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SMOKE INDUCED GERMINATION OF SOME IMPORTANT MEDICINAL PLANTS

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The application of smoke and aqueous smoke solutions stimulates seed germination in a number of plant species. This study highlights the effects of aerosol smoke and smoke solutions on the germination and seedling vigour of four Indian indigenous medicinal plants *i.e. Terminalia chebula, Holorrhina antidysentrica, Clitoria ternatea* and *Gymnema sylvestre.* The vigour index, of one-week-old seedlings of all four species examined, increased with the application of dry smoke and smoke extract dilutions, as compared to control treatments. This investigation shows that the application of smoke technology can be adopted to produce high vigour seedlings. Smoke and aqueous smoke extracts can potentially be used for a variety of applications related to seed technology. These include uses in horticulture, agriculture, ecological management and rehabilitation of disturbed areas.

Keywords: Germination; Medicinal plants; Smoke; Vigour.

Introduction

Plant-derived smoke plays an important role in breaking the dormancy of many species. Smoke is an important factor involved in fire and post-fire germination cues. Farmers have traditionally used fire and smoke in grain drying practices. Fire is a major environmental selective force that influences plant communities in many parts of the world. Reproductive strategies have evolved as adaptations to the various factors generated by, and/ or associated with fire. This is particularly true for seeds, in which strategies have evolved that respond to both the physical and chemical germination cues that may be associated with fires [1-4]. De Lange and Boucher [5] were the first to demonstrate that smoke, and aqueous extracts of smoke, was responsible for the stimulation of seed germination of a threatened fynbos species, *Audouina capitata*. A number of ecosystems around the world, for example chaparral (southern California), kwongan (Australia) and fynbos (South Africa) show a requirement for fire-related cues to stimulate seed germination of many species [6-8]. Following this significant discovery, Brown [9-10] showed that other fynbos species, from several genera and families, also exhibited improved germination with smoke treatments [11]. Many workers showed that smoke is effective on species from a wide range of families, which vary in ecology, reproductive strategy, seed size and morphology [12-14]. However, the promotion of germination by smoke is not limited to species from fire-prone habitats[15]. It has been shown that in addition to the more obvious effects of heat, smoke from burning vegetation is responsible for breaking dormancy and stimulating the germination of some seeds, for example, celery (*Apium graveolens* L.) lettuce (*Lactuca sativa* L.) and many California species *Salvia apiana, Cryptantha clevelandi, Romneya coulteri* [16-18]. Although the chemical identity of the main active compound from smoke has only recently been discovered[4, 19]. The remarkable effect of smoke on seed germination is widely known and utilized in various ways [20-22]. It is possible, therefore, that the use of smoke may play a vital role in the natural rehabilitation and conservation of indigenous vegetation and can potentially be used for a variety of applications related to seed technology [22].

Many medicinal plant species are under severe threat in wild, and are often difficult to find outside of protected areas. As a result, many species are endangered and included in the red data list. If the future demand for medicinal plants is to be met, it is imperative that many of the species utilized in traditional medicines be domesticated and commercially cultivated. The use of plants in the indigenous cultures of developing countries like India are numerous and diverse[23-25]. For many people they still form an important economic basis and are commonly used in medicine. One of the reasons could be that traditional medicine provides people with a good alternative. To meet this increasing demand it is important to develop techniques for efficient low-cost cultivation practices. The successful cultivation of medicinal plants from India is determined to large extent by the germinability of the seeds. The application of smoke and smoke solutions may assist in establishing healthy and vigorous seedlings for cultivation of a number of important medicinal plants. The present study investigated the effects of smoke or smoke solution on germination and seedling vigour under controlled environmental conditions of *Terminalia chebula*, *Holorrhina antidysentrica*, *Clitoria ternatea* and *Gymnema sylvestre*.

Material and Methods

Seed collection- Seeds of *T. chebula*, *H. antidysentrica*, *C. ternatea* and *G. sylvestre* were collected from the Western Ghat Forest, India. Immediately after the collection, seeds were stored in brown paper bags for 2 months at room temperature before being used. Weight was determined by weighing 100 seeds of four replicates. The moisture content of fresh seeds was measured by drying seeds at 110° C. The seeds were weighed repeatedly until a constant weight was reached. The moisture content was expressed as a percentage of fresh weight.

Viability and imbibition studies- Viability was determined using 2,3,5-triphenyl tetrazolium chloride (TTC) solution (ISTA)[26]. The seeds were imbibed for 24 h in water. After cutting longitudinally, so exposing the embryo, they were then soaked in 1% colorless solution of TTC for 24 h at 25±4° C in the dark. Seeds with red-stained embryos were recorded as being viable. In imbibition studies, the seeds were placed in 9 cm disposable Petri dishes on two layers of filter paper (Whatman No.1) moistened with 3.5 ml distilled water and allowed to imbibe at room temperature (25±4° C). At 2 h intervals, for 48 h, the seeds were blotted dry, weighed and returned to the wet filter paper. The amount of water imbibed by seed is expressed as a percentage increase over the initial seed weight.

Germination experiments - For the germination experiments, seeds were placed in 9 cm Petri dishes on two layers of filter paper (Whatman No. 1) moistened with 4.5 ml distilled water or test solution. Each treatment consisted of five replicates of 30 seeds. Experiments were conducted at 25 ± 3.0°C under a 16:8 h light/dark photoperiod provided by cool-white fluorescent lamps. Some treatments were kept under continuous dark conditions using lightproof boxes. Germination was recorded under a green safe light. Germination counts were made daily for 30 days. Germination was considered when the radicle protruded 2 mm. Mean germination time (MGT) was calculated by using the equation: MGT ¼ Pðn ~ dÞ=N, where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the end of the experiment[27].

Aerosol smoke treatments- Seeds were placed in sieves and exposed to cool aerosol smoke for 30 min. This was achieved by placing the sieves inside a chimney, 150 cm above slow burning of a mixture of semi-dry grasses *Aristida setacea* and *Cymbopogon martini* (Graminiaceae). Smoke-treated seeds and untreated (control) seeds were imbibed for 48 h and then rinsed with two washes of 500 ml water, after which they were transferred to new Petri dishes moistened with 3 ml distilled water.

Treatments with smoke solutions - Seeds were surface decontaminated with 0.1% mercuric chloride for 2 min and then rinsed with distilled water. For the smoke water treatments, the seeds were germinated on filter paper moistened with 3 ml of smoke solution (1:500, 1:1000 and 1:2000, pH 7.8, 7.9 and 8.2 respectively) prepared from a mixture of semi-dry grasses of *A. setacea* and *C. martini* in the equal proportion in weight. The filter papers were rewetted when required with distilled water or appropriate smoke solutions during the course of the experiment.

Vigour experiments - The vigour index of one-week-old seedlings was calculated as VI = (shoot length + root length) · percentage germination[28]. To determine whether there is a prolonged vigour stimulus by smoke on germinated seedlings, two-week-old seedlings were grown *in vitro* for a period of 75 days. For each treatment, 30 seedlings were transferred into sterilized tissue culture vials with quartz sand as a substrate. Half-strength Hoaglands solution (HS)[29] was used as a liquid growth medium (7 ml per vial). The following treatments were used: Seedlings germinated with water, grown with HS only (control); Seedlings from aerosol smoke germination treatment, grown with HS only; Seedlings from germination treatments with smoke solutions (1:500, 1:1000 and 1:2000), grown in HS only; and Seedlings germinated with water (control), grown with HS containing smoke solution at dilutions of 1:500, 1:1000 and 1:2000. The substrate was re-moistened with 2 ml HS and/or the respective smoke solution after 35 days from the start of the experiment. After 75 days growth parameters were measured and analyzed.

Statistical analysis - The germination data in each treatment were arcsine transformed and analysis of variance (ANOVA) was conducted. The Least Significant Difference (LSD) at the 5% level was used to test differences between means of percentage germination and means of growth parameters of seedlings of different treatment and the differences contrasted using Duncan's

Table 1. Effects of aerosol smoke and smoke solutions on seed germination (± SE) of indigenous medicinal plants under different light conditions.

Species	Treatment	Germination (%)			MGT (days)[a]
		16:8 h light/dark	Continuous dark	Continuous light	
T.chebula	Control	87.0±1.0a	43.0±0.5c	100±0.0a	8
	Aerosol smoke	98.0±0.5a	*	100±0.0a	6
	1:500	96.0±0.3a	42.0±0.3c	97.8±0.0a	5
	1:1000	90.0±0.6a	47.0±0.2c	95.7±0.4a	5
	1:2000	88.01.3a	66.0±0.5b	92.0±0.6a	5
H. antidysentrica	Control	85.6±0.7a	62.7±0.6b	100±0.0a	4
	Aerosol smoke	94.8±0.6a	*	98.7±0.5a	4
	1:500	92.5±0.3a	48.5±0.5c	*	4
	1:1000	90.8±0.6a	39.8±0.6c	91.0±0.7a	4
	1:2000	89.0±0.4a	46.8±0.6c	90.8±0.4a	4
C.ternatea	Control	81.0±0.8a	92.0±1.5a	100±0.0a	3
	Aerosol smoke	89.7±1.5a	*	*	4
	1:500	84.0±0.8a	82.0±0.5a	*	3
	1:1000	92.0±0.6a	65.0±0.7b	100±0.0a	3
	1:2000	80.1±0.4a	57.9±1.8b	91.0±0.5a	3
G.sylvestre	Control	80.6±0.3a	68.1±1.3b	100±0.0a	3
	Aerosol smoke	89.0±0.7a	66.0±0.8b	*	3
	1:500	95.9±0.4a	*	94.9±0.6a	3
	1:1000	90.7±0.6a	62.7±0.2b	100±0.0a	3
	1:2000	84.8±0.7a	61.6±1.5b	91.5±0.4a	3

Mean percentage values with the same letter for each species are not significantly different ($p \leq 0.05$)

* Not tested in the experiment

[a] Mean germination time under 16:8 h light/dark condition

Table 2. Effects of germination with aerosol smoke and smoke solutions on seedling vigour of indigenous medicinal plants.

Species	Treatment	Vigour[a] index	Height[b] (mm)	Seedling survival (%)
T.chebula	Control	431.5	185b	38
	Aerosol smoke	765.1	221a	87
	1:500	675.9	197b	93
	1:1000	701.0	245a	89
	1:2000	654.6	231a	95
H. antidysentrica	Control	301.0	76a	100
	Aerosol smoke	531.0	153b	100
	1:500	421.0	89a	100
	1:1000	385.8	96a	100
	1:2000	359.0	91a	100
C.ternatea	Control	347.1	86a	78
	Aerosol smoke	485.0	95a	100
	1:500	571.0	93a	95
	1:1000	531.0	79c	97
	1:2000	410.0	80c	100
G.sylvestre	Control	321.0	112a	76
	Aerosol smoke	563.0	95a	100
	1:500	486.0	154a	89
	1:1000	431.9	121a	100
	1:2000	421.0	100c	100

Mean values with the same letter for each species are not significantly different ($p \leq 0.05$)

[a] After 7 days

[b] After 75 days

multiple range test. All statistical analysis was performed using SPSS statistical software package.

Results and Discussion

The results of the germination studies are summarized in Table 1. The moisture content of stored seeds of *T. chebula, H. antidysentrica, C. ternatea* and *G. sylvestre* was 43.2, 36.5, 28.6 and 13.3% respectively. All the four species showed high germination (81–95%) under 16:8 h light/dark in the control and smoke treatments. Continuous light did not affect the germination of *T. chebula, H. antidysentrica, C. ternatea* and *G. sylvestre* seeds in either the control or smoke extract dilution treatments. By treating the seeds with aerosol smoke, the mean germination time for all the species was reduced (Table 1). The calculated vigour index of one-week-old seedlings showed that the application of aerosol smoke and smoke solutions enhanced the seedling vigour of all the species (Table 2). In most cases, aerosol smoke was more effective than aqueous smoke dilutions, showing good growth. *T. chebula* seedlings grown *in vitro* for 75 days with HS containing 1:2000 smoke solution, exhibited a significantly greater growth and total mass than untreated seedlings. Similarly, *C. ternatea* seedlings grown with 1:2000 smoke solution, exhibited a significantly greater leaf and total mass than untreated seedlings. *G. sylvestre* and *H. antidysentrica* seedlings did not show any significant differences when grown for a period of 75 days with HS containing smoke solutions.

At high concentrations, smoke extracts are known to inhibit seed germination[10]. According to Brown and Van Staden [10] more dilute solutions improved the germination in dormant seeds of *Syncarpha vestita* (L.) B. Nord. In this study, the dilutions of the smoke extract used (1:500, 1:1000 and 1:2000) showed no inhibitory effect on germination in *T. chebula*. However, post-germination measurements clearly indicated the ability of smoke to improve seedling vigour. Aerosol smoke treatment and 1:1000 smoke dilutions also increased the survival rate of these seedlings grown *in vitro* (Table 2). Post-germination application of 1:2000 smoke solution resulted in significantly greater seedling mass. This study has revealed that the effects of smoke extend beyond germination stimulation and can also act to enhance seedling vigour. Plant-derived smoke extracts are known to stimulate seed germination in a number of species. A highly active, heat stable, long lasting compound, 3-methyl-2H-furo-(2, 3-c)-pyran-2-one, that stimulates seed germination, was isolated from plant derived smoke water using bioactivity-guided fractionation. The identification of this natural molecule, the major germination cue from smoke, should now rapidly lead to a more comprehensive understanding of the role of the smoke as promoter of seed germination [11, 19]. The physiological mechanism resulting in improved vigour is unknown. However, smoke may protect the seed and seedlings against microbial attack[20], which can result in higher seedling survival. The recent identification of the germination cue from smoke will now allow for research into the physiological action of smoke on seed germination [9, 11]. It will also enable researchers to more carefully unravel the mechanisms and responses of seeds to smoke and whether the effects of enhanced germination are related to the improved vigour as observed in certain cases.

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