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# Herbicidal Activity of Smoke Water

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**Abstract:** Weeds cause more crop yield loss and increase farmers' production costs more than any other agricultural pest worldwide. Natural extracts can be an important alternative to synthetic herbicides, or they can be one of the resources from which to discover new pesticide leads. The phytotoxic potential of smoke water was evaluated regarding germination and initial growth, prospecting for its possible herbicidal activity in weeds. Herbicidal activity was evaluated through germination, initial growth, and seedling vigor index bioassays in the laboratory and emergency with initial development bioassays in a greenhouse with smoke water solutions at 2.5, 5, 10 and 20% *v*/*v*. Experiments with two treatments were analyzed using T-tests for the parametric data and the Mann–Whitney test for the non-parametric data ($p < 0.05$). Experiments with three treatments or more were analyzed with a one-way ANOVA test followed by a Tukey test for the parametric data and a Kruskal–Wallis test followed by a Dunn test for the non-parametric data ($p < 0.05$). Linear regression was used to analyze data from the time–injury curve. The greatest effect on germination suppression (98%) was achieved when the *Amaranthus viridis* seeds were germinated in the laboratory with a 10% smoke water solution. Germination of *Raphanus raphanistrum* and *Digitaria insularis* was reduced by 93 and 75%, respectively, at this concentration. In greenhouse experiments, emergence of *A. viridis* was inhibited 81% by 20% smoke water. In laboratory initial growth experiments, 5% smoke water had the greatest inhibitory effect (94%) on *A. viridis*. *R. raphanistrum* initial growth reduction was 82%, *Urochloa decumbens* was 80%, *D. insularis* was 77% and *Emilia fosbergii* was 70% in the same conditions. In greenhouse development experiments, 70% of the *A. viridis* plants were killed by 5% smoke water treatment. These plants had 88% injury after treatment with 5% smoke water. Therefore, these findings suggest that smoke water solutions have potential as an herbicide, inhibiting the germination and initial growth of monocotyledonous and eudicotyledonous weeds. However, field tests are needed to confirm the potential of smoke water as an herbicide.

**Keywords:** weed; injury; phytotoxic potential; smoke water



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## 1. Introduction

Weeds cause more crop yield loss and increase farmers' production costs more than any other agricultural pest worldwide. Weeds not only compete with plants for survival, but also serve as a shelter, food and reproductive site for pathogens, insects, mites, nematodes, rodents and other agricultural pests [1]. Thus, weed control with herbicides is essential to reduce labor costs and improve the yield of world food production [2]. In recent decades, a large volume of pesticides has been produced [3] and used to protect crops, but the overreliance of a few herbicides and the lack of rotation of their modes of action have led to the selection of herbicide-resistant weeds [4]. There is a trend of weed resistance to various herbicides that has been increasing over time [4]. Farmers are thus seeking alternatives that are ecofriendly and cost-effective [5].

Recently, some studies have reported that smoke water can stimulate or inhibit plant germination and development. The efficacy of plant-derived smoke extends beyond germi-

nation, and it can modulate somatic embryogenesis [6], flowering [7], photosynthesis [8], rooting [9], and yield in plants [10].

Smoke water or liquid smoke can be obtained industrially; it is produced by burning plant matter and capturing smoke in water, followed by decanting and filtration steps. Most plants, including weeds, are stimulated by smoke, especially eudicotyledonous species [11–13]. The use of smoke water has applications in ecological management, rehabilitation of disturbed areas, horticultural industries, crop production, and mainly organic farming systems [14].

Smoke contains hundreds of pyrolytic compounds [15], such as karrikins—molecules composed only of C, H, and O. In addition, they often are bicyclic, one ring being a pyran and the other a lactone, comprising a five-membered butenolide ring [11,16]. The first compound identified in smoke water was 3-methyl-2*H*-furo[2,3-c]pyran-2-one (KAR1), which is generally the most abundant and the most active in stimulating seed germination [11,16]. KARs remain in the soil for about 7 years [11]. KARs interact directly or indirectly with crucial phytohormones (i.e., abscisic acid, gibberellic acid, auxins and ethylene) [16,17]. KAR1 can stimulate germination of some species by concentrations as low as $1 \times 10^{-10}$ mol $L^{-1}$, which is similar in effectiveness to plant hormones [11,12]. However, it is worth noting that not all species responsive to smoke water are necessarily responsive to KAR1 [18], and the inhibitory effect of smoke water on the germination of seeds that respond positively to KAR1 has also been noted [19]. It is suggested that compounds in smoke water may have dual regulatory cues. At higher concentrations, compounds such as 3,3′,5,5′-tetramethylbenzidine (TMB) would inhibit germination, whereas they may stimulate germination at lower concentrations. However, plants respond more intensely to these stimulating compounds at low concentrations [20]. Plant responses to smoke water can vary considerably according to the test species and their respective sensitivity. Therefore, it is important to test different concentrations of smoke water on several plant species.

A better understanding of the role of smoke water in germination and the development of plants may help to develop some strategies for weed control measures. The main objectives of this study were to evaluate the phytotoxic potential of smoke water in different concentrations on germination and the initial growth of weeds in laboratory experiments, as well on emergence and seedling development in greenhouse experiments.

## 2. Materials and Methods

### 2.1. Study Seeds

The following species were evaluated: *Digitaria insularis* (L.) Fedde (sourgrass), *Rottboellia cochinchinensis* (Lour.) Clayton (itchgrass), *Urochloa decumbens* (Stapf) R.D. Webster (signalgrass), *Amaranthus viridis* L. (slender amaranth), *Bidens pilosa* L. (hairy beggartick), *Conyza canadensis* (L.) Cronquist (horseweed), *Emilia fosbergii* Nicolson (Florida tasselflower), *Mucuna pruriens* (L.) DC. (mucuna bean) and *Raphanus raphanistrum* L. (wild radish), the first three being monocotyledons and the other eudicotyledons. All seeds were obtained commercially. In the germination and emergence experiments, the tested species were *A. viridis*; *D. insularis* and *R. raphanistrum*. In the initial laboratory growth experiment, all species were tested. In the greenhouse experiments, the most responsive species in the laboratory were selected.

### 2.2. Test Solutions

The four different concentrations of smoke water used in this study were prepared by diluting 25, 50, 100 and 200 mL of Regen 2000® smoke extract in up to 1000 mL of distilled water (2.5, 5, 10 and 20% *v*/*v*). Higher concentrations were used under greenhouse conditions, as efficacy is usually lower than under laboratory conditions.

### 2.3. Germination Protocol

Four replications of 50 seeds were placed on a sheet of Whatman No. 1 filter paper in 9 cm Petri dishes. The filter paper was moistened with 5 mL of distilled water (control

treatment) or test solutions. The Petri dishes were wrapped in black plastic and placed in a germination chamber in the dark at 25 ± 1 °C for 24 h. Then, the seeds treated with smoke water solutions were transferred to Petri dishes with filter paper moistened with distilled water. The germination chamber was set for a 12 h photoperiod with a photosynthetic photon flux density of 112 ± 5 μmol $m^{-2}$ $s^{-1}$. The filter paper was kept moist throughout the experiment by adding distilled water. Germination was monitored every day for 30 days (d). Seeds were considered germinated when the radicle had emerged 1 mm. Mean germination time (MGT) was calculated by using the following equation: MGT = $\sum (n \times d)/N$, 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. Germination percentage (GP) was calculated by the following equation: GP = seeds germinated/total seeds × 100.

### *2.4. Emergence Protocol*

Five replications of 20 seeds were sown in red latosol in plastic pots (10 cm × 10 cm × 3 cm). Each pot was sprayed with 10 mL of distilled water (control treatment) or test solutions. The plastic pots were placed in a greenhouse. The soil was kept moist throughout the experiment by spraying 10 mL of water daily. The average temperature in the greenhouse was 25 °C. Emergence was monitored every day for 40 d. Seedlings were considered emerged when the shoot emerged 1 mm above the soil. Mean emergence time (MET) was calculated by using the following equation: MET = $\sum (n \times d)/N$, where n = number of seedlings emerged on each day, d = number of days from the beginning of the test, and N = total number of seedlings emerged at the end of the experiment. Emergence percentage (EP) was calculated by the following equation: EP = seeds emerged/total seeds × 100. Plants were evaluated for injury 10, 20, 30 and 40 d after start.

### *2.5. Initial Growth in Laboratory Protocol*

Seeds of weeds were previously germinated to 1 mm of radicle protrusion. Four replications with 12 to 25 germinated seeds were placed on a sheet of Whatman No. 1 filter paper in 9 cm Petri dishes. The filter paper was moistened with 5 mL of distilled water (control treatment) or 5% test solution. The filter paper was kept moist throughout the experiment by adding distilled water or test solution. The Petri dishes were placed in a germination chamber and subjected to a 12 h photoperiod with a photosynthetic photon flux density of 112 ± 5 μmol $m^{-2}$ $s^{-1}$ at 25 ± 1 °C. At the end, the seedlings were removed from the Petri dishes and blotted with the help of filter paper before measuring the root and shoot length. Initial growth was evaluated when the control plants reached approximately 5 cm.

### *2.6. Initial Development in Greenhouse Protocol*

Seeds of weeds were sown on a sheet of Whatman No. 1 filter paper in a 9 cm Petri dish. The filter paper was moistened with 10 mL of distilled water, and the Petri dishes were placed in a germination chamber and subjected to a 12 h photoperiod with a photosynthetic photon flux density of 112 ± 5 μmol $m^{-2}$ $s^{-1}$ at 25 ± 1 °C. When reaching 2 cm of shoot, the seedlings were removed from the Petri dishes and transplanted into plastic bags (10 cm × 20 cm) filled with soil, with 10 plants per treatment (one per bag). Plants were irrigated every day with 25 mL of water and grown in a greenhouse. The temperature varied between 14 and 32 °C, and the humidity varied between 53 and 78%. When the seedlings reached the 4-leaf stage, each one was sprayed with 5 mL of distilled water (control treatment) or test solutions, a sufficient volume to wet the leaf surfaces. After 10 d of spraying, another spraying was carried out with 5 mL of distilled water or test solutions. Plants were evaluated for mortality 10 d after the second spray. Injury was evaluated 10 d after the first and second spraying. The plants received injury scores according to the degree of injury, where 0% meant that they did not differ from the control, and 100% meant plant death.

### 2.7. Seedling Vigor Index in the Laboratory

Seedling vigor index (SVI) was calculated as SVI = [average shoot length (mm) + average root length (mm)] × average percentage germination. Data from the initial laboratory growth experiment were used to calculate the seedling vigor index. Germination data used in calculation not shown.

### 2.8. Statistical Analysis

The germination data of each treatment were arcsine transformed for results expressed in percentage. Experiments with two treatments were analyzed using a T-test for parametric data and a Mann-Whitney test for non-parametric data ($p < 0.05$). Experiments with three treatments or more were analyzed with a one-way ANOVA test followed by a Tukey test for parametric data and a Kruskal–Wallis test followed by a Dunn test for nonparametric data ($p < 0.05$). The data were analyzed using BioEstat statistical package (Release 5.0). Linear regression was used to analyze data from the time–injury curve. The data were analyzed using R statistical package (Release 4.2.2). Values presented in tables and figures are untransformed.

## 3. Results

### 3.1. Smoke Water Effects on Germination

The increase in smoke water concentration significantly decreased the germination percentage of all weeds. The highest germination reduction (98%) was achieved for *A. viridis* seeds germinated in the laboratory with 10% smoke water solution (Table 1). Germination of *R. raphanistrum* and *D. insularis* decreased by 93 and 75%, respectively, at that concentration (Table 1). MGT changed only for *A. viridis* seeds, which had an increase with a 5% concentration of smoke water (Table 1).

**Table 1.** Effect of smoke solutions on seed germination of weeds under a 12 h photoperiod at 25 °C.

| Treatment | Smoke Water (%) | Germination (%) [1] | MGT [2] (Days) [1] |
|---|---|---|---|
| *Amaranthus viridis* | 0 | 45 ±7 a | 2.6 ± 0.7 a |
| | 2.5 | 44 ± 13 a | 4.9 ± 1.0 ab |
| | 5 | 6 ± 5 b | 18.4 ± 4.0 b |
| | 10 | 1 ± 2 b | 20.0 * |
| *Digitaria insularis* | 0 | 36 ± 11 a | 2.7 ± 0.6 a |
| | 2.5 | 20 ± 9 ab | 3.4 ± 0.4 a |
| | 5 | 22 ± 11 ab | 4.6 ± 1.6 a |
| | 10 | 9 ± 3 b | 4.4 ± 1.0 a |
| *Raphanus raphanistrum* | 0 | 70 ± 12 a | 1.0 ± 0 a |
| | 2.5 | 76 ± 15 a | 1.3 ± 0.2 a |
| | 5 | 41 ± 7 b | 1.8 ± 0.4 a |
| | 10 | 5 ± 4 c | 4.2 ± 4.2 a |

[1] Significant differences are indicated in each column by different letter(s) according to one-way ANOVA test followed by Tukey for parametric data and Kruskal–Wallis test followed by Dunn for non-parametric data ($p < 0.05$); standard error (±); ($n = 4$). * Datum obtained from a single replica, once there was seed germination in only one replica. [2] Abbreviation: MGT, mean germination time.

### 3.2. Smoke Solutions on Emergence

In the emergence experiment, only *A. viridis* was significantly inhibited (80.7%) by 20% smoke water (Table 2). At this concentration, the MET was significantly increased for the same species and for *R. raphanistrum* (Table 2).

Smoke water at 20% concentration caused a 50% injury rate after 15 d of sowing and 90% injury rate after 25 d in *D. insularis* (Figure 1).

**Table 2.** Effect of smoke solutions on seed emergence of weeds in a greenhouse.

| Treatment | Smoke Water (%) | Emergence (%) [1] | MET [2] (Days) [1] |
|---|---|---|---|
| *Amaranthus viridis* | 0 | 26 ± 10 a | 8.4 ± 4.0 a |
| | 5 | 17 ± 10 ac | 10.6 ± 7.9 a |
| | 10 | 15 ± 11 ac | 7.2 ± 6.1 a |
| | 20 | 5 ± 6 bc | 25.2 ± 4.9 b |
| *Digitaria insularis* | 0 | 3 ± 34 a | 3.7 ± 1.1 a |
| | 5 | 2 ± 3 a | 3.0 ± 0.7 a |
| | 10 | 4 ± 4 a | 5.0 ± 2.8 a |
| | 20 | 6 ± 2 a | 4.0 ± 0.7 a |
| *Raphanus raphanistrum* | 0 | 55 ± 10 a | 5.1 ± 2.5 a |
| | 5 | 60 ± 14 a | 5.1 ± 3.8 a |
| | 10 | 51 ± 15 a | 4.9 ± 2.0 a |
| | 20 | 40 ± 7 a | 11.6 ± 2.3 b |

[1] Significant differences are indicated in each column by different letter(s) according to one-way ANOVA test followed by Tukey ($p < 0.05$); standard error (±); ($n = 5$). [2] Abbreviation: MET, mean emergence time.

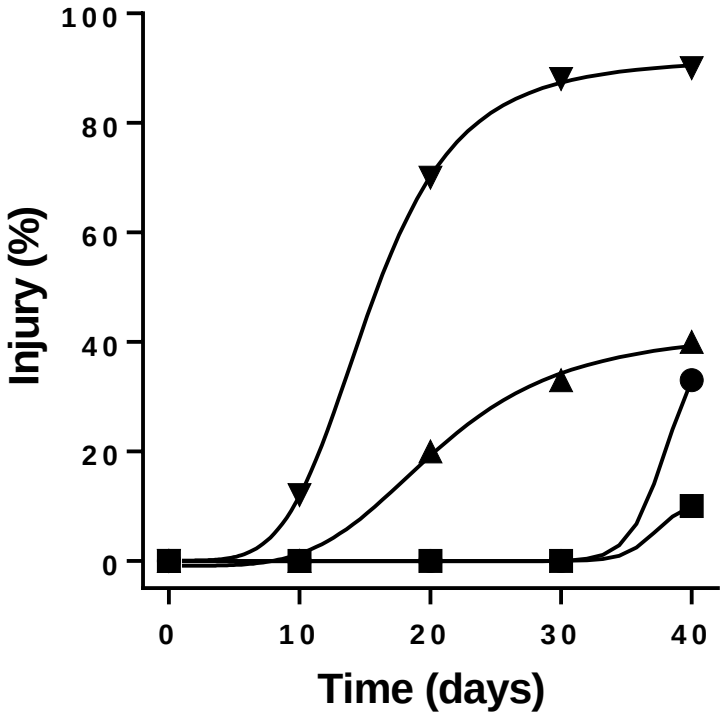


**Figure 1.** Injury rating curve (%) of different concentrations of smoke water in *D. insularis*. ● = 0%, ■ = 5%, ▲ = 10%, and ▼ = 20%. Linear regression was used to analyze data from the time–injury curve. These data were analyzed using R statistical package (Release 4.2.2).

### *3.3. Smoke Solutions Effects on Initial Growth in the Laboratory*

All weeds showed initial growth inhibited by smoke water at 5% concentration, except *M. pruriens*, which showed no effect (Table 3). Smoke water caused the highest inhibition (94%) in the length of root + shoot in *A. viridis*. The other species with initial growth reductions bigger than 70% were *R. raphanistrum* (82%), *U. decumbens* (80%), *D. insularis* (77%) and *E. fosbergii* (70%) (Table 3). In most cases, the roots had a slightly greater reduction in length than the shoots with smoke water (Table 3). *D. insularis*, *R. raphanistrum* and *U. decumbens* showed a reduction in the number of secondary roots when treated with 5% smoke solutions (data not shown).

**Table 3.** Effects of smoke solution on seedling growth of weeds under a 12 h photoperiod at 25 °C.

| Treatment | Smoke Water (%) | Root Length (mm) [1] | Shoot Length (mm) [1] | Root + Shoot Length (Mm) [1] |
|---|---|---|---|---|
| *Amaranthus viridis* (*n* = 25) | 0 | 29.1 ± 8.6 a | 18.2 ± 6.6 a | 47.3 ± 13.7 a |
| | 5 | 2.4 ± 1.7 b | 1.1 ± 0.8 b | 3.5 ± 2.3 b |
| *Bidens pilosa* (*n* = 14) | 0 | 21.1 ± 16.6 a | 16.4 ± 9.7 a | 37.6 ± 22.6 a |
| | 5 | 8.2 ± 6.2 b | 8.8 ± 6.1 b | 17.1 ± 11.0 b |
| *Conyza canadensis* (*n* = 12) | 0 | 5.3 ± 5.6 a | 3.9 ± 4.0 a | 9.2 ± 8.3 a |
| | 5 | 1.6 ± 0.8 b | 0.9 ± 0.5 b | 2.5 ± 1.2 b |
| *Digitaria insularis* (*n* = 46) | 0 | 41.5 ± 13.6 a | 2.7 ± 1.0 a | 44.2 ± 13.9 a |
| | 5 | 8.1 ± 6.3 b | 1.5 ± 0.8 b | 9.5 ± 6.8 b |
| *Emilia fosbergii* (*n* = 14) | 0 | 47.0 ± 11.2 a | 17.1 ± 3.2 a | 64.1 ± 12.7 a |
| | 5 | 14.7 ± 6.6 b | 4.5 ± 1.5 b | 19.2 ± 7.4 b |
| *Mucuna pruriens* (*n* = 113) | 0 | 64.1 ± 56.2 a | 34.3 ± 24.5 a | 98.4 ± 77.0 a |
| | 5 | 56.5 ± 54.2 a | 32.0 ± 22.7 a | 88.4 ± 72.6 a |
| *Raphanus raphanistrum* (*n* = 38) | 0 | 45.3 ± 40.6 a | 25.4 ± 14.4 a | 70.7 ± 49.4 a |
| | 5 | 6.4 ± 4.5 b | 6.5 ± 9.1 b | 12.9 ± 8.5 b |
| *Rottboellia cochinchinensis* (*n* = 41) | 0 | 35.8 ± 22.9 a | 30.1 ± 9.3 a | 65.9 ± 28.5 a |
| | 5 | 21.3 ± 23.0 b | 22.4 ± 8.2 b | 43.7 ± 26.4 b |
| *Urochloa decumbens* (*n* = 47) | 0 | 70.7 ±26.8 a | 7.2 ± 3.0 a | 77.9 ± 28.8 a |
| | 5 | 12.5 ± 14.9 b | 3.9 ± 2.5 b | 16.4 ± 16.6 b |

[1] Significant differences are indicated in each column by different letter(s) according to T test for parametric data and Mann–Whitney test for non-parametric data ($p < 0.05$); standard error (±).

### 3.4. Smoke Solutions on Initial Development in a Greenhouse

For *A. viridis*, injury rating increased significantly after the second spray of 5% smoke water, showing 88% injury (Table 4). *D. insularis* was less responsive, showing injury only with 10% smoke water, while *R. raphanistrum* was not responsive (Table 4).

**Table 4.** Percent injury on weeds with two applications of smoke solutions.

| Treatment | Smoke Water (%) | Injury Rating (%) [1] | |
|---|---|---|---|
| | | Ten Days after the 1st Spray [2] | Ten Days after the 2nd Spray [2] |
| *Amaranthus viridis* | 0 | 0 ± 0 aA | 3 ± 3 aA |
| | 5 | 39 ± 42 bA | 88 ± 24 bB |
| | 10 | 64 ± 35 bA | 83 ± 23 bA |
| *Digitaria insularis* | 0 | 0 ± 0 aA | 0 ± 0 aA |
| | 5 | 10 ± 22 aA | 21 ± 34 acA |
| | 10 | 38 ± 42 aA | 44 ± 46 bcA |
| *Raphanus raphanistrum* | 0 | 0 ± 0 aA | 0 ± 0 aA |
| | 5 | 11 ± 19 aA | 13 ± 23 aA |
| | 10 | 14 ± 19 aA | 16 ± 20 aA |

[1] The injury rate was evaluated 10 d after the first and second spraying. The plants received injury scores according to the degree of injury, where 0% meant that they did not differ from the control, and 100% meant plant death.[2] Lower-case letters indicate difference between treatments; capital letters indicate difference between times. The one-way ANOVA test followed by Tukey test for parametric data and the Kruskal–Wallis test followed by Dunn test for non-parametric data was used ($p < 0.05$); standard error (±); ($n = 10$).

Significant mortality was observed only for *A. viridis* plants; 70% of them died after treatment with 5% smoke water (Table 5).

**Table 5.** Mortality percentage caused by smoke solutions in weeds.

| Treatment | Smoke Water (%) [1] | Mortality Rate (%) [1] |
|---|---|---|
| *Amaranthus viridis* | 0 | 0 ± 0 a |
| | 5 | 70 ± 50 bc |
| | 10 | 60 ± 50 ac |
| *Digitaria insularis* | 0 | 0 ± 0 a |
| | 5 | 10 ± 30 a |
| | 10 | 30 ± 50 a |
| *Raphanus raphanistrum* | 0 | 0 ± 0 a |
| | 5 | 0 ± 0 a |
| | 10 | 0 ± 0 a |

[1] Significant differences are indicated in each column by different letter(s) according to Kruskal–Wallis test followed by Dunn test ($p < 0.05$); standard error (±); ($n = 10$).

### 3.5. Seedling Vigor Index in the Laboratory

The greatest reduction in seedling vigor index was in *A. viridis*; the negative control was 2270.4, while the treated was only 28.0. In *C. canadensis*, the negative control was 82.8, while the treated one was 1.3. In *R. raphanistrum*, the negative control was 4949.0, while the treated one was 528 (Table 6).

**Table 6.** Effect of smoke solutions on seedling vigor index of weeds under a 12 h photoperiod at 25 °C.

| Treatment | Smoke Water (%) | Seedling Vigor Index [1] |
|---|---|---|
| *Amaranthus viridis* | 0 | 2270.4 ± 99.3 |
| | 5 | 28.0 ± 24.5 |
| *Bidens pilosa* | 0 | 1725.0 ± 303.8 |
| | 5 | 799.0 ± 129.4 |
| *Conyza canadensis* | 0 | 82.8 ± 27.2 |
| | 5 | 1.3 ± 0.8 |
| *Digitaria insularis* | 0 | 1524.9 ± 83.8 |
| | 5 | 196.8 ± 51.0 |
| *Emilia fosbergii* | 0 | 3846.0 ± 182.2 |
| | 5 | 902.4 ± 85.2 |
| *Mucuna pruriens* | 0 | 8757.6 ± 309.1 |
| | 5 | 6726.0 ± 1305.0 |
| *Raphanus raphanistrum* | 0 | 4949.0 ± 660.0 |
| | 5 | 528.9 ± 92.9 |
| *Rottboellia cochinchinensis* | 0 | 1318.0 ± 223.1 |
| | 5 | 786.6 ± 222.1 |
| *Urochloa decumbens* | 0 | 2414.9 ± 417.2 |
| | 5 | 459.2 ± 242.7 |

[1] Seedling Vigor Index = [average shoot length (mm) + average root length (mm)] × average percentage germination; standard error (±).

## 4. Discussion

Smoke water caused a remarkable reduction in seed germination. Since seed germination is a crucial stage in plant establishment prior to successful growth and development [21], the results obtained reveal the potential of smoke water in weed control.

Studies have suggested that smoke water contains toxic or germination-inhibiting compounds [13,19], and that the 3,4,5-trimethylfuran-2(5*H*)-one (TMB) isolated from smoke water inhibits germination and reduces the stimulating effect of 3-methyl-2*H*-furo[2,3-c]pyran-2-one (KAR1) when applied simultaneously [22].

The present work confirms the findings of [23], in which high concentrations of smoke water (above 2%) were inhibitory to lettuce germination. Light et al. [20] point out that concentrations of smoke solutions higher than 1% inhibit germination and continuous exposure results pertaining to the gradual inhibition of germination.

In other studies, smoke solutions were inhibitory at higher concentration [24] or prolonged treatment [23], but they stimulated germination in the 1–2% dilution range [14]. Adkins and Peters [14] observed reduced germination and root damage in *Avena fatua* only at the highest concentrations—50 and 100% Regen 2000® smoke water, while we found the same effect at 10% concentration in *A. viridis*, *D. insularis* and *R. raphanistrum*. They found a 40% reduction in *Lamium purpureum* with 10% smoke water, while at the same concentration our reduction varied between 98 and 75%. It is important to highlight that both studies used smoking water of the same brand. These findings suggest that the inhibition of germination of different weeds depends on different concentrations of smoke water and exposure time, making clear the importance of the differential sensitivity of the species.

On the other hand, most studies have reported that smoke water stimulates seed germination at low concentrations. Adkins and Peters [14] reported a strong stimulating effect on weed seeds of three monocot species: *Alopecurus myosuroides*, *A. sterilis*, and *Phalaris paradoxa*; and one eudicot: *Malva neglecta*. Kandari et al. [25] observed that 0.1% smoke water increased 394% the germination of *Solanum viarum*, and Akeel et al. [21] reported a 58% increase in the germination of *Daucus carota* L. when treated with 51.6 μg $L^{-1}$ of smoke water. In the present study, we used higher concentrations and observed inhibition, which shows the importance of the concentration to obtain the desired response. Therefore, it may be thought that different concentrations of smoke solutions have different effects in different environments on different species [15].

Kandari et al. [25] observed that 0.1% smoke water reduced the MGT of *S. viarum* compared to the control. In the present study, however, TMG increased only for *A. viridis* seeds, while the others showed no statistical difference. The fact that *A. viridis* presents a reduction in germination and an increase in the time for the seeds to germinate is an advantage from a management point of view, as the weed species would lose the competitive lead for the crops [26].

In the emergence experiment, only *A. viridis* was significantly inhibited (81%) by 20% smoke water. Emergence was less inhibited than germination due to a probable reduction in the effect of smoke water, which was applied to the soil surface instead of soaking the seeds in the solution. At 20% concentration, MET significantly increased for the same species and for *R. raphanistrum*. Species which can occupy the available space later will be the weakest competitor [26]; in this case, the high MET is an advantage in terms of weed management. In the case of seeds that were able to emerge, smoke water at 20% concentration caused a 50% injury rate after 15 d of sowing and a 90% injury rate after 25 d in *D. insularis*. Therefore, smoke water may play an important role in the pre-emergence control of *A. viridis*.

In the laboratory, all weeds had their initial growth inhibited by smoke water at 5% concentration, except *M. pruriens*. Smoke water caused the greatest inhibition in the length of root + shoot in *A. viridis* (Table 3). The other species with an initial growth reduction bigger than 70% were *R. raphanistrum*, *U. decumbens*, *D. insularis* and *E. fosbergii* (Table 3). In most cases, the roots had a slightly greater reduction in length than the shoots.

However, studies have reported an increase in the length of plants treated with smoke water. Akeel et al. [21] observed that the application of 51.6 μg $L^{-1}$ of smoke water resulted in a 37% maximum increase in carrot length. Yaman and Basaran [15] noted a 63% increase in root length of *Lathyrus sativus* when treated with 75% smoke solution and a 124.3% increase in shoot length when treated at 100%. Kandari et al. [25] reported that smoke water (0.2%) improved (41%) the root length of *S. viarum*, whereas the same smoke water concentration caused a significantly greater (53%) shoot length compared to the control treatment. This phenomenon is called hormesis, where stress caused by

low doses of phytotoxins stimulates growth in plants [27,28]. The plant's growth stage, physiological state and environmental factors can influence the occurrence and magnitude of this phenomenon [28]. Most studies report that the hormetic response occurs when plants have a modest stimulatory response, typically about 30–60% above control values [28,29]. Therefore, most studies that report stimulatory responses caused by smoke water can be considered hermetic responses.

In their initial development in a greenhouse, *A. viridis*' injury rating increased significantly after the second spray of 5% smoke water, resulting in an 88% injury. *D. insularis* was less responsive, and injury was only observed with 10% smoke water, while *R. raphanistrum* was not injured at all. A significant mortality of the *A. viridis* plants (70%) was achieved with 5% smoke water. With the exception of this species, the effect of smoke water on the initial development of the plants was less pronounced in the greenhouse. Some reasons can be considered., such as the fact that plants at a more advanced stage of development may be less sensitive, and/or foliar absorption may have been less efficient than root absorption.

In this study, the smoke water clearly reduced the seedling vigor of the weeds. The greatest reduction was 99% in *A. viridis*, followed by 98% in *C. canadensis* and 90% in *R. raphanistrum*. However, studies have indicated an increase in the seedling vigor index treated with smoke solutions. Kandari et al. [25] observed an increase of 23% in SVI of *S. viarum* treated with 0.2% smoke water, a lower concentration than that tested in this work.

One study showed that the germination and the growth of seedlings in non-fire-prone environments can be enhanced by smoke water, and that stimulating impacts depend on the plant species used to prepare the smoke solutions [30]. In the present study, this variable can be disregarded in comparisons with studies that used Regen 2000®.

The inhibitory effects of smoke solutions are probably due to the acidity of the solution and the presence of numerous organic substances that may act as growth and development retardants [31]. Baldwin et al. [31] tested 233 compounds present in smoke water, of which 16 were inhibitory to germination.

## 5. Conclusions

These findings were discussed with the premise that smoke water may play an important ecological role in the management and control of introduced weeds in arable communities. Furthermore, the data suggest that smoke water has potential as a herbicide by inhibiting the germination and initial growth of monocotyledonous and eudicotyledonous weeds. However, field tests are needed to confirm the potential of smoke water as an herbicide.

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