Plant-derived Smoke Solution and Potassium Nitrate Affect Seed Germination and Seed Vigour in Four Medicinal Plant Species

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Pflanzliche Rauchlösung und Kaliumnitrat beeinflussen Samenkeimung und Triebkraft von vier Arzneipflanzen

1 Introduction

Seed germination as a complex process in higher plants is influenced by many environmental factors such as light, temperature, and nitrates (NELSON et al., 2009). The effect of plant derived smoke on seed germination was first reported by DE LANGE and BOUCHER (1990) in fynbos species, *Audouinia capitata* (L.) Brongn (Bruniaceae). Nitrogen oxides (KEELEY and FOTHERINGHAM, 1998) and butenolide (FLEMATTI et al., 2007; VAN STADEN et al., 2006) have been proposed as the most important compounds of smoke for seed germination stimulation (GOMEZ-GONZALEZ et al., 2008). The combustion of living and dead plant material releases nitrogenous compounds to the soil (FRANCO VIZCAINO and SOSA RAMIREZ, 1997). Numerous studies described smoke as a germination cue. SPARG et al. (2005) suggested that in addition smoke may also positively affect seed vigour. Seed germination of different families and genera of gymnosperms, monocotyledons and dicotyledons not only from the Mediterranean fire-prone ecosystems has been stimulated by smoke (VAN STADEN et al., 2000). Stimulation effects of smoke were found even in commercial crop plants and in indigenous medicinal plants (SPARG et al., 2006; ABDOLLAHI et al., 2011). Findings of these studies and germination problems in majority of medicinal plants led to the present study in which we compare the effects of smoke water (derived from burning maize leaf) and potassium nitrate on germination and seed vigour of *Sanguisorba minor* (Rosaceae), *Pimpinella anisum* (Apiaceae), *Melissa officinalis* (Lamiaceae) and *Nigella sativa* (Ranunculaceae).

2 Materials and Methods

Seeds of the four medicinal plant species *Sanguisorba minor* (Rosaceae, native to western, central and southern Europe,

Zusammenfassung

Die Auswirkungen von Rauchlösungen und Kaliumnitrat auf Keimung und Triebkraft von vier Arzneipflanzen (*Sanguisorba minor, Pimpinella anisum, Melissa officinalis und Nigella sativa*) wurden getestet. Trockene Samen wurden für 1 h eingeweicht in entweder Rauchlösung (1:500), KNO_3-Lösung (150 mM) oder destilliertem Wasser als Kontrolle, gefolgt von einem Keimtest in der jeweils gleichen Lösung. Die Rauchlösung verbesserte bei *S. minor* die Keimfähigkeit (GP), die Keimgeschwindigkeit (GS), die Spross- (SL) und Wurzellänge (RL), die Gesamtlänge des Keimlings (TSL), dessen Frischmasse (SFM), den Triebkraftindex (VI) sowie die Keimrate (GV). Bei *P. anisum* waren GS, SL, VI und GV verbessert und bei *M. officinalis* RL, das Wurzel/Spross-Längenverhältnis (R/S) und SFM. Samen von *N. sativa* zeigten keine Reaktion auf die Rauchlösung. Kaliumnitrat verbesserte bei *P. anisum* GS, SL, VI und GV, bei *M. officinalis* GS, SL, RL, TSL, SFM, VI und GV, bei *N. sativa* SL. Signifikant positive Korrelationen wurden gefunden zwischen Effekten von Rauchlösung und Kaliumnitrat bei den vier Arten für GP ($r = 0{,}58$, $p < 0{,}01$), GS ($R = 0{,}61$, $p < 0{,}01$), R/S ($r = 0{,}55$, $p < 0{,}01$) und GV ($r = 0{,}43$, $p < 0{,}05$).

Schlagworte: Rauchlösung, Triebkraft, *Sanguisorba minor, Pimpinella anisum, Melissa officinalis, Nigella sativa.*

Summary

The effects of smoke water and potassium nitrate on germination and seed vigour were tested in the four medicinal species *Sanguisorba minor*, *Pimpinella anisum*, *Melissa officinalis* and *Nigella sativa*. Dry seeds were soaked in either smoke water (1:500), KNO_3 solution (150 mM) or distilled water as a control for 1 h followed by a germination test in the same solutions. Smoke water enhanced in *S. minor* germination percentage (GP), germination speed (GS), shoot length (SL), root length (RL), total seedling length (TSL), seedling fresh mass (SFM), vigour index (VI) and germination value (GV), in *P. anisum* GS, SL, VI and GV and in *M. officinalis* RL, root/shoot length ratio (R/S) and SFM. Seeds of *N. sativa* showed no response to smoke water. Potassium nitrate enhanced in *P. anisum* GS, SL, VI and GV, in *M. officinalis* GS, SL, RL, TSL, SFM, VI and GV and in *N. sativa* SL. Significant positive correlations were found between the responses of the four species to smoke water and to potassium nitrate for GP ($r = 0.58$, $p < 0.01$), GS ($r = 0.61$, $p < 0.01$), R/S ($r = 0.55$, $p < 0.01$) and GV ($r = 0.43$, $p < 0.05$).

Key words: Smoke water, seed vigour, *Sanguisorba minor, Pimpinella anisum*, *Melissa officinalis, Nigella sativa.*

northwest Africa and southwest Asia,), *Pimpinella anisum* (Apiaceae, native to the eastern Mediterranean region and southwest Asia), *Melissa officinalis* (Lamiaceae, native to southern Europe and the Mediterranean region) and *Nigella sativa* (Ranunculaceae, native to southwest Asia) were collected from the medicinal plants garden of the Hamedan, Iran. These species do not have seed dormancy or have low level of seed dormancy and their germination ability at harvest time is 82 % for *S. minor*, 90 % for *P. anisum*, 99 % for *M. officinalis* and 100 % for *N. sativa*. Seeds of the four plant species were allowed to dry to 12 to 15 % moisture and stored for about 3 months at room temperature (20–30° C) hermetically sealed in aluminum foil packets until the start of the experiment.

Smoke water was prepared by the method outlined by Baxter et al. (1994). It was generated by continuously bubbling smoke from smoldering maize (*Zea mays*, cv. SC704) leaf material (stage of tassels fully emerged) in a 50 l metal drum through 2000 ml distilled water for 45 min. The metal drum was connected to a container by metal tubes (containing 2000 ml distilled water) and a vacuum machine, respectively. One milliliter of the concentrated solution with smoke was diluted in 500 ml distilled water and used for experimentation. Based on a previous study of Brown and Van Staden, (1997) showing no significant effects of the pH of smoke extracts, the smoke solution was used without pH adjustment and stored at room temperature until the start of the experiment. Seeds were surface disinfected with 2.5 % sodium hypochlorite solution for 10 min and then rinsed twice with distilled water. Seeds were then soaked for 1 h in either smoke water, potassium nitrate solution (KNO3) (150 mM) or in distilled water for control. After this soaking period, seeds were allowed to dry and immediately placed in 9 cm circular Petri dishes on top of two layers of filter paper (Whatman No. 1) moistened with the three different solutions according to the treatment. Petri dishes were placed horizontally. Each treatment consisted of six replicates of 20 seeds each for *S. minor* and *N. sativa* and 30 seeds each for *P. anisum* and *M officinalis*. Seeds were incubated at 25 ± 0.5 °C under a 16:8 h light/dark period. The filter paper was remoistened when required with a few drops of distilled water. The number of germinated seeds (root longer than 1 mm) was recorded for each Petri dish in daily intervals up to the seventh day after starting the test. On the seventh day seedling size and the shoot and root mass were measured. Germination percentage, germination speed, shoot length, root length, root/shoot length ratio, total seedling length, seedling fresh mass, vigour index, and germination value were recorded or calculated after 7 d as follows:

Germination percentage (GP) is the proportion of germinated seeds after 7 days.

The germination speed (GS) was calculated according to Maguire's equation (Maguire, 1962): ($M = n_1/t_1 + n_2/t_2 + \ldots + n_n/t_n$; where $n_1, n_2, \ldots, n_n$ are the number of emerged seeds at times $t_1, t_2, \ldots, t_n$ (in days).

Seedling vigour index (VI) was calculated as: VI = (shoot length + root length) × GP (Dhindwal et al., 1991).

Germination value (GV) was calculated using the formula:

$$GV = \frac{\sum_{i=1}^{N} DGi}{N} . \frac{GP}{10}$$

where GP = Germination percentage; DG = Daily germination speed obtained by dividing the cumulative germination percentage at that day by the number of days since sow-

Table 1: Germination and seed vigour parameters (means ± SE) of the four medicinal species after germination in water (control), smoke water (1:500) and KNO3 (150 mM) and in a16:8h light/dark photoperiod and at 25 ± 0.5°C.
Tabelle 1: Keimung und Triebkraft (Mittelwert ± SE) der vier Medizinalpflanzen nach Keimung in Wasser (Kontrolle), Rauchlösung (1:500) und KNO_3 (150 mM) bei 16/8 h hell/ dunkel und 25 ± 0,5 °C.

Germination and seed vigour parameters						Treatment	Plant species
GV	**VI**	**SFM (mg)**	**R/S**	**GS**	**GP**		
13.79 ± 1.10 b	316.22 ± 49.45 b	20.7 ± 1.2 b	1.16 ± 0.08 a	3.05 ± 0.19 b	75.00 ± 3.42 b	**Control**	***Sanguisorba minor***
16.63 ± 1.43 a	554.81 ± 65.25 a	24.7 ± 1.7 a	1.06 ± 0.07 a	3.61 ± 0.24 a	85.83 ± 4.17 a	**SW**	
11.09 ± 1.27 b	229.55 ± 42.89 b	21.3 ± 1.3 b	1.08 ± 0.04 a	2.62 ± 0.25 b	68.33 ± 5.43 b	**KNO_3**	
10.87 ± 0.61 b	371.54 ± 29.84 b	7.8 ± 0.2 a	2.77 ± 0.32 a	5.39 ± 0.26 b	88.33 ± 3.82 a	**Control**	***Pimpinella anis*sum**
12.82 ± 0.52 a	516.63 ± 20.86 a	7.7 ± 0.4 a	1.97 ± 0.27 a	6.18 ± 0.18 a	96.11 ± 2.18 a	**SW**	
13.46 ± 0.64 a	570.11 ± 50.05 a	8.0 ± 0.7 a	1.91 ± 0.26 a	6.34 ± 0.22 a	96.11 ± 2.00 a	**KNO_3**	
20.35 ± 0.53 b	237.22 ± 25.48 b	9.7 ± 0.6 b	1.56 ± 0.25 b	8.38 ± 0.20 b	99.44 ± 0.56 a	**Control**	***Melissa officinalis***
18.79 ± 0.62 c	286.13 ± 5.96 b	11.0 ± 1.0 a	2.13 ± 0.07 a	7.84 ± 0.20 c	98.89 ± 0.70 a	**SW**	
22.74 ± 0.18 a	408.89 ± 21.06 a	11.8 ± 0.4 a	0.98 ± 0.07 c	9.24 ± 0.08 a	100 a	**KNO_3**	
56.36 ± 0.31 a	1107.22 ± 38.79 a	111.8 ± 4.9 a	1.98 ± 0.11 a	14.60 ± 0.16 a	100 a	**Control**	***Nigella sativa***
52.66 ± 1.35 b	1113.89 ± 64.94 a	110.5 ± 10.8 a	2.04 ± 0.18 a	12.66 ± 0.77 b	100 a	**SW**	
50.68 ± 1.10 b	1130.97 ± 45.99 a	99.2 ± 3.9 a	1.73 ± 0.13 a	12.26 ± 0.33 b	99.17 ± 0.83 a	**KNO_3**	

SW: smoke water, GP: germination percentage, GS: germination speed, R/S: root/shoot length ratio, SFM: seedling fresh mass, VI: vigour index, GV: germination value.
Mean values with the same letter for each species are not significantly different ($p < 0.05$).

ing; N = the total number of daily counts, starting from the date of first germination;10 = Constant (DJAVANSHIR and POURBEIK, 1976).
The experiment was arranged in a completely randomized design with three treatments, smoke water (1:500), KNO_3 (150 mM) and control in 6 replications. The results were analyzed using SPSS 16 for windows (SPSS Inc., an IBM Company, USA). Analysis of variance (ANOVA) was conducted and Duncan's Multiple Range Test was used to test the differences between means. Pearson's correlation coefficients were calculated with the differences between the two solutions and the water control. Significance tests were made for $\alpha = 0.05$.

3 Results

The results of germination and seedling growth parameters of four medicinal plants species are presented in Table 1 and Figure 2. Smoke water had significant positive effects on germination percentage in comparison to the control and potassium nitrate in *S. minor*. Smoke had no significant effect on germination percentage in *P. anisum*, *N. sativa* and *M. officinalis* seeds. Similarly, KNO_3 did not show any significant effect in all species compared to control. There was a significant positive correlation ($P < 0.05$) between the responses of the four species to smoke water and potassium nitrate for germination percentage (Table 2). Smoke water increased the germination speed in *S. minor* and *P. anisum* while had negative effects on germination speed in *N. sativa* and *M. officinalis*. KNO_3 revealed a positive effect on germination speed in *P. anisum* and *M. officinalis,* a negative effect in *N. sativa* and no significant effect in *S. minor*. For germination speed, there was a significant correlation between the responses of the four species to smoke water and KNO_3 (Table 2).

Smoke water showed a significant positive effect on seedling fresh mass (Table 1) in *S. minor* and *M. officinalis*, but no significant effects in *P. anisum* and *N. sativa*. Potassium nitrate only had a significant positive effect in *M. officinalis*. For seedling fresh mass there was no significant correlation between the responses of the four species to smoke water and KNO_3 (Table 2).

Smoke water showed significant positive effects on shoot length (Figure 1) in *S. minor* (3.14 ± 0.32 cm) and *P. anissum* (1.91 ± 0.22 cm) but no significant effect in *M. officinalis* and *N. sativa*. Potassium nitrate had a significant positive effect on shoot length (Figure 1) in *P. anissum* (2.11 ± 0.23 cm), *M. officinalis* (2.08 ± 0.13 cm), and *N. sativa* (4.19 ± 0.14 cm) but not in *S. minor*. In addition, smoke

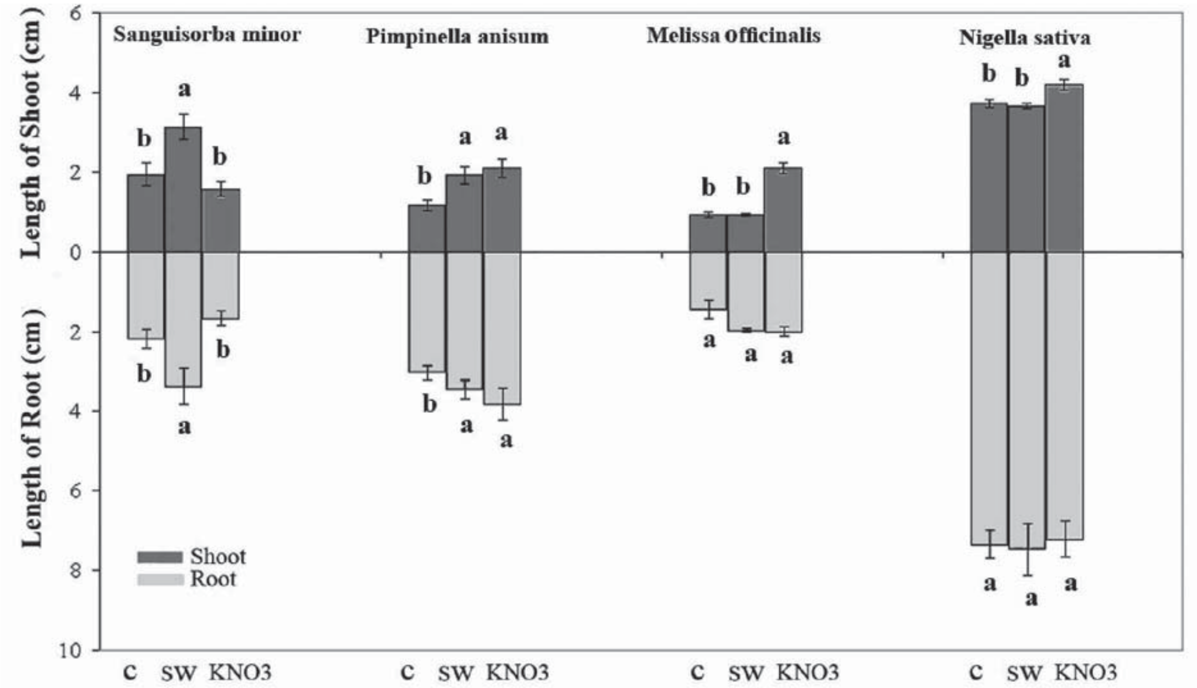

Figure 1: Shoot length, root length and total seedling length in four medicinal species after 7 days of germination in water (control), smoke water (1:500) and KNO_3 (150 mM). Seeds were germinated in a 16:8h light/dark photoperiod and at 25 ± 0.5°C. Error bars are ± SE of the mean. An asterisk above a column indicates a significant difference from the control (P < 0.05, r=6).

Abbildung 1: Sprossänge, Wurzellänge und Gesamtlänge des Keimlings der vier Arzneipflanzen nach 7 Tagen Keimung in Wasser (Kontrolle), Rauchlösung (1:500) und KNO_3 (150 mM). Die Samen wurden bei 16/8 h hell/dunkel und 25 ± 0,5 °C gekeimt. Fehlerbalken sind ± SE der Mittelwerte. Ein Sternchen über einer Spalte zeigt einen signifikanten Unterschied von der Kontrollgruppe (p <0,05, r = 6).

water showed significant positive effects on root length (Figure 1) in *S. minor* (3.37 ± 0.46 cm) and *M. officinalis* (1.97 ± 0.04 cm) but not in *P. anisum* and *N.sativa*. Potassium nitrate only had a significant positive effect on root length in *M. officinalis* (2.01 ± 0.12 cm). Smoke water showed significant positive effects on total seedling length (Figure 1) in *S. minor* (6.51 ± 0.77 cm) but not in the other species. Potassium nitrate enhanced the total seedling length only in *M. officinalis* (4.09 ± 0.21 cm). Smoke water enhanced the root/shoot ratio (Table 1) in *M. officinalis* whereas potassium nitrate reduced it in this species. In the other species no significant effects were found. For shoot length, root length and total seedling length there were no significant correlations between the responses of the four species to smoke water and KNO_3 (Table 2). Only for root/shoot ratio there was a significant correlation (Table 2).

Smoke water showed significant positive effects on vigour index and germination value in *S. minor* and *P. anisum* but no significant effect on vigour index and negative effect on germination value in *M. officinalis* and *N. sativa* (Table 1). Potassium nitrate enhanced both vigour index and germination value in *M. officinalis* whereas de-

Table 2: Pearson's correlation coefficients describing the relationships between the relative changes in the germination and seed vigour parameters (relative to the control) caused by smoke water (1:500) and KNO3 (150 mM) across the four medicinal species

Tabelle 2: Pearson-Korrelationskoeffizienten der Beziehungen zwischen den relativen Veränderungen in der Keimung und Triebkraft (bezogen auf die Kontrolle) durch Rauchlösung (1:500) und KNO_3 (150 mM) bei den vier Arzneipflanzen.

	KNO_3								
Smoke water	GP	GS	SL (cm)	RL(cm)	TSL(cm)	R/S	SFM(g)	VI	GV
	0.577^{**}	0.606^{**}	$-0.227^{n.s}$	$-0.087^{n.s}$	$-0.196^{n.s}$	0.547^{**}	$0.200^{n.s}$	$-0.02^{n.s}$	0.429^{*}

Correlation coefficients are presented separately for germination percentage (GP), germination speed (GS), shoot length (SL), root length (RL), total seedling length (TSL), root/shoot length ratio (R/S), seedling fresh mass (SFM), vigour index (VI) and germination value (GV).
* P < 0.05, ** P<0.01, $^{n.s}$ P>0.05

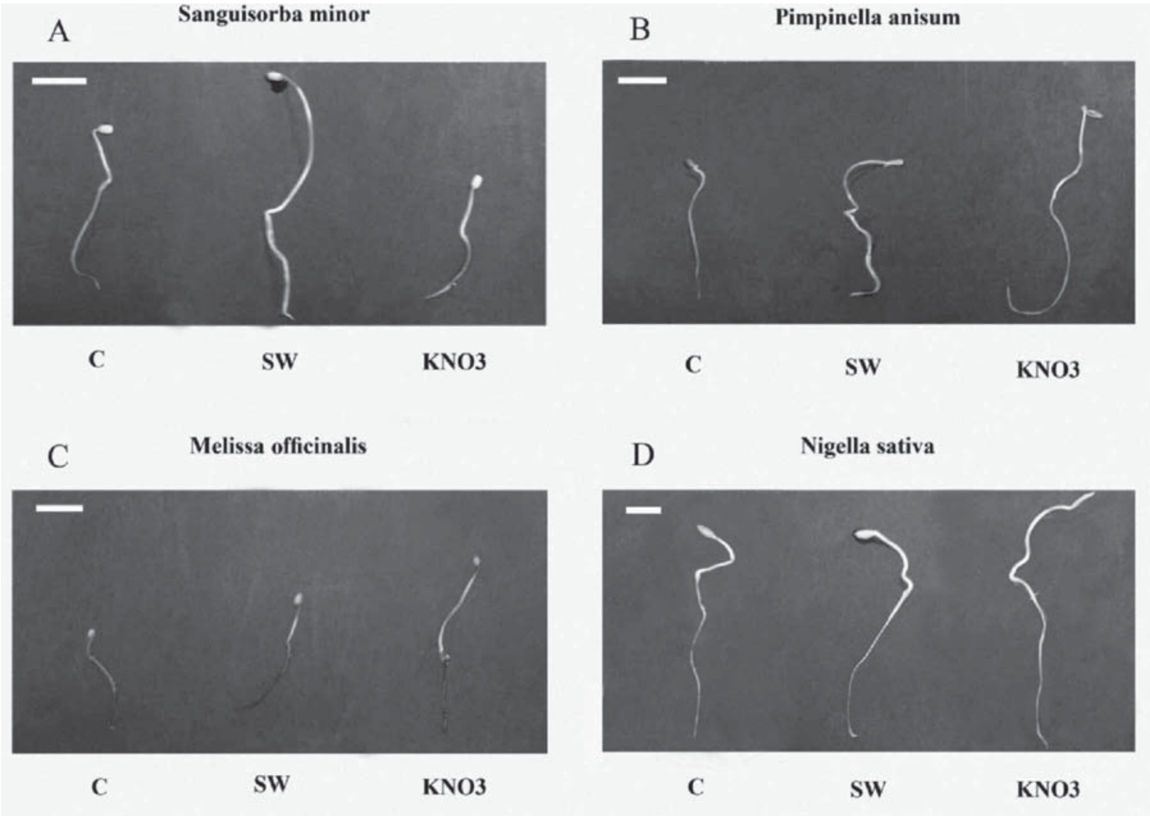

Figure 2: A comparison of seedling size for (A) *Sanguisorba minor* (B) *Pimpinella anisum* (C) *Melissa officinalis and* (D) *N. sativa* after 7 days of germination in water (control), smoke water (1:500) or KNO_3 (150 mM). Seeds were germinated in a 16:8h light/dark photoperiod and at 25 ± 0.5°C. C = control; SW= smoke water; scale bars = 1 cm.

Abbildung 2: Vergleich der Keimlingsgröße für (A) *Sanguisorba minor* (B) *Pimpinella anisum* (C) *Melissa officinalis* und (D) *Nigella sativa* nach 7 Tagen Keimung in Wasser (Kontrolle), Rauchlösung (1:500) oder KNO_3 (150 mM). Die Samen wurden bei 16/8 h hell/dunkel und 25 ± 0,5 °C gekeimt. C = Kontrolle; SW = Rauchlösung; Maßstabsbalken = 1 cm.

creased the germination value in *N. sativa* and had no significant effect on vigour index in this species (Table 1). For vigour index there was no significant correlation between the responses of the four species to smoke water and KNO_3 (Table 4). Only for germination value there was a significant correlation (Table 2).

The effect of smoke water and KNO_3 on seedling growth of the four species in seventh day after germination start was shown in Figure 2.

4 Discussion

This research demonstrates that smoke water can both positively and negatively affect seed germination and also seed vigour of a number of important medicinal species. Among the four species studied, the greatest positive effects of smoke water were found in *S. minor* and *P. anisum*. This is consistent with previous studies showing a great variability of species response to the smoke treatment (BROWN, 1993; BROWN et al., 1994; CROSTI et al., 2006; DIXON et al., 1995; MERRITT et al., 2006; MORRIS, 2000; RAZANAMANDRANTO et al., 2005; REYES and TRABAUD 2009; THOMAS et al., 2003; TIGABU et al., 2007). At high concentrations, smoke extract is found to inhibit seed germination (BROWN and VAN STADEN, 1997). According to BROWN and VAN STADEN (1997), more dilute solutions improved the germination percentage in dormant seeds. In this study, the dilution of the smoke water used (1:500) showed no inhibitory effect on germination and seed vigour in *S. minor* and *P. anisum*. However, germination speed and germination value in *M. officinalis* and *N. sativa* were reduced in comparison to control. Seedling vigour is an important parameter for the establishment of a plant, and in this study, the effect of smoke water on seedling vigour in *S. minor* and *P. anisum* was highly significant ($p < 0.05$, Table 1). This certainly provides evidence for the stimulatory effect of smoke water on seedling vigour and growth. In a study on *Erica* sp. and species of *Asteraceae*, it was mentioned that young seedlings showed more vigour when treated with smoke (Brown, 1993). A similar effect was reported for smoke-treated seeds of the fire-climax grass *Themeda triandra* (Baxter and Van Staden, 1994). In other studies, smoke water increased seedling length in grasses (Blank and Young, 1998) and indigenous medicinal plants (Sparg et al., 2005; Abdollahi et al., 2011). The importance of smoke in agriculture has been investigated in crops like lettuce (Drewes et al., 1995), celery (Thomas and Van Staden, 1995), tomato, okra, bean and maize (Van Staden et al., 2006). In a study (Modi, 2002) using an indigenous storage method of maize cobs it was reported that smoke pretreated seeds produced significantly more vigorous seedlings than untreated seeds. The smoke water treatments exhibited stimulatory effects on the rooting of maize seedlings. Kernels treated with smoke water developed more roots than the untreated control (Van Staden et al., 2006). In a study by Taylor and Van Staden (1996), it was shown that a smoke extract stimulated root formation in *Vigna radiata* (L.), indicating that smoke components may play an important role in promoting rooting. The results of this study are similar to the above mentioned studies. Germination value (GV) is another germination parameter investigated in this study that is a composite value combining both germination speed and total germination. Germination value provides an objective means of evaluating the results of a germination test. In this study, smoke water enhanced this parameter in *S. minor* and *P. anisum* and reduced it in *M. officinalis* and *N.sativa*. In another part of this research we investigated the effect of potassium nitrate on seed germination and seedling growth. Nitrate has been proposed as the active component of charred wood-stimulated germination (Thanos and Rundel, 1995) and as a germination factor derived from ash extracts (Henig-Sever et al., 2000). Seed germination following fire in several California chaparrals species has been related to the post fire presence of water-soluble nitrogenous compounds in soils (Thanos and Rundel, 1995) and several Western Australia species had their germination enhanced in the presence of KNO_3 (Bell, 1999). Based on these studies, we applied potassium nitrate (150 mM) in comparison with smoke water for study of germination and seedling growth in four medicinal species. To determine the relation between responses to smoke water and to potassium nitrate across the species, Pearson's correlation coefficients were used. These correlations were significant for germination percentage, germination speed, root/shoot ratio, and germination values. Concerning shoot, root and seedling length and seedling vigour, there was no significant correlation between response to smoke and potassium nitrate. In this study, the effect of smoke water on germination and seedling growth parameters in *S. minor* was greater than potassium nitrate while the effect of smoke water on mentioned traits in *P. anisum* and *N. sativa* was equal to potassium nitrate and in *M. officinalis,* the effect of smoke water on some germination parameters was less than potassium nitrate (Table 1). Last findings show that the effect of smoke water on the same germination parameters in the four plant species is similar to potassium nitrate (high correlation coefficients) and these results support the findings of previous studies related to the post fire presence of water-soluble nitrogenous compounds in soils increasing the seed germination following fire in several species (Thanos and Rundel, 1995). This study revealed that the effects of smoke water extend beyond the germination stimulation of some medicinal seeds and that it can also act to enhance seedling vigour and accelerate plant growth. This suggests that in previous studies, where germination parameters of many species had not responded to smoke treatments, these species may show some responses during seedling growth stages.

5 Conclusion

On the base of this study, we suggest that the smoke water may be potentially used as a growth stimulant in various plant species. Furthermore, it is clear that the use of smoke water to pre-treat the seeds of medicinal plants is a major progress in the field of agriculture. Hence, there is an urgent need to investigate the effects of this compound on yield under field conditions.

Aberrations

SW, Smoke water
GP, Germination percentage
GS, Germination speed
SL, Shoot length
RL, Root length
TSL, Total seedling length
R/S, root/shoot length ratio
VI, Vigour index
GV, Germination value

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