



Plant-Derived Smoke Solutions as a Strategy to Alleviate ODAP Toxicity in Hydroponic Grass Pea

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ABSTRACT

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This study was performed to investigate how to smoke solution affects dry matter loss, crude protein (CP) and toxic compound ODAP contents of grass pea in hydroponic media during seven days period. Three doses of smoke solutions (1, 5 and 10%, V: V) by diluting with tap water were applied to seeds as priming agent, and tap water was used as a control (0%). The experiment was arranged according to the split-plot design with three replications. Compared to seed, significant change was detected in hydroponic sprouts depending on the day in terms of dry matter loss and chemical content. Grass pea sprouts had higher CP and lower ODAP content than seed, even in control treatment. Furthermore, smoke solution increased the efficiency of system by increase CP, decrease ODAP content and also decreases dry matter loss, with significant effect on doses. CP of sprouts increased day by day and was the highest on day 6 (36.27%) at the 10% dose of smoke solution followed by 5% on day 7 (36.09%). ODAP decreased up to day 6 and then began to increase, but it was lower than control at all doses of solution during to experiment. This study showed that grass pea is suitable crop for hydroponic forage produce and that smoke solution can make a significant contribution to yield and quality of sprouts, especially at the dose of 10%. However, when the yield, nutritional value, and safe use were evaluated together, it was observed that the best time for harvesting was the end of the 6th day and delaying did not provide a significant advantage and even occurred undesirable results in terms of ODAP and dry matter.

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Introduction

Developments in animal production increase the need for green feed day by day. Green feeds are essential for productivity, reproductive performance and product quality in animals, especially dairy animals (Dung et al., 2010). However, restrictions on water resources, the pressure on land for other crops and climatic problems make difficult to meet increasing demand. This situation leads the sector to more efficient and economical methods for green feed production, such as hydroponic forage.

The hydroponic forage technique, which is based on germination of grain for a certain period (mostly seven days) instead of being used directly in animal feeding, has attracted attention in recent years. Hydroponic systems could generate high yield per unit area using limited land, water, and no soil (Saha et al., 2016; Naik et al., 2017). The hydroponic forage technique enables the production of large of green feed from many forage seeds in a small area without use of chemicals and artificial growth promoters (Girma and Gebremariam, 2018). The hydroponic system

eliminates many of the problems or costs are seen in conventional methods such as soil preparation, weed control, post-harvest losses, labor requirements, harvest and fertilizers cost. It is possible to obtain 8-10 kg hydroponic forage in 7-8 days from one kg normal grain (Reddy, 2014). Hydroponic sprouts are rich in compounds such as minerals, crude protein, neutral detergent fiber, acid detergent fiber (Fazaeli et al., 2012; Kide et al., 2015; Mehta and Sharma, 2016), vitamin A, vitamin E, vitamin C, thiamine, riboflavin, niacin, biotin, free folic acid, β -carotene (Finney, 1982; Cuddeford, 1989) and bioactive enzymes (Chavan and Kadam, 1989), which have a great impact on reproduction and product quality in animals (Naik et al., 2015). Additionally, germination neutralizes the anti-nutritional factors such as phytate in the grains and enhances the beneficial enzymes (Girma and Gebremariam, 2018). However, soaking process activates enzymes that convert starch to simple sugar and results in 7-47% loss in DM from the original seed for a period of 6-

7 days due to respiration (Sneath and McIntosh, 2003; Dung et al., 2010). In hydroponic forage system, barley, oats, rye, triticale, wheat and maize grains (Naik et al., 2013; Heins et al., 2015) can be grown successfully. Recently, it was showed that grass pea (*Lathyrus sativus* L.) seeds are also suitable for hydroponic system and produce higher protein ratio and yield than wheat and barley (Dogrusoz, 2021).

Grass pea (*Lathyrus sativus* L.) is a legume, is the most cultivated species of the genus *Lathyrus* as pulse, vegetable and forage (Campbell, 1997) with the high seed protein content ranging from 26 to 30% in dry matter (Hanbury et al., 2000). It has also remarkable tolerance to various stress factors such as drought, flooding, salinity, insects and diseases, and adapts to wide range of soils (Girma et al., 2011). Despite its superior properties, grass pea has been reported to have toxic metabolites that limits the uses it in animal feed. The major factor restricting grass pea uses is the presence of the toxin β -L-oxalyl-2,3-diaminopropionic acid (ODAP), which can cause paralysis if it is consumed over long periods (Vaz Patto and Rubiales, 2014). It is found mainly in seedlings and seeds, and less in other tissues (Zhao et al., 1999). Since grass pea harvested in hydroponic system is physiologically at the seedling stage, its ODAP content may be high. In this sense, it is very important to know the ODAP content of grass pea in hydroponic production to safe use. Also, the daily change in ODAP content can be a useful factor in determining the optimum harvest time. As a common result of numerous studies, the most important constraint in the use of grass pea is the presence of ODAP. However, there are some promising developments in this regard. A recent study on 21-day-old grass pea seedlings reported that certain doses of plant-derived smoke solution improved growth, protein content, and reduced the toxic compound ODAP (Basaran et al., 2019). The promotive effects of plant-derived smoke and smoke products on germination and subsequent growth processes documented on many plants have been reported in different plants (De Lange and Boucher, 1990; Van Staden et al., 2004; Ghebrehiwot et al., 2012; Aslam et al., 2014; Govindaraj et al., 2016; Waheed et al., 2016).

These previous studies were the source of inspiration for the current study and, in this context, the objective of this study was to investigate how smoke solutions affect the protein and ODAP contents of hydroponic grass pea.

Material and Method

As a plant material, “Gürbüz 2001” variety of grass pea that registered in Türkiye was used. A smoke solution was derived from oat straw. Oat straw was completely burned and, smoke was collected in the water using a special system consisting of furnace, vacuum machine and large jar (Basaran et al., 2019). In total, 2 kg of oat straw smoke was kept through 4 L of distilled water. The liquid was then filtered through filter papers and used as the stock solution. Three different concentrations (1%, 5% and 10%) were prepared from the stoke solution by diluting with tap water.

Grass pea seeds were left to swell for 24 hours in smoke solutions and also in tap water as a control, then washed with tap water and placed back in the trays, and irrigation in the following days was done with tap water according to the need of the seedlings. The trial was continued for a total

of 7 days under hydroponic media ($22 \pm 2^\circ\text{C}$ constant temperature, 75% relative humidity and 14/10-hour light/dark conduction) and samples were taken at the end of each day for analysis. The experiment was arranged according to the split plot design with three replications with concentrations in the main plots and days in the sub plots.

In the trial lasted 7 days, treatments were daily taken and three characteristics were examined for each day. These characteristics; dry weight/fresh weight ratio (DFWR), crude protein (CP) and toxic compound ODAP (β -L-oxalyl-2,3-diaminopropionic acid) were determined in grass pea sprouts. For dry weight/fresh weight, after fresh weight was taken, the samples were dried at 70°C for 24 hours and weighed again. CP was determined by the Kjeldahl procedure by using the formula of N content $\times 6.25$ (Basaran et al., 2013). Quantitative determination of ODAP was determined by the o-phthalaldehyde (OPT) method according to Rao (1978). Experimental data were subjected to analysis of variance and differences between the means were determined using Duncan’s multiple comparison test. In addition, only the control treatments were analyzed according to the random block design in order to examine the daily changes of the examined features.

Results and Discussion

The daily change in dry weight/fresh weight ratio (DFWR) for only control treatments of the hydroponic grass pea in the environment is given in Figure 1. The effect of the day on DFWR was significant ($P < 0.01$). Over the seven days period, the DFWR decreased steadily from 56.25% to 26.05%. This result also points to dry matter decrease in grass pea during the germination process. However, the reduction in dry matter ratio cannot be as high as the reduction in DFWR. Because germinating grass pea seeds turned into sprouts with high water content day by day. Therefore, increasing water content of sprouts was a factor in the decrease in DFWR.

In hydroponic conditions, smoke solutions affected DFWR in grass pea and, the doses \times day interaction was significant ($P < 0.01$) (Figure 2). In the treatments where smoke solution was applied, the DFWR decreased continuously as with the control. However, this reduction was not as high as in the control. Smoke solution treatments reduced the dry matter loss in grass pea sprouts compared to the control, especially at the dose of 5%. The doses of 1% and 10% had similar DFWR to the control for first 4 days but higher afterward. On days 6 and 7, all the solution doses had higher DFWR than control (Figure 2).

The daily change in the crude protein content (CP) of hydroponic grass pea under the control treatment is given in Figure 3. Compared to seed (28.71%), CP increased significantly ($P < 0.01$) with germination and was highest at seven days of sprouts (35.01%). Compared to the control, the CP of hydroponic grass pea was significantly improved by the smoke solutions at certain doses (Figure 4). As determined in the control, the CP under solution treatments increased depending on time and, the day \times dose interaction was significant ($P < 0.01$). Among the solutions, the dose of 5% exhibited higher CP than the control on all days. Moreover, it was higher than the other solution doses on days 1, 3, 4 and 7. Exceptionally, a different situation

occurred on day 6 and the highest CP was detected at the 10% dose of the smoke solution.

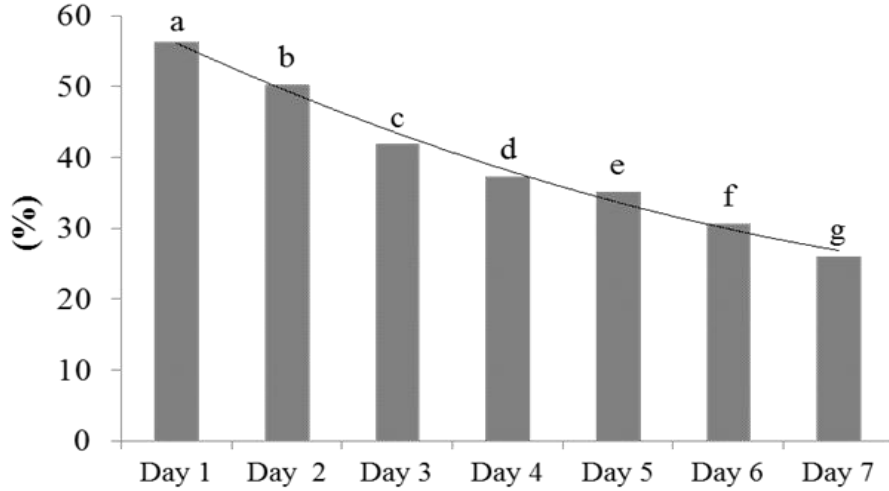


Figure 1. Daily change in dry weight/fresh weight of hydroponic grass pea sprouts under control treatment.

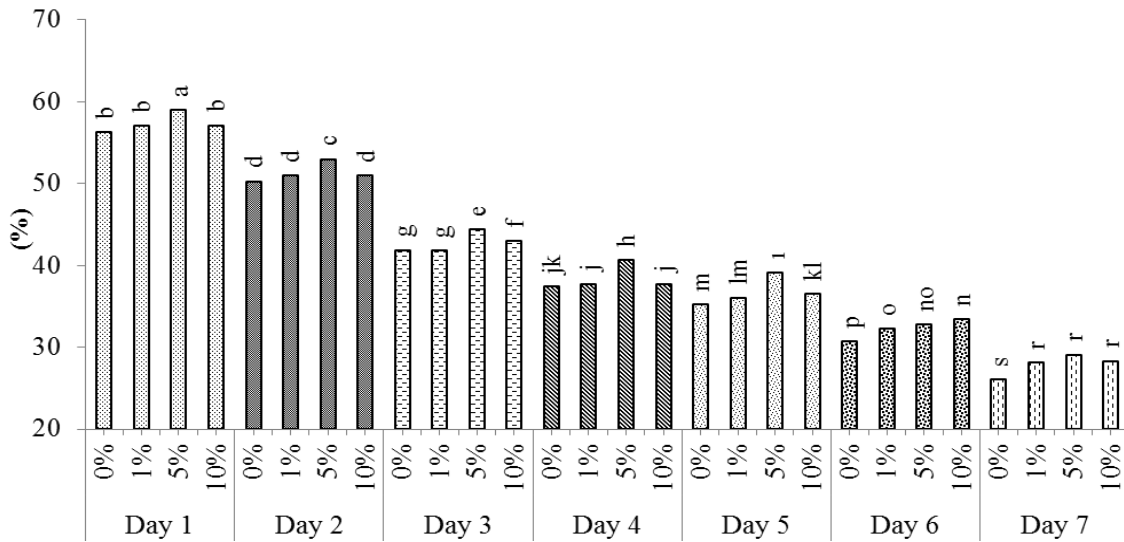


Figure 2. The effect of smoke solutions to daily change in dry weight/fresh weight ratio of hydroponic grass pea sprouts

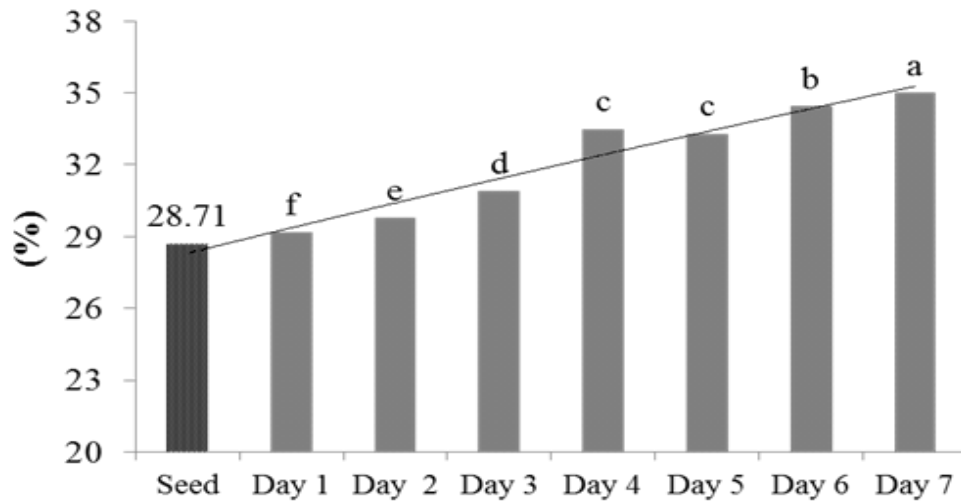


Figure 3. Daily change in crude protein content of hydroponic grass pea sprouts under control treatment.

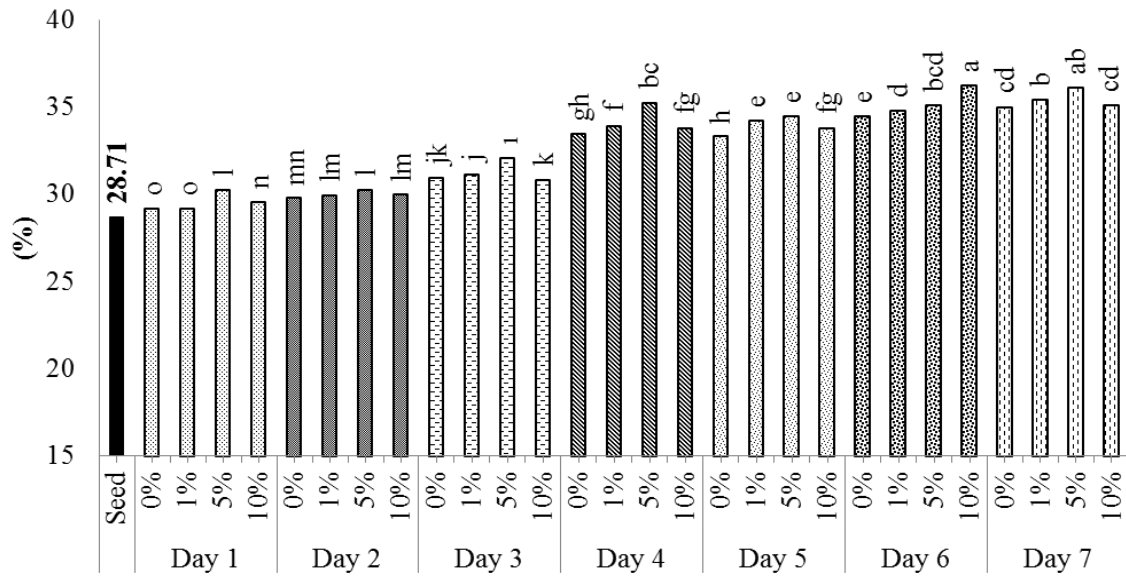


Figure 4. The effect of smoke solutions to daily change in crude protein content of hydroponic grass pea sprouts

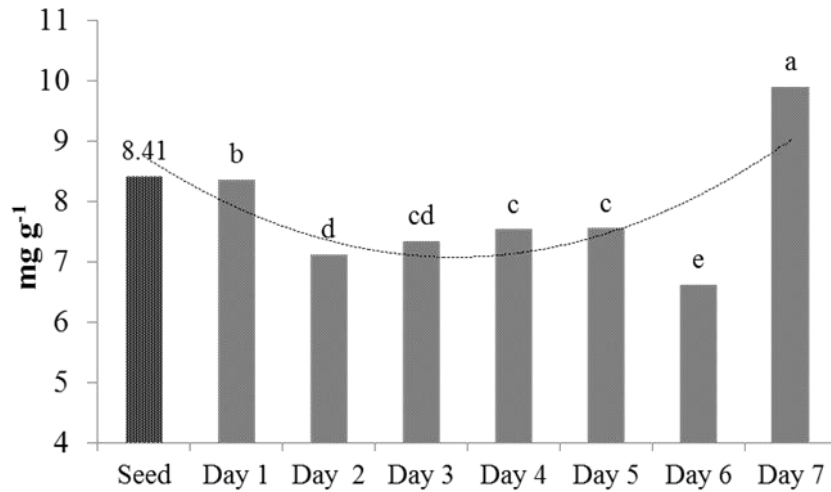
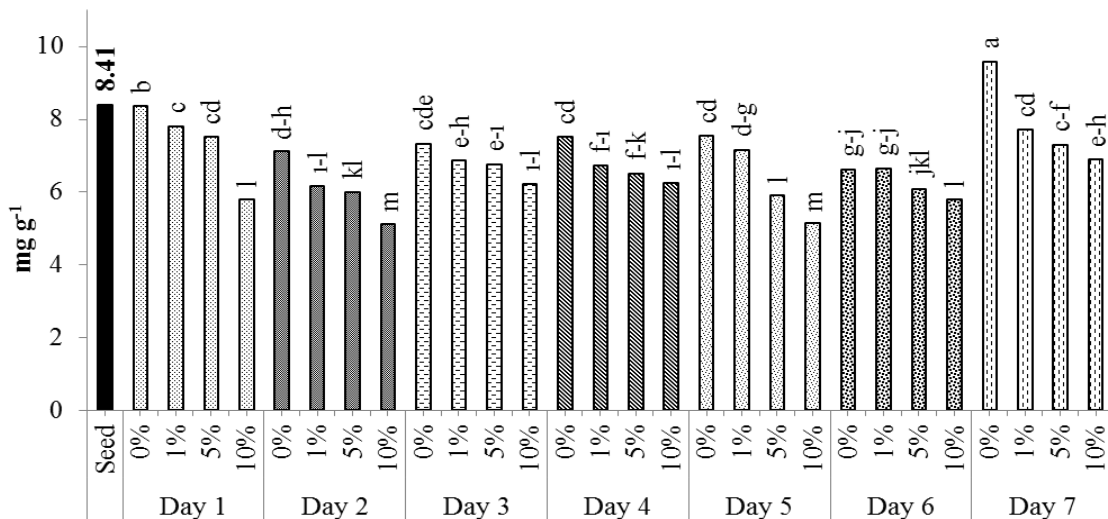


Figure 5. Daily change in ODAP content of hydroponic grass pea sprouts under control treatment



The ODAP content of grass pea seed was determined to be 8.41 mg g⁻¹. The result of the control treatment indicated that the effect of the day on the toxic compound ODAP in hydroponic grass pea was also significant (P<0.01) (Figure 5). Under hydroponic conditions, the

ODAP content of grass pea decreased significantly and was lower than that of the seed up to day 6. However, it increased significantly on day 7 and measured higher (9.89 mg g⁻¹) than that of the seed (Figure 5). The effect of smoke solutions was effective on ODAP. Smoke solution

treatment resulted in lower ODAP content than control at all doses (Figure 6). On the other hand, day x dose interaction was also found significant ($P < 0.01$). The effect of the doses on different days was interestingly similar and the ODAP content decreased the increase in dose. The lowest content of ODAP was determined in the 10% dose of solution on both separate and all days (Figure 6).

This study demonstrated that grass pea is an important alternative for hydroponic forage production, especially with its high protein content of sprouts (35-36%). Mostly grains such as barley, corn, wheat and sorghum are used for hydroponic feed production worldwide. In fact, many plants are suitable for this system, but it is essential that the plant to be used grows in that region and can be obtained easily and economically (Kumar et al., 2018). Grass pea is the most cultivated species of the *Lathyrus* genus and an important annual legume crop cultivated in several drought-prone areas over three continents (Campbell, 1997). In view of the climatic change pressure and concerns about sustainability, this plant can be made a great crop for drought-prone and marginal environment (Lambein et al., 2019). Drought tolerance, low input requirement, high grain yield, nutrient content and pod shattering resistance made grass pea an ideal crop for seed production (Basaran and Acar, 2013) and, its seeds are quality animal feed with 18–34% protein content (Rizvi et al., 2016). In this sense, grass pea may be a suitable option in the production of hydroponic forage worldwide. Dogrusoz et al. (2020) found that grass pea is superior to barley and wheat in the hydroponic environment in terms of dry matter ratio and protein increase and recommended that sprouts should be harvested at the end of 7th day for the highest protein content.

Although numerous advantages, the toxic ODAP in its content major obstacle to use of the grass pea (Yan et al., 2006). The concentration of ODAP in the seed can vary from 0.2 mg to 24 mg g⁻¹ (Kumar et al., 2011; Crews and Clarke, 2014) but the highest in seedlings (Zhao et al., 1999). So, ODAP in hydroponic grass pea is more critical than was in its seed or fodder. The attempts to improve genetically zero-ODAP grass pea have been unsuccessful (Kumar et al., 2011). Additionally, strong genotype x environment interaction has been observed for the production of ODAP and, increases particularly under drought stress (Fikre et al., 2010; Polignano et al., 2009). These results show that agronomic treatments are still the most viable way to reduce the ODAP content.

In a previous study, the observations made on 21-day-old grass pea seedlings showed that smoke solutions positively affect the seedling development and protein content as well as reduce the ODAP content (Basaran et al., 2019). Smoke solutions contain some bioactive metabolites such as butenoloid karrikinolide 1, karrikinolide 2 and burnt cellulose that are responsible for stimulating seed germination (Flematti et al., 2004; Van Staden et al., 2004; Gupta and Hrdlička, 2020). Therefore, smoke solutions can be expected to increase efficiency in the hydroponic media regarding growth and chemical content. Because, the efficiency of the hydroponic system depends on the germination and sprouting performance of the seeds during the first 7 - 8 days (Naik et al., 2015).

Results of this study have shown that hydroponically grown grass pea exhibited higher CP and lower ODAP

content compared to its seed. And smoke solutions improved the efficiency of the hydroponic system with higher CP and lower ODAP content and dry matter loss. In other words, the smoke solution contributed to hydroponic grass pea becoming a better quality and safer fresh fodder. The smoke solution significantly reduced dry matter loss in grass pea sprouts at all doses but especially by 5% (Figure 2). Dry matter loss in hydroponic forage is one of the common disadvantages mainly due to starch losses in the sprouting period (Fazaeli et al., 2012; Putnam et al., 2013). The seeds usually contain 85-87% dry matter while hydroponic feed is lower and ranges from 80 to 85% but, this disadvantage may be compensated by the increase in CP (Ndaru et al., 2020). The hydroponic technique resulted increase in CP, vitamin, mineral and crude fiber content (Lorenz, 1980; Rahman et al., 2019).

CP was improved by sprouting and high on all days with respect to seed (28.71%). The change in the CP of sprouts increased significantly depending on the day. In the control treatment, the CP of the grass pea sprouts was 29.19% on the first day and reached 35.01% on the seventh day (Figure 3).

Smoke solutions significantly promoted CP in grass pea sprouts compare to control (Figure 4). Moreover, this effect was observed over the 7 days, with the importance of dose x day interaction. Considering the experimental period, the CP of the grass pea sprout was the highest on the 6th day at the 10% solution dose (36.27%) and on the seventh day at 5% doses (36.09%). Previously, the positive effect of smoke solution on CP in grass pea (Basaran et al., 2019) and maize (Waheed et al., 2016) was reported and it was associated with an increase in chlorophyll content (Aslam et al., 2019). Also, it was reported that smoke solutions improved photosynthetic pigments, total nitrogen and total soluble proteins (Jamil et al., 2014), and alleviated the inhibitory effects of stressors such as heavy metal, drought, salinity, and high/low temperature (Akhtar et al., 2017) and have some kind of fertilizer effect on plant that increases the growth and stress tolerance (Khatoun et al., 2020). Verschaeve et al., (2006) stated that smoke solutions are safe in agriculture without any toxic effects on crops. However, the effect of smoke solution highly depends on doses and, adverse effect of high doses on seed germination has been determined (Daws et al., 2007; Light et al., 2002), which has been attributed to an inhibitor trimethyl butenolide in smoke solution (Light et al., 2010; Gupta and Hrdlička, 2020).

The hydroponic system itself reduced the ODAP content even in the control treatment, with lower values than the initial seed (8.41 mg g⁻¹) (Figure 5), and this effect significantly increased when the smoke solutions are applied (Figure 6). But the reduction in ODAP is only valid for the first 6 days. On day 7, the ODAP content of the sprouts started to increase in all treatments, even higher than of the seed in the control treatment. This situation indicates that time is a critical factor in ODAP. As a matter of fact, in the control treatment, it was the lowest on day 6 (6.62 mg g⁻¹) while the highest on day 7 (9.89 mg g⁻¹) (Figure 6). When smoke solutions were applied significantly lower ODAP contents were noted in grass pea sprouts compared to control moreover it was case on all the days. The decrease in ODAP was positively affected by the increase in dose and, the lowest content of ODAP was

detected the dose of 10% all days of experiment. This result indicates that the change in ODAP during sprouting is a result of biochemical and physiological mechanisms depend on time, and also that how importance of harvest time of sprouts. That the smoke solution causes a further reduction in ODAP can be attributed to its germination, growth-promoting, and stress-reducing effect, as described above.

Conclusion

The development of the livestock sector, especially dairy farming, constantly increases the demand for fresh feed. Increasing pressure on land and water resources, climatic and geographic difficulties make it difficult to meet this demand. This situation has forced the sector to seek more effective and sustainable feed production systems.

Hydroponic production can make a significant contribution to quality and sustainable fresh forage supply. The most basic component in the hydroponic system is the plant used. This study showed that grass pea can be used successfully in hydroponic system. When plant-derived smoke solutions were used, a significant improvement occurred in the efficiency and quality of hydroponic grass pea. In addition, the decrease in the ODAP content makes a significant contribution in terms of safe consumption. Doses of the solution and harvest time were also found to be important for total gain and mitigating the risk of ODAP toxicity. Plant-derived smoke solutions are organic growth promoter and not cause any hazard to nature. As a result, this study has shown that the integration of grass pea with smoke solution in hydroponic system can make a significant contribution to more efficient and environmentally-friendly green feed production.

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