**INFLUENCE OF SMOKE-WATER AND WATER IMBIBITION ON GERMINATION AND PHYTOCHEMICAL CONTENT OF TWO *TULBAGHIA* SPECIES WITH ORNAMENTAL AND MEDICINAL VALUE**

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Abstract

*Tulbaghia* species are well-known for their medicinal, horticultural and ornamental potential. In an attempt to stimulate their cultivation, the current study investigated the effect of different soaking duration and smoke-water dilutions on germination, seedling growth and phytochemical content in *Tulbaghia ludwigiana* and *Tulbaghia violacea*. In addition to differences in their moisture content and imbibition rates, *Tulbaghia ludwigiana* seeds germinated faster than those of *Tulbaghia violacea*. Even though high germination rates were observed in most cases, there was no significant difference in germination rates when the treated seeds were compared to the control. At post-germination stage, SW (1:1000) and 48 h soaking produced the longest shoots and fresh weights in *Tulbaghia ludwigiana*. Root number (SW 1:1500) and fresh weight (12 h soaking and SW 1:1500) was also significantly higher in *Tulbaghia violacea*. *Tulbaghia ludwigiana* treated with SW (1:500) with varying soaking durations significantly increased the phenolic, flavonoid and condensed tannin content compared to the control. Similar treatments also improved the phenolic and flavonoid content in *Tulbaghia violacea* seedlings. These findings suggest the potential of SW and soaking treatment in enhancing the cultivation of the two investigated *Tulbaghia* species.

Keywords: Alliaceae; Conservation; Phenolics;Secondary metabolites; *Tulbaghia* species; Wild garlic

**INTRODUCTION**

Globally, the increasing popularity and uses of medicinal plants is well-known. The recent surge in demand for medicinal plants has been attributed to the therapeutic potential of medicinal plants and their abundant secondary metabolite reservoirs ([Lubbe and Verpoorte 2011](#_ENREF_14)). Although the numerous benefits derived from the plant species cannot be overemphasized, the aftermath effect of incessant harvesting mainly manifested in the depletion of wild populations remain major concerns ([Affolter and Pengelly 2007](#_ENREF_1), [Jäger and Van Staden 2000](#_ENREF_9)). Convincing evidence indicates that the continuous reliance on plant material harvested from wild stocks cannot meet the escalating demand for both local and international markets ([Affolter and Pengelly 2007](#_ENREF_1)). Inevitably, it is imperative to devise means to guarantee a sustained supply of renewable resources ([Canter et al. 2005](#_ENREF_7)). As a possible solution, effective propagation and breeding methods of plant species (especially for medicinal plants) could alleviate pressure on wild population as well as providing sufficient plant materials to both local and global markets ([Jäger and Van Staden 2000](#_ENREF_9)).

Recently, the cultivation of medicinal plants has gained more attention globally ([Canter et al. 2005](#_ENREF_7), [Lubbe and Verpoorte 2011](#_ENREF_14)). Although some breakthroughs have been achieved, challenges such as low germination rates and unpredictability of phytochemical content of plant species remain major concerns ([Canter et al. 2005](#_ENREF_7), [Kulkarni et al. 2006](#_ENREF_13)). Therefore, researchers are channeling more efforts towards overcoming these problems. For instance, stratification and artificial emulation of environmental conditions required for seed germination such as soaking or chilling enhance seed germination rates ([Baskin and Baskin 1998](#_ENREF_4), [Canter et al. 2005](#_ENREF_7)). Several factors are known to affect rate of seed germination ([Baskin and Baskin 1998](#_ENREF_4), [Nonogaki et al. 2010](#_ENREF_16)). The stimulatory effect of supplements such as smoke-water (SW) and karrikinolide on germination of several plant species is well-documented ([Kulkarni et al. 2011](#_ENREF_11)). It has also been established that SW increases the phytochemical contents in *planta* ([Aremu et al. 2012](#_ENREF_2), [Kulkarni et al. 2013](#_ENREF_10)). Logically, the availability of essential information on the seed germination and general growth requirements of widely used ornamental and medicinal plant species remain valuable from a conservation perspective.

The medicinal, ornamental and horticultural potential of the genus *Tulbaghia* is well documented ([Aremu and Van Staden 2013](#_ENREF_3)). Many species of *Tulbaghia* have attained great importance in indigenous folk medicine in southern Africa. Consequently, the often over-collection inevitably place the species in danger of extinction, thus the protection of the genus is of paramount interest to researchers ([Vosa 2007](#_ENREF_22)). [Kulkarni et al. (2005](#_ENREF_12)) investigated the effect of temperature and watering frequencies on seed germination and seedling growth of *Tulbaghia violacea*. In the current study, we evaluated the effect of soaking duration and smoke-water (SW) treatments on seed germination and phytochemical content of *Tulbaghia ludwigiana* and *Tulbaghia violacea*.

**MATERIALS AND METHODS**

Source of smoke-water and seeds

Stock SW solution as prepared by [Baxter et al. (1994](#_ENREF_5)) was used in the current study. *Tulbaghia violacea* Harv. and *Tulbaghia ludwigiana* Harv. seeds were obtained from Silverhill Seed Nursery, Cape Town and African Bulbs, Napier, South Africa, respectively.

Tests for seed viability, moisture content and imbibition

The viability, moisture content and imbibition of both *Tulbaghia* species were determined using the methods as outlined by [Kulkarni et al. (2006](#_ENREF_13)). For each experiment, four replicates of 25 *Tulbaghia* species seeds were used. Seed viability was determined using 2,3,5-triphenyl tetrazolium chloride (TTC) solution. Seeds were placed on Petri dishes on filter paper moistened with distilled water. After 24 h, 1% TTC was added to the seeds in the Petri dishes and incubated in the dark at room temperature for 24 h. Seeds were classified as viable if a red-stained embryos was observed. Moisture content was determined by placing seeds in an oven at 110 °C. Once a constant mass was achieved, the moisture content of the seeds was determined ([Kulkarni et al. 2006](#_ENREF_13)). For imbibition tests, the seeds were placed in Petri dishes with two layers of filter paper (Whatman No.1) moistened with 5 ml distilled water and allowed to imbibe at room temperature. At 3 h intervals, for 48 h, the seeds were blotted dry, weighed and returned to the wet filter paper. The amount of water imbibed by the seeds was expressed as a percentage increase over the initial seed weight ([Kulkarni et al. 2006](#_ENREF_13)).

Decontamination of seeds and germination experiments

For decontamination,the seeds were soaked in 70% ethanol for 60 s followed by 0.2% fungicide (Benlate®, Du Pont de Nemours Int., South Africa) for 2 min and 3.5% commercial bleach for 5 min. The seeds were thoroughly rinsed with distilled water. Soaking treatment of the seeds was conducted by complete immersion of 240 seeds for different time durations (0, 6, 12, 24 and 48 h). Thereafter, the soaked seeds were germinated *in vitro* on 1/10th strength Murashige and Skoog (MS) medium ([Murashige and Skoog 1962](#_ENREF_15)) supplemented with varying SW dilutions (0, 1:500, 1:1000, 1:1500). Four replicates of 15 seeds were used for each SW treatment or control.

*Tulbaghia* species were cultured under 24 h light conditions with a photosynthetic photon flux (PPF) of 45 µmol m-2 s-1 at 25 ± 2 °C. A seed was considered as germinated when the radicle protruded 2 mm. Germination counts for all experiments were made daily for 15 days. Mean germination time (MGT) was calculated as outlined by [Kulkarni et al. (2013](#_ENREF_10)). After 15 days, seedlings were harvested and the growth parameters were recorded.

Phytochemical content quantification

Phytochemical contents in the seedlings of *Tulbaghia* species were determined using colorimetric methods. Oven-dried plant materials (100 mg) were extracted with 10 ml of 50% methanol in an ice-bath for 20 min and filtered using Whatman No 1 filter paper. Thereafter, phenolics, flavonoids and condensed tannin content were quantified ([Aremu et al. 2012](#_ENREF_2)). Total phenolics, flavonoids, condensed tannins and iridoids were expressed in mg gallic acid equivalents (GAE), mg catechin equivalents (CE) and µg/mg cyanidin chloride equivalents (CCE) per g DW, respectively. Three replicates were used for the phytochemical assays.

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS software package for Windows (SPSS®, version 10.0 Chicago, USA). Where there was statistical significance (*p* ≤ 0.05), the mean values were further separated using Duncan’s Multiple Range Test.

**RESULTS**

Seed viability, moisture content and imbibition

Both *Tulbaghia* species demonstrated a high level (≥95%) of seed viability (Fig. 1). The moisture content of *Tulbaghia violacea* (approximately 41%) was higher than that for *Tulbaghia ludwigiana* seeds (31%). As depicted in Fig. 2, there was a continuous increase in water uptake in both species. *Tulbaghia ludwigiana* seeds generally had better absorption compared to *Tulbaghia violacea*. After 72 h, fresh weights of approximately 200% and 140% were observed for *Tulbaghia ludwigiana* and *Tulbaghia violacea*, respectively.

Effect of soaking and smoke-water treatments on germination of *Tulbaghia* species

The MGT ranged from 1.6-3.5 days in *Tulbaghia ludwigiana* and 2.1-4.3 days for *Tulbaghia violacea* seedlings (Fig. 3A). While soaking and SW enhanced the MGT in *Tulbaghia ludwigiana*, there was no noticeable improvement for *Tulbaghia violacea*. Both species demonstrated a high germination percentage, about 76-98% in *Tulbaghia ludwigiana* and 85-100% in *Tulbaghia violacea*. Generally, soaking and/or addition of SW had no significant stimulatory effect on the observed germination rate (%) for both *Tulbaghia* species (Fig. 3B)*.* Nevertheless, SW (1:500) slightly improved the germination rate in both species when compared to the control.

Effect of soaking and smoke-water treatments on growth of *Tulbaghia* species

The effect of different soaking durations and SW solutions on the growth of *Tulbaghia ludwigiana* and *Tulbaghia violacea* seedlings is presented in Tables 1 and 2, respectively. *Tulbaghia ludwigiana* seedlings soaked for 48 h and supplemented with SW (1:1000) had the longest shoot length and highest fresh weight (Table 1). While the treatments had no significant stimulatory effect on leaf number, root and shoot length in *Tulbaghia violacea* seedlings, the root number and plant fresh weight were significantly higher with various soaking and SW treatments compared to the control seedlings (Table 2).

Effect of soaking and smoke-water treatment on phytochemical content in *Tulbaghia* species

Generally, soaking and application of SW enhanced the phytochemical content in both *Tulbaghia* species (Fig. 4). In terms of phenolic content, the highest concentration was detected in *Tulbaghia ludwigiana* (14 mg GAE g-1 DW) soaked for 24 h and treated with SW (1:1000) while 6 h soaking and SW (1:500) was best with *Tulbaghia violacea* seedlings (8 mg GAE g-1 DW). Flavonoids (Fig. 4B) and condensed tannin (Fig. 4C) content was highest in 24 h soaked and SW (1:500)-treated *Tulbaghia ludwigiana*. In *Tulbaghia violacea* seedlings, highest concentration of flavonoids and condensed tannins was observed in un-soaked seeds supplemented with SW 1:500 and 12 h soaked seedlings, respectively.

**DISCUSSION**

Members of the genus *Tulbaghia* are known to possess characteristic seed-coat, which in turn plays a significant role in their ecology and distribution ([Vosa 2003](#_ENREF_21)). Both *Tulbaghia ludwigiana* and *Tulbaghia violacea* are classified as type ‘A’ seeds, imbibing water rapidly (2-5 h). Based on the current findings, *Tulbaghia ludwigiana* seeds have lower moisture content and imbibed larger quantities of water compared to *Tulbaghia violacea*. The importance of the initial water uptake by seeds is well documented ([Nonogaki et al. 2010](#_ENREF_16)). As a crucial stage, it determines whether a seed will germinate or not. Generally, soaking of seeds is aimed at shortening the lag phase during germination ([Sabongari and Aliero 2004](#_ENREF_18)). As postulated by [Nonogaki et al. (2010](#_ENREF_16)), soaking allows for rapid hydrolysis of complex sugar into simple units allowing for better utilization for synthesis of auxins and proteins. Inevitably, the auxins soften cell walls to facilitate growth and the proteins are available for production of new tissues. Soaking of seed to enhance germination could either be responsive ([Sabongari and Aliero 2004](#_ENREF_18)) or ineffective ([Himanen et al. 2012](#_ENREF_8)) in enhancing germination rate. There was no significant stimulatory effect of soaking on the MGT and germination rate of both *Tulbaghia* species in the current study. With SW (1:500 and 1:1500) however, the MGT in *Tulbaghia ludwigiana* was significantly shortened to 1.6 days when compared to the control devoid of SW (3.3 days). Apart from the apparent synergistic effect from soaking and addition of SW in the investigated *Tulbaghia* species, there are cases whereby the combinatory or individual effect of soaking and SW treatment were detrimental or indifferent in the current study. Further studies are required to better elucidate the various interactions of the applied treatment which could possibly enhance the beneficial effects for seed germination.

Although previous studies have demonstrated high (˃ 90%) germination rate for *Tulbaghia violacea* ([Kulkarni et al. 2005](#_ENREF_12), [Sparg et al. 2005](#_ENREF_20)), 100% germination was achieved with seeds treated with SW (1:1500) and soaked for 6 or 48 h in the current study. As in the current study, the lack of significant positive responses of SW on medicinal plant seed germination has been observed by other researchers ([Kulkarni et al. 2013](#_ENREF_10), [Sparg et al. 2005](#_ENREF_20)). In some of such cases ([Sparg et al. 2005](#_ENREF_20)), a favorable response of SW was reported at post-germination stages. A similar positive effect was observed in *Tulbaghia ludwigiana* seedlings soaked for 48 h and treated with SW (1:1000). As previously reported that SW had a greater effect on root systems ([Aremu et al. 2012](#_ENREF_2)), this was evident in the current study as clearly observed in SW (all dilutions)-treated *Tulbaghia violacea* treated with (6 h) or without soaking had more roots than the control seedlings.

The ability of SW to significantly increase phytochemical content in both investigated species is valuable. Recently, the stimulatory effect of SW on phytochemical content in several species ([Aremu et al. 2012](#_ENREF_2), [Kulkarni et al. 2013](#_ENREF_10), [Zhou et al. 2011](#_ENREF_23)) were reported. Based on molecular evidence, it is has been postulated that SW up-regulate the phenylpropanoid-pathway and flavonoid-related genes thereby enhancing phenolic biosynthesis ([Soós et al. 2010](#_ENREF_19)). In the current study, phenolic content in 24 h soaked and SW (1:1000)-*Tulbaghia ludwigiana* was approximately 2-fold higher than the control seedlings. Similarly, about 2.4-fold more flavonoids was detected in *Tulbaghia ludwigiana* when soaked for 24 h and supplemented with SW (1:500) compared to the control. The function of secondary metabolites such as phenolic compounds in plant survival is well known. For instance, these compounds act as signalling molecules ([Peer and Murphy 2007](#_ENREF_17)) as well as for defence against plant herbivores and pathogens ([Bennett and Wallsgrove 1994](#_ENREF_6)). It is logical to assume that the accumulation of secondary metabolites in SW-treated seedlings will be vital for successful field acclimatization of the seedlings.

In summary, even though soaking and SW application had no significant effect on the germination rate of both *Tulbaghia* species, there was a marked favourable effect during the post-germination stage whereby shoot length of *Tulbaghia ludwigiana* and fresh weight in both species were improved. Phytochemical content of both species was also higher following treatment with SW and/or soaking. Taken together, the current findings provide an avenue for enhancing the cultivation of two *Tulbaghia* species known for their medicinal, horticultural and ornamental potential.

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**Figure legends**

Fig. 1: Moisture content (%) and seed viability (%) of *Tulbaghia ludwigiana* and *Tulbaghia violacea*

Fig. 2: Water uptake by seeds of *Tulbaghia ludwigiana* and *Tulbaghia violacea*

Fig. 3: Effect of different soaking time and smoke-water treatments on the germination of *Tulbaghia ludwigiana* and *Tulbaghia violacea* seeds. A = Mean germination time (days) and B = germination rate (%). For each *Tulbaghia* species, bars (mean value ± SE) with different letter(s) indicate significant differences (*p* ≤ 0.05) based on Duncan’s Multiple Range Test.

Fig. 4: Effect of different soaking time and smoke-water treatments on the phytochemical content of *Tulbaghia ludwigiana* and *Tulbaghia violacea* seeds. A = Total phenolic (mg GAE g-1 DW), B = flavonoids (mg CE g-1 DW) and C= condensed tannins (µg or mg CCE g-1 DW). For each *Tulbaghia* species, bars (mean value ± SE) with different letter(s) indicate significant differences (*p* ≤ 0.05) based on Duncan’s Multiple Range Test. GAE = Gallic acid equivalents. CE = Catechin equivalents, CCE = cyanidin chloride equivalents, DW = dry weight.



Fig. 1

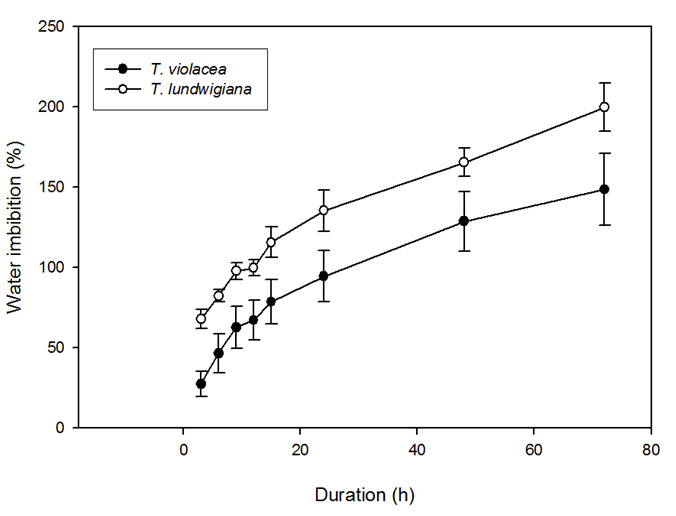


Fig. 2



Fig. 3



Fig. 4

Table 1

Effect of soaking time and smoke-water treatments on the growth of *Tulbaghia ludwigiana* seedlings

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | | Growth parameters | | | | | | | | | | | | | | | |
| SW conc | Time (h) | Shoot length (mm) | | | | Root number (#) | | | | Root length (mm) | | | | Plant fresh weight (mg) | | | |
| 0 | 0 | 38.2 | ± | 1.99 | cde | 1.4 | ± | 0.09 | abcd | 36.5 | ± | 2.85 | abc | 49.0 | ± | 2.82 | bcd |
| 6 | 28.5 | ± | 1.66 | fgh | 1.1 | ± | 0.06 | def | 27.0 | ± | 2.28 | efg | 34.8 | ± | 2.33 | def |
| 12 | 33.4 | ± | 2.69 | defg | 1.1 | ± | 0.06 | def | 25.1 | ± | 2.83 | efg | 39.5 | ± | 3.26 | bcdef |
| 24 | 39.8 | ± | 2.41 | bcd | 1.2 | ± | 0.08 | bcdef | 35.5 | ± | 2.47 | abcd | 50.9 | ± | 3.53 | bcd |
| 48 | 31.6 | ± | 3.52 | efg | 1.0 | ± | 0.05 | f | 28.3 | ± | 2.93 | cdef | 39.9 | ± | 3.74 | bcdef |
| SW 1:500 | 0 | 40.6 | ± | 1.98 | bcd | 1.2 | ± | 0.08 | bcdef | 40.7 | ± | 2.23 | a | 49.8 | ± | 2.90 | bcd |
| 6 | 34.1 | ± | 3.42 | def | 1.1 | ± | 0.06 | ef | 35.2 | ± | 3.65 | abcd | 41.8 | ± | 3.07 | bcdef |
| 12 | 40.6 | ± | 2.52 | bcd | 1.2 | ± | 0.07 | cdef | 27.9 | ± | 2.88 | def | 37.4 | ± | 2.68 | bcdef |
| 24 | 43.3 | ± | 2.07 | abc | 1.5 | ± | 0.11 | a | 36.2 | ± | 2.58 | abc | 54.0 | ± | 2.48 | b |
| 48 | 46.5 | ± | 2.02 | ab | 1.3 | ± | 0.09 | abcde | 39.8 | ± | 2.49 | a | 54.0 | ± | 2.56 | b |
| SW 1:1000 | 0 | 40.9 | ± | 1.40 | bcd | 1.4 | ± | 0.09 | abc | 37.5 | ± | 2.32 | ab | 54.3 | ± | 1.96 | b |
| 6 | 38.5 | ± | 2.43 | cde | 1.5 | ± | 0.12 | ab | 25.3 | ± | 2.86 | efg | 41.9 | ± | 3.14 | bcdef |
| 12 | 35.1 | ± | 3.31 | def | 1.3 | ± | 0.10 | abcdef | 32.9 | ± | 2.63 | abcdef | 46.4 | ± | 4.35 | bcde |
| 24 | 39.7 | ± | 1.82 | bcd | 1.2 | ± | 0.07 | cdef | 33.4 | ± | 2.89 | abcde | 53.6 | ± | 2.51 | bc |
| 48 | 49.9 | ± | 2.61 | a | 1.1 | ± | 0.06 | cdef | 37.7 | ± | 2.31 | ab | 57.5 | ± | 2.40 | a |
| SW 1:1500 | 0 | 22.7 | ± | 2.67 | hi | 1.2 | ± | 0.07 | bcdef | 22.2 | ± | 2.39 | fgh | 36.4 | ± | 2.85 | cdef |
| 6 | 17.5 | ± | 1.81 | i | 1.1 | ± | 0.05 | ef | 16.0 | ± | 1.16 | h | 24.8 | ± | 2.25 | f |
| 12 | 21.5 | ± | 2.63 | hi | 1.1 | ± | 0.07 | def | 19.0 | ± | 1.45 | gh | 31.6 | ± | 2.18 | ef |
| 24 | 26.5 | ± | 2.86 | gh | 1.2 | ± | 0.08 | bcdef | 30.0 | ± | 3.03 | bcdef | 43.9 | ± | 2.85 | bcde |
| 48 | 16.0 | ± | 1.89 | i | 1.1 | ± | 0.06 | cdef | 18.9 | ± | 2.03 | gh | 31.4 | ± | 2.20 | ef |

SW conc = smoke-water concentration

In each column, different letter(s) following mean values indicate significant differences (*p* ≤ 0.05) based on Duncan’s Multiple Range Test.

Table 2

Effect of soaking time and smoke-water treatments on the growth of *Tulbaghia violacea* seedlings

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | | Growth parameters | | | | | | | | | | | | | | | | | | | |
| SW conc | Time (h) | Shoot length (mm) | | | | Root number (#) | | | | Root length (mm) | | | | Plant fresh weight (mg) | | | | Leaf number | | | |
| 0 | 0 | 60.4 | ± | 1.82 | a | 1.0 | ± | 0.00 | e | 36.9 | ± | 2.80 | abc | 73.4 | ± | 3.50 | cdef | 2.2 | ± | 0.07 | abcd |
| 6 | 55.0 | ± | 1.65 | ab | 1.2 | ± | 0.07 | bcde | 30.6 | ± | 1.79 | bcde | 76.2 | ± | 2.51 | bcde | 2.0 | ± | 0.05 | d |
| 12 | 48.8 | ± | 2.50 | c | 1.0 | ± | 0.00 | e | 32.6 | ± | 1.67 | abcde | 67.2 | ± | 3.42 | ef | 2.0 | ± | 0.03 | cd |
| 24 | 60.1 | ± | 1.96 | a | 1.4 | ± | 0.09 | ab | 33.7 | ± | 2.56 | abcde | 82.0 | ± | 2.75 | abcd | 2.2 | ± | 0.09 | abcd |
| 48 | 59.0 | ± | 1.82 | ab | 1.2 | ± | 0.07 | bcde | 29.4 | ± | 2.32 | cde | 67.0 | ± | 2.26 | ef | 2.2 | ± | 0.07 | abcd |
| SW 1:500 | 0 | 58.8 | ± | 1.80 | ab | 1.1 | ± | 0.06 | de | 28.3 | ± | 1.97 | de | 63.3 | ± | 2.77 | f | 2.0 | ± | 0.00 | d |
| 6 | 52.5 | ± | 2.06 | bc | 1.4 | ± | 0.09 | ab | 29.5 | ± | 2.67 | cde | 78.7 | ± | 4.48 | bcd | 2.3 | ± | 0.09 | a |
| 12 | 60.1 | ± | 2.04 | a | 1.1 | ± | 0.05 | e | 29.8 | ± | 2.17 | cde | 83.4 | ± | 2.79 | abc | 2.2 | ± | 0.07 | abcd |
| 24 | 58.2 | ± | 1.89 | ab | 1.1 | ± | 0.06 | de | 35.6 | ± | 2.91 | abcd | 71.9 | ± | 2.44 | def | 2.1 | ± | 0.05 | bcd |
| 48 | 57.9 | ± | 2.42 | ab | 1.1 | ± | 0.06 | cde | 30.6 | ± | 2.11 | bcde | 71.5 | ± | 3.09 | def | 2.0 | ± | 0.03 | cd |
| SW 1:100 | 0 | 61.5 | ± | 1.57 | a | 1.2 | ± | 0.07 | bcde | 38.1 | ± | 2.67 | ab | 77.4 | ± | 2.49 | bcde | 2.1 | ± | 0.06 | bcd |
| 6 | 55.9 | ± | 1.88 | ab | 1.3 | ± | 0.09 | abc | 33.8 | ± | 2.98 | abcde | 85.2 | ± | 4.65 | ab | 2.4 | ± | 0.09 | a |
| 12 | 55.4 | ± | 1.79 | ab | 1.1 | ± | 0.06 | cde | 26.8 | ± | 1.32 | e | 84.2 | ± | 3.33 | ab | 2.2 | ± | 0.07 | abcd |
| 24 | 58.7 | ± | 2.49 | ab | 1.3 | ± | 0.09 | abcd | 34.4 | ± | 3.16 | abcde | 76.8 | ± | 3.45 | bcde | 2.1 | ± | 0.05 | bcd |
| 48 | 57.8 | ± | 2.02 | ab | 1.1 | ± | 0.06 | de | 32.7 | ± | 2.40 | abcde | 72.6 | ± | 2.80 | def | 2.1 | ± | 0.05 | bcd |
| SW 1:1500 | 0 | 58.7 | ± | 2.14 | ab | 1.4 | ± | 0.09 | a | 28.0 | ± | 2.79 | de | 73.2 | ± | 3.56 | cdef | 2.2 | ± | 0.08 | abc |
| 6 | 59.9 | ± | 1.95 | a | 1.4 | ± | 0.09 | ab | 40.1 | ± | 2.24 | a | 86.1 | ± | 2.73 | ab | 2.3 | ± | 0.08 | ab |
| 12 | 61.5 | ± | 2.11 | a | 1.1 | ± | 0.05 | e | 29.5 | ± | 2.40 | cde | 90.6 | ± | 3.67 | a | 2.2 | ± | 0.08 | abc |
| 24 | 60.9 | ± | 1.68 | a | 1.2 | ± | 0.07 | bcde | 40.0 | ± | 2.02 | a | 80.4 | ± | 3.50 | abcd | 2.3 | ± | 0.08 | ab |
| 48 | 60.8 | ± | 1.63 | a | 1.0 | ± | 0.00 | e | 34.8 | ± | 1.95 | abcde | 81.2 | ± | 2.35 | abcd | 2.1 | ± | 0.05 | bcd |

SW conc = smoke-water concentration

In each column, different letter(s) following mean values indicate significant differences (*p* ≤ 0.05) based on Duncan’s Multiple Range Test.