic technique, a study on molecular aspects of seed germination in lettuce reported that abscisic acid, seed maturation, and dormancy-related transcripts were up-regulated by trimethyl butenolide and suppressed by KAR1( Soos et al., 2012). This study clarified that increased seed germination by KAR1 might be due to suppression of abscisic acid and dormancy-related transcripts by KAR1 present in smoke. Another investigation explaining the physiology of breaking seed dormancy in response to smoke water and KAR1 treatment was carried out by Gupta et al., 2019. It was demonstrated that smoke water and KAR1 significantly promoted the lettuce seed germination by reducing abscisic acid level and increasing the activity of hydrolytic enzymes, which supports the mobilization of stored food reserves. Elsadek and Yousef, (2019) provided evidence of smoke water actively breaking dormancy present in gladiolus.

**Effect of smoke on seedling growth :** In addition to promoting germination,smoke treatment enhance seedling vigor as karrikin treated seeds are found to have more carbohydrate content, leading to stronger and more resilient plants. Ibrahim et al., (2022) have found that smoke application increased shoot and root length, as well as fresh weight in wheat seedlings under heavy metal stress. For vegetable growers, the most significant vectors of productivity is faster-growing seedlings, Meanwhile foliar application of smoke water and a butenolide in okra [*Abelmoschus esculentus* (L.) Moench] and tomato (*Lycopersicon esculentum Mill*.) seedlings imparted positive effects on shoot/root length, shoot fresh/dry weight, number of leaves, total leaf area and stem thickness and also remarkably increased absolute growth rate(AGR) per week. This indicates that the foliar application of smoke-water or butenolide may be a feasible and inexpensive technique for enhancing seedling growth of vegetable crops (Kulkarni et al., 2007). Sunmonu et al., ([2016](https://link.springer.com/article/10.1007/s00344-021-10473-5#ref-CR139)) have found that seedling growth improvement in smoke treated seeds are due to breakdown of starch found in seed as well as increased activity of amylase enzyme in roots and above ground parts.

**Effect of smoke on Antioxidant defense modulation system:** Khatoon et al., (2020) demonstrates the role of plant-derived smoke on the morphological, physiological, and biochemical mechanisms of plant growth under stress conditions. Plant-derived smoke regulates the germination and growth of wheat by scavenging ROS directly, increasing antioxidant enzyme activity, and stimulating the expression of stress-responsive genes. Further recorded that there was an approximately fourfold increase in the positive effector of germination, increase in root and shoot length and a decrease in overall boron accumulation in the wheat seedling, leading to a reduction in B-triggered oxidative injury that takes place due to boron toxicity. Plant-derived smoke can alleviate oxidative stress caused by heavy metals such as arsenic (As) and mercury (Hg) by modulating the cellular antioxidative defense system. Heavy metal stress can inhibit seed germination and seedling growth, reduce photosynthetic pigments, and increase the levels of harmful compounds like H2O2. Application of PDS can reverse these effects, enhancing seed germination rate, shoot/root length, and fresh weight while decreasing the levels of H2O2 and lipid peroxidation (Ibrahim et al., 2022). PDS can modulate the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) under heavy metal stress (Ibrahim et al., 2022). In conclusion, the role of smoke or aqueous smoke has been effectively established for abiotic stress like salinity, drought, or heavy metal toxicity, as it regulates redox homeostasis and also regulates the genes responsible for stress regulation and response.

1. **CONCLUSION**

Plant-derived smoke and its active components, have significant potential in agriculture and horticulture. These compounds can stimulate seed germination, promote seedling growth, and enhance crop productivity, even under adverse conditions. Recent advancements have focused on understanding the mechanisms of action, optimizing application methods, and exploring the potential uses. While challenges remain, such as method of application-concentration, timing, and delivery method, Species response therefore continued research and development in this area promise to provide sustainable and effective solutions for improving crop production and promoting ecological diversity (Erickson et al., 2017).

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

1. Abdollahi, M. R., Mehrshad, B. and Moosavi, S. S. (2011). Effect of method of seed treatment with plant derived smoke solutions on germination and seedling growth of milk thistle (Silybum marianum L.). *Seed Science and Technology*, 39(1), 225-229.
2. Akeel, A., Khan, M. M. A., Jaleel, H., & Uddin, M. (2019). Smoke-saturated water and karrikinolide modulate germination, growth, photosynthesis and nutritional values of carrot (Daucus carota L.). *Journal of Plant Growth Regulation*, 38, 1387-1401.
3. Antala, M., & Brestic, M. (2020, December). Karrikins Reduce the Hypocotyl Length of Rapeseed (Brassica napus napus L.) under Continuous Red Light. In *Biology and Life Sciences Forum* (Vol. 4, No. 1, p. 19). MDPI.
4. Antala, M., Sytar, O., Rastogi, A., & Brestic, M. (2019). Potential of karrikins as novel plant growth regulators in agriculture. *Plants*, *9*(1), 43.
5. Aslam, M. M., Jamil, M., Khatoon, A., Hendawy, S. E., Al-Suhaibani, N. A., Malook, I., & Rehman, S. U. (2017). Physiological and biochemical responses of maize (Zea mays L.) to plant derived smoke solution. *Pak. J. Bot*, *49*(2), 435-443.
6. Baldwin, I. T., & Morse, L. (1994). Up in smoke: II. Germination of Nicotiana attenuata in response to smoke-derived cues and nutrients in burned and unburned soils. *Journal of chemical ecology*, *20*, 2373-2391.
7. Banerjee, A., Tripathi, D. K., & Roychoudhury, A. (2019). The karrikin ‘calisthenics’: can compounds derived from smoke help in stress tolerance?. *Physiologia plantarum*, *165*(2), 290-302.
8. Bethke, P. C., Libourel, I. G., Reinöhl, V., & Jones, R. L. (2006). Sodium nitroprusside, cyanide, nitrite, and nitrate break Arabidopsis seed dormancy in a nitric oxide-dependent manner. *Planta*, *223*, 805-812.
9. Bogatek, R., & Lewak, S. (1991). Cyanide controls enzymes involved in lipid and sugar catabolism in dormant apple embryos during culture. *Physiologia Plantarum*, *83*(3), 422-426.
10. Brown, N. A., & van Staden, J. (1997). Smoke as a germination cue: a review. *Plant growth regulation*, *22*, 115-124.
11. Chiwocha, S. D., Dixon, K. W., Flematti, G. R., Ghisalberti, E. L., Merritt, D. J., Nelson, D. C., ... & Stevens, J. C. (2009). Karrikins: a new family of plant growth regulators in smoke. *Plant science*, *177*(4), 252-256.
12. Chumpookam, J., Lin, H. L., Shiesh, C. C., & Ku, K. L. (2012). Effect of smoke-water on seed germination and resistance to Rhizoctonia solani inciting papaya damping-off. *Horticulture NCHU*, *37*(1), 13-29.
13. Cohn, M. A., & Hughes, J. A. (1986). Seed dormancy in red rice: V. Response to azide, hydroxylamine, and cyanide. *Plant Physiology*, *80*(2), 531-533.
14. Commander, L. E., Merritt, D. J., Rokich, D. P., Flematti, G. R., & Dixon, K. W. (2008). Seed germination of Solanum spp.(Solanaceae) for use in rehabilitation and commercial industries. *Australian Journal of Botany*, *56*(4), 333-341.
15. Daws, M. I., Davies, J., Pritchard, H. W., Brown, N. A., & Van Staden, J. (2007). Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regulation*, *51*, 73-82.
16. De Lange, J. H., & Boucher, C. (1990). Autecological studies on Audouinia capitata (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany*, *56*(6), 700-703.
17. Dixon, K. W., Merritt, D. J., Flematti, G. R., & Ghisalberti, E. L. (2009). Karrikinolide–a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration and agriculture. *Acta Horticulturae*, *813*(2009), 155-170.
18. Dixon, K. W., Roche, S., & Pate, J. S. (1995). The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia*, *101*, 185-192.
19. Downes, K. S., Lamont, B. B., Light, M. E., & Van Staden, J. (2010). The fire ephemeral Tersonia cyathiflora (Gyrostemonaceae) germinates in response to smoke but not the butenolide 3-methyl-2 H-furo [2, 3-c] pyran-2-one. *Annals of Botany*, *106*(2), 381-384.
20. Elsadek, M. A., & Yousef, E. A. (2019). Smoke-water enhances germination and seedling growth of four horticultural crops. *Plants*, *8*(4), 104.
21. Erickson, T. E., Muñoz-Rojas, M., Kildisheva, O. A., Stokes, B. A., White, S. A., Heyes, J. L., ... & Merritt, D. J. (2017). Benefits of adopting seed-based technologies for rehabilitation in the mining sector: a Pilbara perspective. *Australian Journal of Botany*, *65*(8), 646-660.
22. Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2004). A compound from smoke that promotes seed germination. *Science*, *305*(5686), 977-977.
23. Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2009). Identification of alkyl substituted 2 H-furo [2, 3-c] pyran-2-ones as germination stimulants present in smoke. *Journal of Agricultural and Food Chemistry*, *57*(20), 9475-9480.
24. Flematti, G. R., Goddard-Borger, E. D., Merritt, D. J., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2007). Preparation of 2 H-furo [2, 3-c] pyran-2-one derivatives and evaluation of their germination-promoting activity. *Journal of Agricultural and Food Chemistry*, *55*(6), 2189-2194.
25. Flematti, G. R., Merritt, D. J., Piggott, M. J., Trengove, R. D., Smith, S. M., Dixon, K. W., & Ghisalberti, E. L. (2011). Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. *Nature Communications*, *2*(1), 360.
26. Ghebrehiwot, H., Kulkarni, M., Bairu, M., & Van Staden, J. (2013). Plant-derived aerosol-smoke and smoke solutions influence agronomic performance of a traditional cereal crop, Tef. *Experimental Agriculture*, *49*(2), 244-255.
27. Gupta, S., Kulkarni, M., Doležalova, I., Novák, O., Van Staden, J., & Doležal, K. (2024). Foliar smoke-water application improves growth and changes the leaf mineral composition of radish, spinach and turnip seedlings. *South African Journal of Botany*, *167*, 527-534.
28. Gupta, S., Plačková, L., Kulkarni, M. G., Doležal, K., & Van Staden, J. (2019). Role of smoke stimulatory and inhibitory biomolecules in phytochrome-regulated seed germination of Lactuca sativa. *Plant physiology*, *181*(2), 458-470.
29. Ibrahim, M., Nawaz, S., Iqbal, K., Rehman, S., Ullah, R., Nawaz, G., ... & Peluso, I. (2022). Plant-derived smoke solution alleviates cellular oxidative stress caused by arsenic and mercury by modulating the cellular antioxidative defense system in wheat. *Plants*, *11*(10), 1379.
30. Indriati, A., & Saparita, R. (2020, March). Utilization of liquid smoke corn cobs for germination tomato (Solanum lycopersicum) seeds. In *IOP Conference Series: Earth and Environmental Science* (Vol. 462, No. 1, p. 012049). IOP Publishing.
31. Iqbal, M., Asif, S., Ilyas, N., Raja, N. I., Hussain, M., Shabir, S., ... & Rauf, A. (2016). Effect of plant derived smoke on germination and post germination expression of wheat (Triticum aestivum L.). *American Journal of Plant Sciences*, *7*(06), 806.
32. Jäger, A. K., Strydom, A., & Van Staden, J. (1996). The effect of ethylene, octanoic acid and a plant-derived smoke extract on the germination of light-sensitive lettuce seeds. *Plant Growth Regulation*, *19*, 197-201.
33. Kamran, M., Khan, A. L., Ali, L., Hussain, J., Waqas, M., Al-Harrasi, A., ... & Lee, I. J. (2017). Hydroquinone; a novel bioactive compound from plant-derived smoke can cue seed germination of lettuce. *Frontiers in chemistry*, *5*, 30.
34. Keeley, J. E., & Fotheringham, C. J. (1998). Mechanism of smoke‐induced seed germination in a post‐fire chaparral annual. *Journal of Ecology*, *86*(1), 27-36.
35. Keeley, J. E., & Fotheringham, C. J. (2000). Role of fire in regeneration from seed. In *Seeds: the ecology of regeneration in plant communities* (pp. 311-330). Wallingford UK: Cabi Publishing.
36. Khatoon, A., Rehman, S. U., Aslam, M. M., Jamil, M., & Komatsu, S. (2020). Plant-derived smoke affects biochemical mechanism on plant growth and seed germination. *International Journal of Molecular Sciences*, *21*(20), 7760.
37. Kulkarni, M. G., Ascough, G. D., & Van Staden, J. (2007). Effects of foliar applications of smoke-water and a smoke-isolated butenolide on seedling growth of okra and tomato. *HortScience*, *42*(1), 179-182.
38. Kulkarni, M. G., Light, M. E., & Van Staden, J. (2011). Plant-derived smoke: old technology with possibilities for economic applications in agriculture and horticulture. *South African Journal of Botany*, *77*(4), 972-979.
39. Kulkarni, M. G., Sparg, S. G., Light, M. E., & Van Staden, J. (2006). Stimulation of rice (Oryza sativa L.) seedling vigour by smoke‐water and butenolide. *Journal of agronomy and crop science*, *192*(5), 395-398.
40. Light, M. E., Burger, B. V., Staerk, D., Kohout, L., & Van Staden, J. (2010). Butenolides from plant-derived smoke: natural plant-growth regulators with antagonistic actions on seed germination. *Journal of Natural Products*, *73*(2), 267-269.
41. Light, M. E., Daws, M. I., & Van Staden, J. (2009). Smoke-derived butenolide: towards understanding its biological effects. *South African Journal of Botany*, *75*(1), 1-7.
42. Light, M. E., van Staden, J., & Bornman, C. H. (2004). The potential of smoke in seed technology. *South African Journal of Botany*, *70*(1), 97-101.
43. Merritt, D. J., Flematti, G. R., Ghisalberti, E. L., & Dixon, K. W. (2007, June). Karrikinolide–a Phytoreactive Compound Derived from Smoke with Applications in Horticulture, Ecological Restoration and Agriculture. In *VI International Symposium on New Floricultural Crops 813* (pp. 155-170).
44. Nelson, D. C., Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Smith, S. M. (2012). Regulation of seed germination and seedling growth by chemical signals from burning vegetation. *Annual review of plant biology*, *63*(1), 107-130.
45. Nelson, D. C., Flematti, G. R., Riseborough, J. A., Ghisalberti, E. L., Dixon, K. W., & Smith, S. M. (2010). Karrikins enhance light responses during germination and seedling development in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences*, *107*(15), 7095-7100.
46. Oracz, K., Bouteau, H. E. M., Farrant, J. M., Cooper, K., Belghazi, M., Job, C., ... & Bailly, C. (2007). ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *The Plant Journal*, *50*(3), 452-465.
47. Oracz, K., El-Maarouf-Bouteau, H., Kranner, I., Bogatek, R., Corbineau, F., & Bailly, C. (2009). The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. *Plant physiology*, *150*(1), 494-505.
48. Roche, S., Koch, J. M., & Dixon, K. W. (1997). Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restoration Ecology*, *5*(3), 191-203.
49. Shah, F. A., Wei, X., Wang, Q., Liu, W., Wang, D., Yao, Y., ... & Wu, L. (2020). Karrikin improves osmotic and salt stress tolerance via the regulation of the redox homeostasis in the oil plant Sapium sebiferum. *Frontiers in Plant Science*, *11*, 216.
50. Soos, V., Sebestyén, E., Posta, M., Kohout, L., Light, M. E., Van Staden, J., & Balázs, E. (2012). Molecular aspects of the antagonistic interaction of smoke‐derived butenolides on the germination process of G rand R apids lettuce (L actuca sativa) achenes. *New Phytologist*, *196*(4), 1060-1073.
51. Sparg, S. G., Kulkarni, M. G., & Van Staden, J. (2006). Aerosol smoke and smoke‐water stimulation of seedling vigor of a commercial maize cultivar. *Crop science*, *46*(3), 1336-1340.
52. Stevens, J. C., Merritt, D. J., Flematti, G. R., Ghisalberti, E. L., & Dixon, K. W. (2007). Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2 H-furo [2, 3-c] pyran-2-one under laboratory and field conditions. *Plant and Soil*, *298*, 113-124.
53. Sunmonu, T. O., Kulkarni, M. G., & Van Staden, J. (2016). Smoke-water, karrikinolide and gibberellic acid stimulate growth in bean and maize seedlings by efficient starch mobilization and suppression of oxidative stress. *South African Journal of Botany*, *102*, 4-11.
54. Troumbis, A. Y. (2021). Meta-analysis using a Lehmann classifier of obligate seeding species germination in fire-prone Mediterranean Type environments.
55. Van Staden, J., Jager, A. K., Light, M. E., & Burger, B. V. (2004). Isolation of the major germination cue from plant-derived smoke.
56. Van Staden, J., Kulkarni, M. G., & Ascough, G. D. (2007). The promotion of tomato and okra seedling growth by foliar applications of smoke? water and a smoke-isolated butenolide. *South African Journal of Botany*, *73*(2), 318-318.
57. Verschaeve, L., Maes, J., Light, M. E., & van Staden, J. (2006). Genetic toxicity testing of 3-methyl-2H-furo [2, 3-c] pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *611*(1-2), 89-95.
58. Zhou, J., Teixeira da Silva, J. A., & Ma, G. (2014). Effects of smoke water and karrikin on seed germination of 13 species growing in China. *Central European Journal of Biology*, *9*, 1108-1116.