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# Using smoke solutions in grass pea (*Lathyrus sativus* L.) to improve germination and seedling growth and reduce toxic compound ODAP

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**Abstract:** The effects of smoke solutions derived from wheat straw on the germination, seedling growth characteristics, and biochemical content of grass pea (*Lathyrus sativus* L.) were examined in this study. The smoke solutions were applied to petri and pot media at different concentrations. Both experiments were conducted in a randomized plot design with 3 replications. In the pot experiments, the smoke solutions were applied through the initial irrigation at 5 different concentrations (1%, 5%, 10%, 20%, and 40%) while 6 concentrations were used for petri experiments (0.5%, 1%, 2%, 4%, 8%, 10%) and results were compared to a control (distilled water). The smoke solutions increased root and shoot growth but decreased the germination rate and speed of grass pea in both media. In general, there were positive outcomes for the investigated traits with moderate concentrations of solution, but negative outcomes with low and high concentrations. The smoke solution treatments improved the shoot dry weighs, K, and protein contents, but decreased the acid detergent fiber and neutral detergent fiber contents and the toxic component oxalyldiaminopropionic acid (ODAP). Accordingly, the smoke solutions stimulated the development of grass pea and promising outcomes were achieved in terms of high protein and low ODAP contents, which are the main targets of grass pea breeding studies. It was concluded, based on the present findings, that smoke solutions derived from wheat straw could be used as an environmentally friendly practice in grass pea culture to increase yield and quality. However, it should be kept in mind that concentration was a critical issue for ensuring the expected outcomes.

**Key words:** Germination, grass pea, ODAP, root, shoot, smoke solution

## 1. Introduction

Some crops are highly tolerant to salinity, drought, and other environmental stressors. Thus, researchers have recently focused on these tolerant crops to ensure agricultural sustainability and food safety under the current trends of global warming and climate change (Massawe et al., 2016). In this sense, grass pea (*Lathyrus sativus* L.) has been classified among such tolerant crops. Grass pea (Fabaceae) is the most cultivated species of the genus Lathyrus and is grown as pulse, vegetable, and forage (Campbell, 1997). With remarkable tolerance to various stress factors, such as drought, flooding, salinity, insects, and diseases, and its good adaptation to a wide range of soil types and marginal areas, it offers great potential as a sustainable food supply (Girma et al., 2011).

The fossilized grass pea seeds discovered by archeobotanical excavations suggest that its use as food dates back to 8200–7500 BC in Turkey and Iraq (Rybiński et al., 2006). Hence, grass pea is considered to be among the earliest crops domesticated by humans. Despite quite a deep history, notable yield stability, protein content, and high adaptation to adverse agricultural conditions (Aletor et al., 1994; Basaran et al., 2016; Zhelyazkova et al., 2016), grass pea cultivation has not been widespread and has even decreased throughout the world (Kumar et al., 2011).

The major factor restricting grass pea cultivation is the presence of the toxin β-L-oxalyl-2.3-diaminopropionic acid (β-ODAP), mainly in seedlings and seeds, and less so in other tissues (Zhao et al., 1999). The toxin can cause irreversible paralysis in humans and animals when consumed in large quantities for long periods (Mehta et al., 1994; Wang et al., 2000). After the first detection of the toxin by Rao et al. (1964), all efforts to determine ODAP-free grass pea genotypes have been unsuccessful (Hanbury et al., 1999; Kumar et al., 2011). However, in many studies, a strong genotype × environment interaction was identified for ODAP synthesis in grass pea and increasing compound quantities were reported under different stress conditions (Yan et al., 2006; Polignano et al., 2009). Therefore, the determination of appropriate

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growing conditions or agronomic processes could provide significant contributions to reducing the level of the toxin in grass pea.

More recently, the positive effects of smoke and smoke product-derived plant materials on germination and plant growth have been reported (Govindaraj et al., 2016). It was reported that smoke components encouraged germination (De Lange and Boucher, 1990) and played an important role in the breakdown of dormancy (Light et al., 2002, 2004; Van Staden et al., 2004; Ghebrehiwot et al., 2011). It was also reported in previous studies that smoke promoted seedling growth (Demir et al., 2012; Ghebrehiwot et al., 2012), chemical stress tolerance (Aslam et al., 2014; Waheed et al., 2016), and enzyme activity (Demir et al., 2012).

Plant-derived smoke solutions can be produced from all plants. In the present study, wheat straw was used. In Turkey, wheat is grown in large areas, and the straw remaining in the field after harvest poses problems for tillage and plant growing. In some regions, burning is the common practice to deal with straw, which results in many ecological threats to the soil, microorganisms, and wildlife. Hence, usage of the wheat straw for smoke solutions is an environmentally friendly system and may contribute to solving this ecological problem (straw burning), which threatens agricultural sustainability in the wheat belt in Turkey.

In the literature, no comprehensive studies about the potential effects of smoke treatments on grass pea were found. Thus, the current study was conducted to determine the effects of straw-derived smoke solutions on the germination, seedling growth, biochemical content, and especially ODAP content of grass pea.

## 2. Materials and methods

In this study, seeds of Gürbüz-2001, the first registered grass pea variety in Turkey, were used as the plant material. For the smoke solutions, wheat straw was collected from the province of Yozgat and the burning process was carried out using a special mechanism (Figure 1). Germination and seedling growth tests were performed under fully controlled climate room conditions at the Agriculture Faculty of Yozgat Bozok University in 2018.

### 2.1. Preparation of the smoke solution

A total of 5 kg of wheat straw was burned and the smoke was passed through 5 L of distilled water (Ghebrehiwot et al., 2009) using a special system. This process was maintained until the straw was completely burned. The liquid was then filtered through filter papers and used as the stock solution.

### 2.2. Preliminary experiment

In order to determine the appropriate concentration of smoke solution, initially, the effects of 8 different concentrations of stock solution (1%, 5%, 10%, 20%, 40%, 60%, 80%, and 100%) and distilled water (as the control) on the germination of grass pea in petri media were tested. It was observed that there was a serious adverse effect at concentrations greater than 10%. These data are not provided here. Based on these results, new concentrations were generated, but they were different for the petri and pot media. Higher concentrations were generated for the pot experiments, since the solution may have been less active in soil. The concentrations of smoke solution were set as 0.5%, 1%, 2%, 4%, 8%, and 10% for the petri experiments and as 1%, 5%, 10%, 20%, and 40% for the pot experiments.



**Figure 1.** Special system used to obtain the smoke solution.

### 2.3. Petri experiments

The sterilized grass pea seeds were kept in the smoke solutions for 18 h at room temperature and then washed thoroughly with distilled water 3 times. The prepared seeds were placed over rough filter papers in petri dishes with a diameter of 90 mm (20 seeds/petri dish). Following supplementation of 3 mL of distilled water, the petri dishes were isolated with Parafilm and then taken into the germination room. All of the treatments were carried out in 3 replicates in the dark and a constant temperature of 20 °C for 7 days. Counting was performed every day and seeds with roots of ≥2 mm were recorded as germinated (Hardegree and Van Vactor, 2000). Germination rates, root lengths, and shoot lengths were determined at the end of day 7. The germination speed and rate was determined with the following equations:

Germination speed: $= \Sigma(G_1/D_1 + G_2/D_2 + \ldots + Gn/Dn)$,

where $G$ is the number of germinated seeds on counting day and $D$ is counting day (Czabator, 1962).

Germination rate (%) = number of seeds germinated at the end of day 7/total number of seeds × 100.

### 2.4. Pot experiments

The field soil taken from the experimental fields of Yozgat Bozok University was dried, passed through a 4-mm sieve, and placed into plastic pots (20 × 30 × 5 cm) to 3 cm in height. After the seeds were placed (25 seeds/pot),

the pots were filled with the same soil at a 2-cm thickness over the seeds. Following seeding, the pots were irrigated with the smoke solutions up to field capacity and placed in the climate room. Subsequent irrigations were performed with distilled water. The experiments were conducted in 3 replicates.

The prepared pots were allowed to germinate and grow at a constant temperature of 25 °C and light/dark photoperiod of 16:8. In addition to the germination speed and rate, at the end of day 21, the average shoot length, root length, dry weight, ODAP, and protein content were determined. Protein and ODAP were investigated in plant samples dried at 65 °C for 24 h and ground to pass through a 1-mm sieve. Crude protein was determined using near infrared reflectance spectroscopy (Foss 6500) (Foss NIRSystems, Inc., Silver Spring, MD, USA) with IC-0904FE software. The amount of ODAP was determined by the o-phthalaldehyde method according to Rao (1978). Experimental data were subjected to analysis of variance according to a randomized plot design and the differences between the means were determined using Duncan's multiple comparison test.

## 3. Results

### 3.1. Petri experiment

The smoke solutions had significant effects ($P < 0.05$) on the germination and seedling development of grass pea. The greatest germination speed was obtained from the control solution, but it was placed in the same statistical group as 1% and 10% smoke solutions (Figure 2a). The lowest germination speed was detected with 8% smoke solution. Although there was an increase in the germination speed at the greatest concentration (10%), the values were still lower than in the control. The germination rate was also influenced by the smoke solutions (Figure 2b). The greatest germination rate (100%) was obtained from 10% smoke solution, but all of the treatments, except for 1%, were placed in the same statistical group.

The root and shoot lengths of the smoke solution treatments were also significantly different from each other. The root length reached the greatest value (7.03 cm) with 4% smoke solution, but it was placed in the same statistical group as the other treatments, except for the 2% smoke solution (Figure 3a). While the greatest shoot lengths were observed with 0.5% and 1% smoke solution (4.88 and 5.58 cm, respectively), the lowest values were observed with 4% and 10% (3.87 and 3.85 cm, respectively) (Figure 3b). However, an increase in concentration caused a decrease in shoot and root length.

### 3.2. Pot experiment

The effects of the smoke solutions on germination and seedling growth characteristics of grass pea were also found to be significant in the pot media (Figure 4). The smoke solutions generally decreased germination speed (Figure 4a) and germination rate (Figure 4b). The greatest germination speeds were observed with 5% smoke solution (15.28) and the control (14.33) and the differences between the other treatments were not significant. The germination rate was higher with the control (100%) than with all of the smoke solution concentrations. However, with the exception of 40% (90.67%) smoke solution, the other concentrations were in the same statistical group as the control in terms of germination rate.

Greater root and shoot lengths were determined with the smoke solutions than with the control. The root length was the highest with 10% and 5% smoke solution (16.44 and 16.03 cm, respectively), while the lowest (13.71 cm) was seen in the control (Figure 5a). The greatest shoot length (23.86 cm) was observed with 1% smoke solution, but it was almost the same with the control and medium concentrations (5% and 10%) (Figure 5b). Significantly lower root lengths were observed with 20% (21.74 cm) and 40% (21.62 cm) smoke solution. It was observed that smoke solutions at medium and low concentrations had a positive effect on the root and shoot lengths in the pot media, but such an effect significantly decreased with increasing concentrations, especially over 10% (Figure 5).

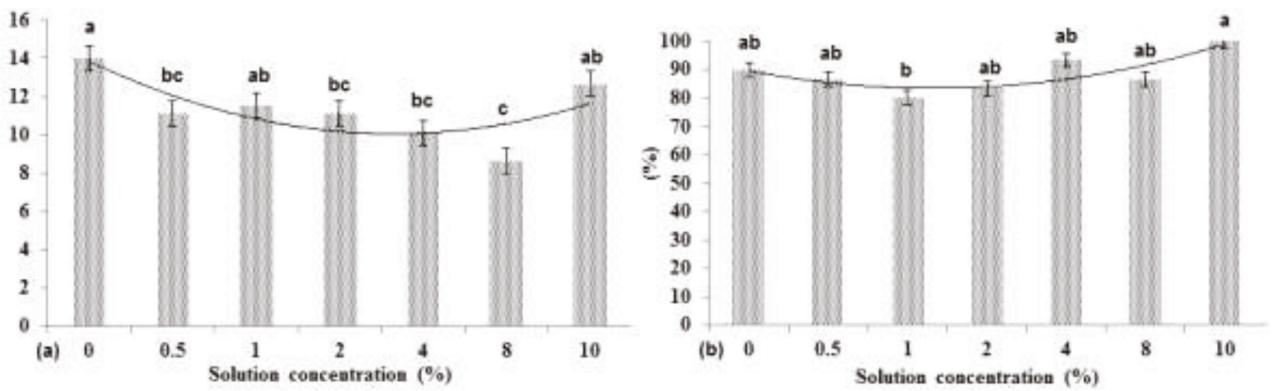



**Figure 2.** Effects of the smoke solutions on the germination speed (a) and germination rate (b) of grass pea in the petri media ($P < 0.05$).

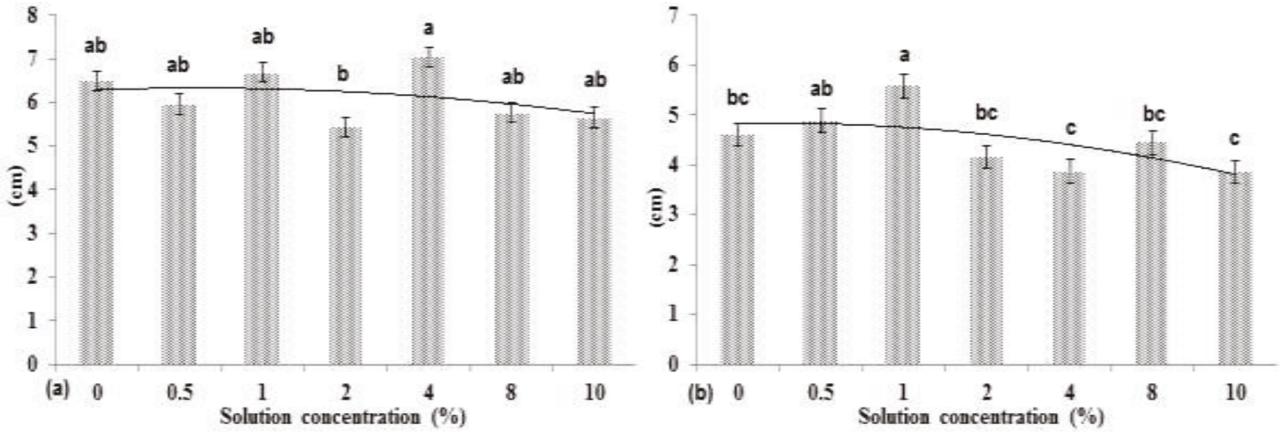



**Figure 3.** Effects of the smoke solutions on the root length (a) and shoot length (b) of grass pea in the petri media ($P < 0.05$).

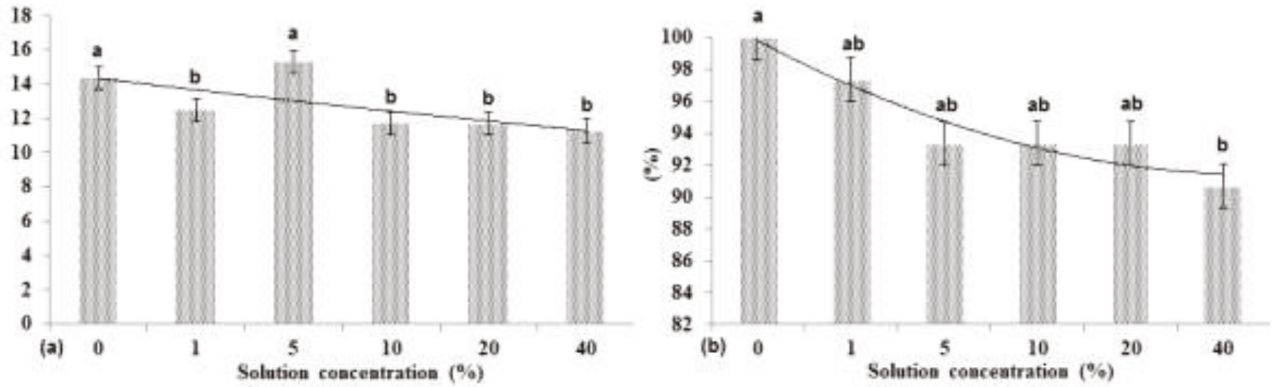



**Figure 4.** Effects of the smoke solutions on the germination speed (a) and germination rate (b) of grass pea in the pot media ($P < 0.05$).

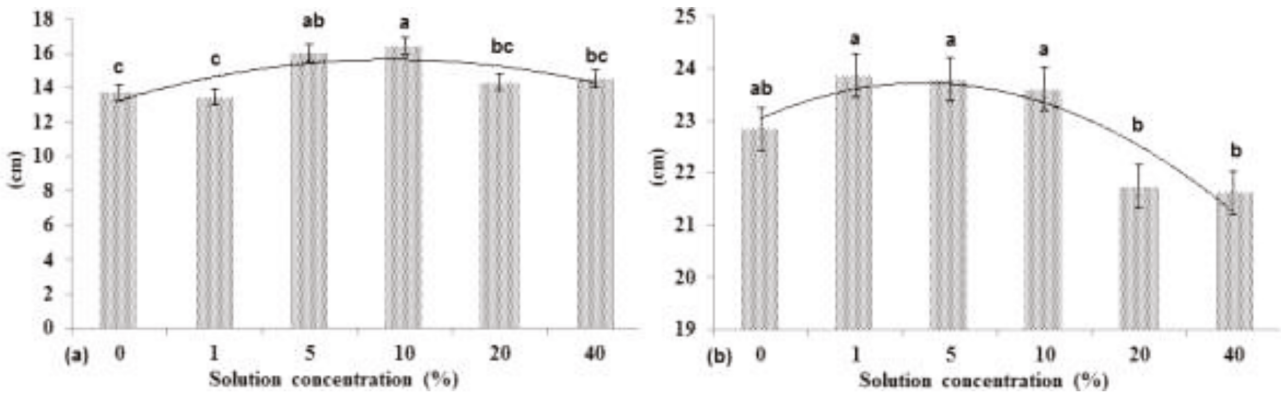



**Figure 5.** Effects of the smoke solutions on the root length (a) and shoot length (b) of grass pea in the pot media ($P < 0.05$).

Root and shoot dry weights also varied among the treatments but with no statistical significance. The root dry weight was higher in the control and was negatively influenced by all of the solutions depending on the increase (Figure 6a). On the other hand, the smoke solutions had positive impacts on shoot dry weight and all of the treatments had greater values than the control. However, shoot dry weight reached a peak with 10% smoke solution and began to decrease at higher concentrations (Figure 6b).

The smoke solutions (except for 1%) had significant positive effects on shoot width when compared to the control (Figure 7a). Accordingly, the greatest shoot width was recorded with 40% (1.84 mm) smoke solution, followed by 5%, 10%, and 20%, respectively.

The number of branches was also significantly influenced by the smoke solutions and reached the highest value (2.66) with 5% smoke solution (Figure 7b). However, 1%, 5%, and 10% smoke solution were placed in the same statistical group as the control. Concentrated smoke solutions limited the number of branches in grass pea and the lowest values (2.03 and 1.84) were recorded with 20% and 40% smoke solution, respectively.

Effects of the smoke solutions on the number of nodules were also significant, with the lowest number of nodules in the control (Figure 8a). The highest number of nodules (7.79) was recorded with 10% smoke solution; however, the difference in the number of nodules among the concentrations of smoke solution was not significant. The smoke solutions did not result in significant differences ($P < 0.05$) in the shoot protein content; however, they yielded greater protein contents than the control (Figure 8b).

Effects of the smoke solutions on the cell membrane components, acid detergent fiber (ADF) and neutral detergent fiber (NDF), and mineral contents (K, P, Ca, and Mg) of the grass pea seedlings were not significant (Table). When compared to the control, the smoke solutions decreased the ADF and NDF contents in the seedlings but increased the K and P contents. Among the smoke solutions, 10% yielded greater K content (3.51%). However, with the exception of 40% smoke solution for Ca, the smoke solutions yielded lower Ca and Mg contents than the control (Table).

Effects of the smoke solutions on the root and shoot ODAP contents were relatively similar (Figures 9a and 9b). In both organs, the ODAP content initially increased with the smoke solutions, reached the highest value with 5%, and started to decline at higher concentrations. It was determined that lower concentrations increased the shoot ODAP contents (Figure 9a). The greatest shoot ODAP content (9.05 mg $g^{-1}$) was recorded with 5% smoke solution, but it decreased with increasing concentrations, and the ODAP content with 10%, 20%, and 40% smoke solution was lower than that of the control (8.16 mg $g^{-1}$).

With all of the smoke solutions, especially 5%, 10%, and 20%, the root ODAP contents were higher than the control (1.60 mg $g^{-1}$) (Figure 9b). On the other hand, it appeared that there was a significant reduction in the root ODAP contents with 40% smoke solution, but the values were still greater than those of the control.

## 4. Discussion

Today, the growing need for agricultural products, climate change scenarios, and the contamination of natural resources with excessive chemical use require new approaches for supplying safe food. In this sense, sustainability in agriculture has become the main target and environmentally friendly practices have gained great significance instead of synthetic chemicals. Some neglected crops have also begun to come back into focus, especially to ensure food safety against global climate change (Massawe et al., 2016). One of these crops is grass pea (*Lathyrus sativus* L.), due to its great tolerance of biotic and abiotic stresses and wide range of environments.

Despite all of these superior properties, the toxic compound ODAP, found in seeds and green tissues, restricts the cultivation of the plant. There are significant changes in the ODAP concentrations of grass pea genotypes, ranging from 0.11% to 2.5% in seeds (Kumar et al., 2011). Moreover, strong genotype × environment interaction effects on ODAP have been determined (Kumar et al., 2011). In this sense, the identification of cultural practices that reduce ODAP content is of great importance for the agriculture of the plant.

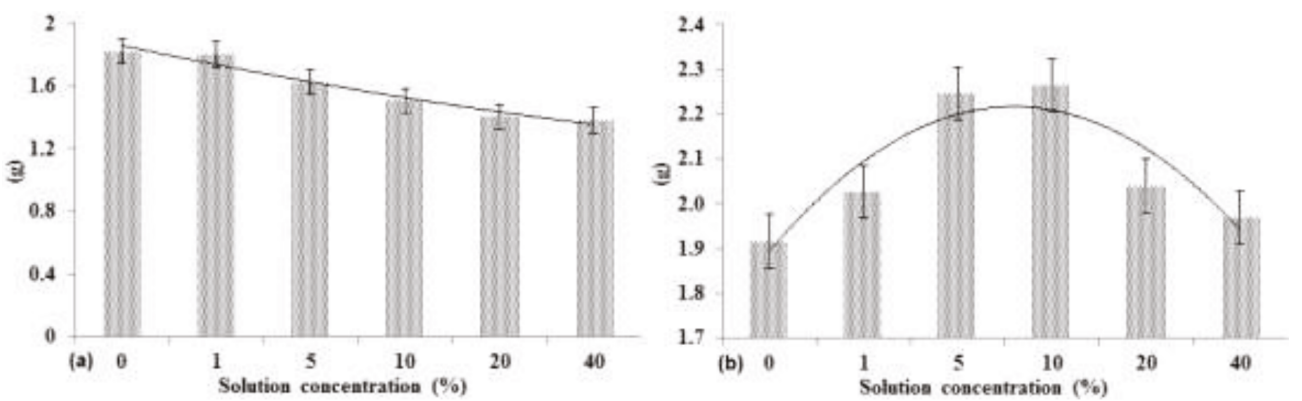



**Figure 6.** Effects of the smoke solutions on the root dry weight (a) and shoot dry weight (b) of grass pea in the pot media.

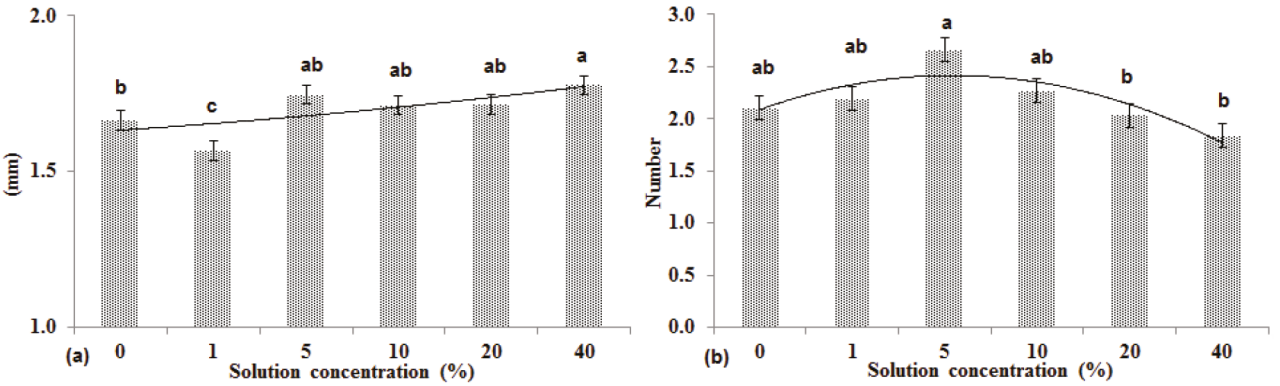



**Figure 7.** Effects of the smoke solutions on the shoot width (a) and branch number (b) of grass pea in the pot media ($P < 0.05$).

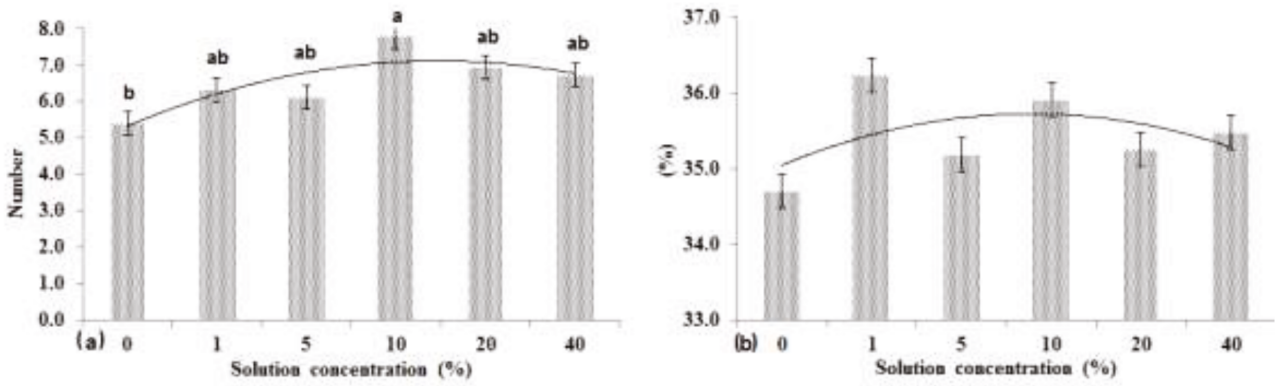



**Figure 8.** Effects of the smoke solutions on the nodule number (a) and shoot protein content (b) of grass pea in the pot media ($P < 0.05$).

**Table.** Effects of the smoke solutions on the ADF, NDF, and mineral matter contents of the grass pea seedlings in the pot media (end of day 21).

| Smoke solution (%) | ADF (%) | NDF (%) | K (%) | P (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|
| Control | 20.83 | 32.68 | 3.27 | 0.59 | 1.13 | 0.34 |
| 1 | 18.56 | 29.12 | 3.40 | 0.61 | 1.11 | 0.31 |
| 5 | 19.88 | 30.84 | 3.30 | 0.61 | 1.12 | 0.31 |
| 10 | 18.83 | 28.99 | 3.51 | 0.61 | 1.12 | 0.31 |
| 20 | 19.23 | 28.80 | 3.42 | 0.60 | 1.12 | 0.32 |
| 40 | 20.27 | 29.78 | 3.42 | 0.60 | 1.14 | 0.33 |

The use of organic fertilizers and herbicides in agriculture is recently among the important applications. Therefore, the demand for natural resources for sustainable agriculture has been increasing steadily (Iqbal et al., 2017). In this study, it was observed that the smoke solutions derived from wheat straw significantly affected the germination, seedling growth, and chemical composition of grass pea. The smoke solutions caused significant increases in the seedling growth in both petri and pot media depending on the concentrations. However, germination characteristics were depressed with the smoke solutions. In general, moderate concentrations were positive, while at low and high concentrations, negative effects were observed in the investigated traits of grass pea. Thus, it was concluded that the concentration of smoke solution played a critical role.

In both media, the smoke solutions adversely affected the germination rate and speed, but increased the root and

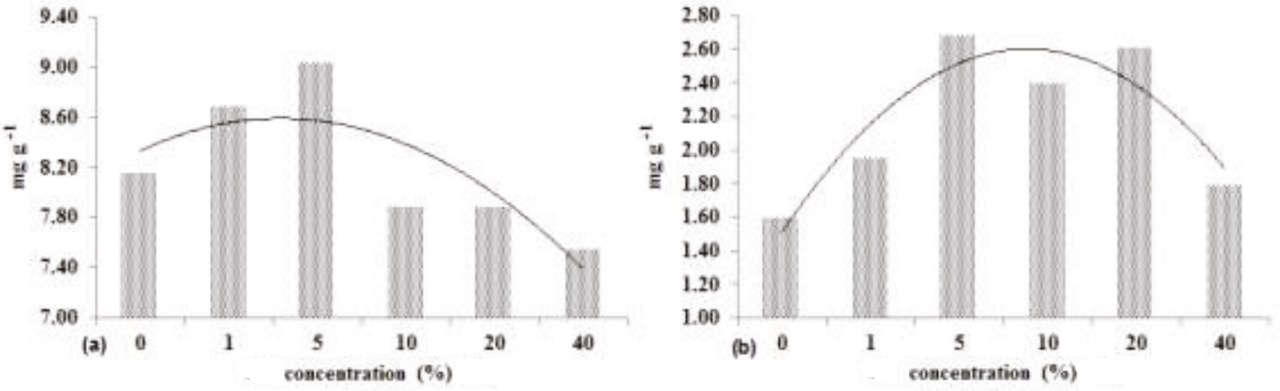



**Figure 9.** Effects of the smoke solutions on the ODAP content of the shoots (a) and roots (b) of grass pea in the pot media.

shoot lengths at different levels. The results revealed that the smoke solutions had negative effects at high doses, especially above 10%. Dry matter accumulation in the roots was negatively affected by all of the concentrations, while it was affected positively in the shoots. Moreover, the smoke solutions significantly increased the shoot width. This means that the smoke solutions led to shorter and thicker grass pea seedlings. Ghebrehiwot et al. (2013) reported that smoke improved the stem thickness of teff (*Eragrostis tef*) and suggested that smoke treatments might have significant contributions to overcome lodging problems in cereals. Similarly, Kulkarni et al. (2007) indicated that seedling growth in okra and tomato was positively influenced by smoke solutions and reported significant increases in shoot length, root length, dry weight, and number of leaves of okra, especially at a concentration of 1:500.

The number of branches was also significantly affected by the smoke solutions and such effects were negative at high concentrations, while not so much at low concentrations. Branch number is an important trait for grass pea (Campbell, 1997) due to its high positive association with seed yield (Basaran et al., 2013). In terms of nodule formation on the roots, one of the most important features of legumes, promising results were found with all concentrations of solution.

The positive and stimulating effects of smoke solutions on germination and plant development were also demonstrated in many previous studies (Ghebrehiwot et al., 2012; Kamran et al., 2013; Govindaraj et al., 2016). Smoke solutions were found to be ineffective in some plants (Catav et al., 2012) or toxic, particularly at high concentrations. Smoke contains some compounds that inhibit germination and thus has different effects on different species (Drewes et al., 1995). Similarly, morphological, physiological, and biochemical characteristics were influenced when smoke was applied to wheat seeds; some characteristics, such as root length, shoot length, leaf area, and chlorophyll content, were reported to increase significantly and the wheat genotypes had different responses to smoke solutions (Iqbal et al., 2017). The same authors also determined that short-term smoke application (1–2 h) yielded better results than long-term applications(3–4 h). In this sense, while the present findings on germination were different from the findings of earlier studies, the results on seedling development complied with the findings of previous studies. Negative findings in the present study may be attributed to the wheat straw. Ren and Bai (2016) reported that the germination and radical length of *Conyza canadensis* (L.) Cronquist increased after priming in concentrated smoke solutions derived from alfalfa, but decreased with the same concentrated smoke solutions made from wheat straw.

The smoke solutions did not significantly affect the fibrous structure (ADF and NDF contents) and mineral matter (K, P, Ca, and Mg) contents of grass pea. However, when compared to the control, the smoke solutions yielded lower NDF contents and greater K and P contents. Waheed et al. (2016) determined that priming with smoke solution increased the germination and root and shoot weights of maize seeds, and also alleviated the negative effects of salt stress and decreased Na contents, while increasing the photosynthetic activity and protein, Ca, and Mg contents.

The differences in the protein and ODAP contents of the grass pea seedlings were not found to be significant among treatments. However, when compared to the control, the smoke solutions yielded greater protein content in the shoots. The ODAP content in the shoots was higher than in the control with 1% and 5% smoke solution and it was lower at high concentrations (10%, 20%, and 40%). The increase in protein contents with the smoke solutions may be attributed to the improved chlorophyll content, as noted in previous studies (Ghebrehiwot et al., 2013; Waheed et al., 2016), and increased number of

nodules. On the other hand, the decrease in ODAP can be explained by the reduced negative effects of stressors by smoke solutions, as reported by Aslam et al. (2014) and Waheed et al. (2016). As a result, considering the fact that high protein and low ODAP contents are two of the most important goals of grass pea breeding, the healing effects of the smoke solutions on seedling growth, protein, and ODAP contents were very important and promising for grass pea culture.

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