J. Phytol. Res. **21**(1) : 71-75, 2008

EFFECT OF SMOKE ON SEED VIGOUR RESPONSE OF SELECTED MEDICINAL PLANTS

RAVINDRA B. MALABADI* and S. VIJAY KUMAR**
Division of Plant Biotechnology, Department of Botany, Karnatak University, Pavate Nagar, Dharwad-580003, Karnataka state, India.
**Department of Biotechnology, Madanpalle Institute of Technology and Science, Madanpalle-517325, Chitoor District, Andhra Pradesh, India
*Present address: Forest Biotechnology Laboratory (6.06), Instituto de Tecnologia Quimica e Biologica (ITQB), Universidade Nova de Lisboa, Av. Da Republica, Apartado 127, 2781-901, Oeiras, Portugal. E-mail:malabadi@itqb.unl.pt

This study highlights the influence of aerosol smoke and smoke solutions on the germination and seed vigour response of few selected medicinal plants of Belgaum district of Karnataka. All the four medicinal plants selected for this study have been intensively used by the local traditional healers for the management of various diseases. The overall germination percentage is very high when seeds were treated with different concentrations of smoke saturated water solutions including aerosol smoke against control. The vigour index of all the medicinal plants under study increased with the applicability of dry smoke and smoke solutions. Hence, priming seeds with smoke or smoke solutions will certainly improve the seedling vigour response and plays an important role in the preservation of medicinal plants at a very low cost. This procedure is less expensive and applicable to most of the plant species.

Keywords : Belgaum; Germination; Medicinal plants; Smoke; Vigour index.

Introduction

The Indian subcontinent, with the history of one of the oldest civilization harbors many traditional health care systems. In India, the history of health care goes back to 5000 years B. C., when health care needs and diseases were noted in ancient literature like *Rig-Veda* and *Atharva-Veda*. Later, the texts like *Charak Samhita* and *Sushruta Samhita* were documented in about 1000 years BC where use of plants and polyherbal formulations were highlighted for health care. Evolution of *Ayurveda* and plant-based remedies for health care through day-to-day life experiences is a part of cultural heritage of India[1]. Plants and plant derived products are of health care system since ancient human civilizations.

Due to traditional health care system used by rural communities in India, the demand for medicinal plants is increasing and sustainable harvesting can no longer meet these needs. Some of the medicinal plants were illegally uprooted from the indigenous forests and are traded at informal markets across the country. Such circumstances have suggested the necessity for growth of medicinal plants in India. Improving overall growth and performance of medicinal plants is an important goal to improve productivity. This is driven by the need to provide herbal medicines for a steady growing Indian population as well as the potential for commercial benefits. Therefore, enhancement of seed vigour is a useful approach to meeting this goal. In technical terms, seed vigour is defined as the qualitative term encompassing 'the sum of those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence[2]. Traditional agronomic methods of seed vigour measurement include germination percentage, shoot weight, shoot height, and root length. There are many factors which influence the vigour including certain biochemical characteristics. In addition to traditional seed vigour parameters, the synthesis of secondary metabolites, such as phenolics, has proved to be useful in the evaluation of plant growth[3].

Seed priming (osmoconditioning) is a common agronomic technique shown to cause an increase seed vigour, as well as synchronize and accelerate germination, improve stress resistance, and enhance overall plant growth and productivity. This treatment effectively initiates germination related processes in the seeds, but prevents the emergence of the radicle. The procedure involves soaking seeds in osmotic solutions for a period

of time before planting. Plant derived smoke plays an important role in breaking the seed dormancy of many species [4-16]. The applications of smoke and smoke solutions may assist in establishing healthy and vigorous seedlings. At the same time, the demand of medicinal plants is very high, and there is very little to no information readily available on propagation practices. Consequently, at this stage, seed propagation remains the most feasible and the cheapest option. This study therefore, aims to establish the optimum conditions for seed germination and seedling growth of medicinal plants selected for this study.

Materials and Methods

Seed collection- Seeds of *Acacia pennata* (Mimosaceae), *Basella alba* (Basellaceae), *Celastrus asiatica* (Celastraceae), and *Cleome gynandra* (Cleomaceae) were collected from the Belgaum district, Karnataka state, India. These plants were selected since they were used as herbal medicines by the local traditional healers[17]. 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)[18]. 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 *P*ðn ~ 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 [19].

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 *Aristida setacea* and *Cymbopogon martini* (Graminiaceae) in the equal proportion in weight[4]. 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) · percent-age germination[20]. 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)[21] 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

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	
A. pennata	Control	81.0±1.0a	33.0±0.6c	100±0.0a	6
	Aerosol smoke	88.0±0.3a	*	100±0.0a	3
	1:500	96.0±0.5a	42.0±0.1c	77.8±0.0a	4
	1:1000	90.0±0.2a	37.0±0.4c	75.7±0.4a	4
	1:2000	88.0±1.0a	56.0±0.2b	82.0±0.6a	5
B. alba	Control	75.6±0.3a	32.7±0.7b	100±0.0a	7
	Aerosol smoke	84.8±0.8a	*	88.7±0.5a	6
	1:500	82.5±0.2a	38.5±0.3c	*	5
	1:1000	91.8±0.3a	39.0±0.2c	81.0±0.2a	5
	1:2000	80.0±0.2a	41.0±0.5c	80.8±0.5a	5
C. asiatica	Control	91.0±0.4a	90.0±1.5a	100±0.0a	7
	Aerosol smoke	80.7±1.2a	*	*	3
	1:500	80.0±0.4a	82.0±0.9a	*	5
	1:1000	90.0±0.2a	75.0±0.2b	100±0.0a	4
	1:2000	80.0±0.7a	67.9±1.4b	81.0±0.3a	4
C. gynandra	Control	86.6±0.2a	66.1±1.6b	100±0.0a	6
	Aerosol smoke	83.0±0.5a	50.0±0.3b	*	3
	1:500	85.9±0.3a	*	90.9±0.6a	4
	1:1000	91.7±0.2a	62.3±0.9b	100±0.0a	4
	1:2000	80.8±0.5a	61.9±1.0b	81.5±0.4a	4

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 (%)
A. pennata	Control	331.5	175b	41
	Aerosol smoke	565.1	201a	77
	1:500	575.9	187b	83
	1:1000	601.0	225a	89
	1:2000	554.6	201a	85
B. alba	Control	201.0	66a	56
	Aerosol smoke	431.0	103b	90
	1:500	521.0	79a	92
	1:1000	485.8	90a	91
	1:2000	459.0	90a	89
C. asiatica	Control	307.1	76a	48
	Aerosol smoke	585.0	90a	90
	1:500	471.0	90a	75
	1:1000	431.0	79c	87
	1:2000	400.0	90c	93
C. gynandra	Control	381.0	102a	46
	Aerosol smoke	503.0	98a	97
	1:500	406.0	104a	89
	1:1000	481.9	101a	93
	1:2000	461.0	97c	94

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

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 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. Germination rate, shoot weight, and root length are all vital indices of seed vigour. Germination rate is particularly is vastly important as an increase in germination rate is usually followed by an improvement of overall seedling performance[22]. On the basis of the results presented in Table 1, it was noticed that all the medicinal plant species showed higher rate of germination under 16:8h light/dark in the control and smoke treatments. Continuous light did not affect the germination of four plant species under study (Table 1). 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). Further in most of the cases the aerosol smoke was more effective than aqueous smoke solutions showing good growth of seedlings. The physiological mechanism resulting in improved vigour is unknown. However, smoke may protect the seed and seedlings against microbial attack, 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 [9-16]. 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[4, 9-16]. The present study indicated that the seeds of all the medicinal plants under study showed higher average germination rates, shoot weights, and root lengths when compared to their control counterpart (Table 1). Seedling survival rate of all the medicinal plant species under study was also found high as compared to control (Table 2).

The major implications of this study are that aerosol smoke and smoke solutions linked enhancement of seed vigour response is closely might be linked to phenolic enhancement. The phenolic enhancement closely follows enhancement in growth of seedlings[22]. Nutrient levels did not have a significant effect on shoot or root length (Table 2). On the otherhand, application of aerosol smoke and smoke solutions, and also application of HS to the seedlings, significantly increased seedling fresh weight, leaf area and aerial shoot length. It has been demonstrated that abiotic stresses can be crucial in increasing the yield of secondary metabolites in medicinal plants. But in the present study, we have not measured any phenolic content of the seedlings. At the higher concentrations, smoke extracts are known to inhibit seed germination[9-16]. The post germination application of 1:2000 smoke solution resulted in significantly greater seedling mass. Therefore, these results suggest that priming seed with potential exogenous smoke and smoke solution treatment has significant agronomic implications through the improved seed vigour as reflected in growth responses. It is conceivable that with further research it will be possible to extend this seed priming technique to other medicinal plant systems to improve both seed vigour and overall plant productivity.

Acknowledgement

We are greatful to our all friends for their every help during the collection of plant materal from the study area of Belgaum district, Karnataka state, India.

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