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Growth performance, tolerance and vigor dynamics of *Salvia candidissima* subsp. *occidentalis* Hedge against heavy metal contamination

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Abstract: In this study, ecotoxicological and ecophysiological effects of some different heavy metal compounds ($CdCl_2$, $PbCl_2$, and $CuCl_2$) on *Salvia candidissima* subsp. *occidentalis* Hedge (Lamiaceae) were examined. Seeds of this plant were exposed to three different concentrations of $CdCl_2$ (2, 6, 10 ppm), $PbCl_2$ (50, 100, 500 ppm), and $CuCl_2$ (20, 60, 150 ppm). The results indicated that increasing $CdCl_2$ and $PbCl_2$ concentrations had no specific inhibitory impacts on seed germination rates, growth performance, biomass, and seedling vigor index, but increasing concentrations of $CuCl_2$ had significant inhibitory effects on these parameters. The metal tolerance index of all applications showed that all heavy metal treatments reduce this value.

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1. INTRODUCTION

Genus *Salvia* L. has 100 different species in Turkey, *Salvia candidissima* Vahl is a species which belongs to Group D and has two subspecies (Davis, 1985; Sen-Utsukarci et al., 2019). *Salvia candidissima* subsp. *occidentalis* Hedge is one of these subspecies. It differs from the other subspecies with smaller leaves, thick indumentum and white flowers (Figure 1 a, b). This taxon is widespread and common for Inner Anatolia, Turkey (Davis, 1985).

Genus *Salvia* has rich chemical compounds like the other members of its family. *Salvia* species is important as medicinal plants. There are some pharmacological studies about *S. candidissima* (Ulubelen and Topcu, 1998), but not any other studies about its ecological or ecophysiological characteristics. From the aboveground parts of *S. candidissima*, 3-oxosalvipisone, was acquired with 11β-hydroxymanoyl oxide, 8,13-diepimanoyl oxide, spathulenol, salvigenin, crysoeriol, diosmetin and *o*,*p*-dimethoxybenzoic acid (Ulubelen *et al.,* 1995). From the roots of *S. candidissima* subsp. *candidissima*, new diterpenes, new steroidal ester and α-amyrin acetate were insulated (Ulubelen *et al.,* 1997). In addition to diterpenoids, 11-hydroxy-12-methoxyabieta-8,11,13-trien and 1-oxosalvipisone, 14-oxopimaric acid, ferruginol, horminone, 7-acetylhorminone, cryptanol, montbretyl 12-methyl ether,

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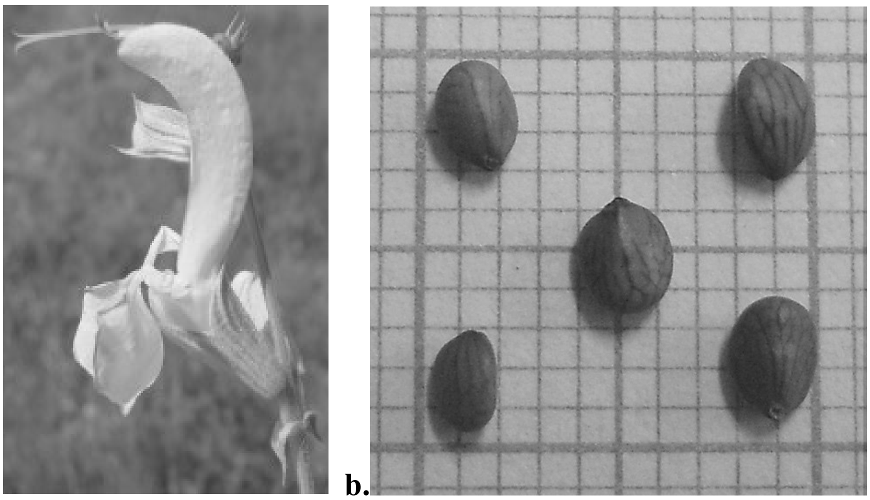
microstegiol, 1-oxoaethiopinone and salvipisone were obtained (Ulubelen *et al.,* 1992a), a new diterpene, candidissiol was insulated from the underground parts of *S. candidissima* (Ulubelen *et al.,* 1992b).

In consequence of increasing industrial human actions, releasing heavy metals to the nature (water, air and soil) has become a scientific matter. All components of the ecosystem are affected of heavy metal contamination in different levels. Heavy metal contamination of agricultural areas is a worldwide issue (Angelova *et al.,* 2017). Heavy metals are not degradable by biological pathways and permanent in the ecological environment continually (Singh & Prasad, 2011). Heavy metals can join the food chain after they are taken from the soil by plants. A deep understanding of metal uptake, transport, and defense processes in plants is required to improve metal uptake under metal-limiting conditions to increase vegetative productivity. (Gangwar *et al.,* 2014).

For plant species, cadmium is not an essential but a toxic element that can be actively extracted from the soil, high concentrations of cadmium can have adverse effects on the production and standard of the crop, as well as harm humans via the food chain (He *et al.,* 2008). Lead is an inherently consisting heavy metal element and main human origin basis of Pb contains metal mining activities, gem-dressing, and molding; pile fabrication; removal of lead containing outcomes without control processes, such as used up piles and computer products, etc. (Scheuhammer *et al.,* 2008). Copper element is an essential micronutrient for the life of plants, but it is thought to have a toxic effect by causing abiotic stress at high concentrations (Gill *et al.,* 2012).

The aims of this study were to observe the reactions of *S. candidissima* subsp. *occidentalis* (Fig 1) against pollution factors such as different heavy metal concentrations, like germination characteristics, shoot and root growth and production as biomass, seedling growth, seedling vigor index and metal tolerance index.

Figure 1. a. Flower and, **b.** Seeds of *Salvia candidissima* subsp. *Occidentalis.*



a. **b.**

2. MATERIAL and METHODS

The plant samples were collected from study area (Borabey Pond environs, Eskisehir Technical University) which is located at Eskisehir, Turkey. *S. candidissima* subsp. *occidentalis* seeds were used in this study. The seed germination experiments were performed out in MLR-350 Model Sanyo (Japan) plant growth chamber. During germination experiments, a photoperiod with 8 hours light/16 hours dark and stable temperature (+25°C) were applied (Yucel, 2000). Based on literature data and hazard levels, different concentrations of $CdCl_2$ (2, 6, 10 ppm), $PbCl_2$ (50, 100, 500 ppm) and $CuCl_2$ (20, 60, 150 ppm) were applied. Germination experiments were carried out in petri dishes (9 cm diameter) with two layers of circular filter papers and 25 seeds were sowed at each petri dish. Seed beds were filled with 9 ml of solutions including different heavy metal concentrations. Control groups were prepared with distilled water. The seed sowed beds kept into plant growth cabinet for 10 days. In order to accept the seed as germinated, the root tip must contact the germination bed (Yucel & Yilmaz, 2009). In the course of the experiments, controls were made every day at a specific time. End of the tenth day, hypocotyl and radicle lengths were measured by using rulers, then hypocotyls and radicles were separated to weigh each of them. For observing wet amounts of hypocotyl and radicle, fresh shoots and roots were measured directly without any application by ruler and for dry amounts of hypocotyl and radicle, shoots and roots were dried at 105°C, for 48 hours at sterilizer. Seedling vigor index (SVI) was calculated with respect to Murthy and Tejavathi (2016). Metal tolerance index (MTI) is calculated according to Turner and Marshall (1972).

The data obtained from experiments were tested statistically with the SPSS Statistics 20 package program, One-Way ANOVA Test with a p-value less than 0.05 considered statistically significant.

3. RESULTS

Seed germination experiments showed that increasing $PbCl_2$ and $CdCl_2$ concentrations has no significant effect on seed germination percentage (F=0.681; df=9.20; p>0.05), seedling growth (For hypocotyl F=10.079; df =9.20; p>0.05, for radicle F =11.558; df =9.20; p>0.05), biomass (For hypocotyl F =1.452; df =9.20; p>0.05, for radicle F =1.824; df=9.20; p>0.05) and SVI (F =1.815; df=9.20; p>0.05) value of *S. candidissima* subsp. *occidentalis,* but increasing $CuCl_2$ concentrations had an inhibitory effect of all ecophysiological parameters of *S. candidissima* subsp. *occidentalis* (Table 1).

Table 1. *Ecophysiological parameters observed after different heavy metal applications.*

Application		% Germination	Hypocotyl length (cm)	Radicle length	Hypocotyl biomass (mg)	Radicle biomass	SVI	MTI
Control		31	2.52	5.35	7.50	2.27	83.48	1.000
$CdCl_2$	2 ppm	39	2.80	4.99	5.77	2.63	113.67	0.708
	6 ppm	35	2.04	2.74	5.93	1.90	73.81	0.523
	10 ppm	44	2.84	3.70	12.13	4.03	126.19	0.586
$PbCl_2$	50 ppm	36	2.41	5.15	5.53	2.50	92.72	0.814
	100 ppm	44	2.25	4.10	9.50	2.90	104.46	0.760
	500 ppm	39	2.35	2.71	8.27	2.23	93.47	0.518
$CuCl_2$	20 ppm	44	2.12	1.49	11.57	2.97	93.62	0.233
	60 ppm	24	1.73	1.14	6.03	0.93	42.53	0.170
	150 ppm	24	1.02	0.60	5.77	0.87	26.70	0.097

Biomass data observed from this research showed that increasing $PbCl_2$ and $CdCl_2$ concentrations had no significant effect on biomass of *S. candidissima* subsp. *occidentalis,* but increasing $CuCl_2$ concentrations had an inhibitory effect on biomass of *S. candidissima* subsp. *occidentalis* (Table 1).

Results about root and stem growth of seedlings showed that increasing $CdCl_2$ concentrations had no significant effect on root and stem growth of seedlings of *S. candidissima* subsp. *occidentalis,* but increasing $PbCl_2$ and $CuCl_2$ concentrations had an inhibitory effect on root and stem growth of seedlings of *S. candidissima* subsp. *occidentalis* (Table 1).

MTI values of all heavy metal applications showed that all treatments decreased metal tolerance index value (F=48.845; df=9.30; p<0.05). MTI parameter is more critical than other ecophysiological data. The sensitivity of experiments can be examined through the metal tolerance index.

4. DISCUSSION

In this study, an examination of the ecophysiological effects of various concentrations of heavy metals which are $CdCl_2$ (2, 6, 10 ppm), $CuCl_2$ (20, 60, 150 ppm) and $PbCl_2$ (50, 100, 500 ppm) on *S. candidissima* subsp. *occidentalis* were investigated. Determining the "seed germination behavior" is essential to protect the natural habitats and gene pools of plant species, and supplying the incrasing request for plant-based outcomes can only be probable by conserving and growing natural plants (Yucel & Yilmaz, 2009).

It is known that elements and different solutions introduced to seeds have an impact on germination and seedling growth, especially, some macro or micro nutrition elements such as potassium or boron stimulates it. However, seed germination is lagged and detentioned concerning some toxic element concentrations like high percentages of iron or sulphur (Katkat and Kaçar, 2009).

Petrescu *et al.* (2014) observed that the cadmium decreased seed germination in *Salvia officinalis*, germination percentages were 81.00% (10 ppm), 59.66% (50 ppm) and 21.33% (100 ppm), compared to control 93.33%. Contrary to that, our observations showed that increasing $CdCl_2$ (2, 6, 10 ppm) and $PbCl_2$ (50, 100, 500 ppm) concentrations had no significant effect on seed germination of *S. candidissima* subsp. *occidentalis,* but increasing concentrations of $CuCl_2$ (20, 60, 150 ppm) had a significant inhibitory effect on seed germination of *S. candidissima* subsp. *occidentalis.* We can say that *S. candidissima* subsp. *occidentalis* could tolerate effect of $CdCl_2$ (up to 10 ppm) and $PbCl_2$ (up to 500 ppm) during seed germination stage.

The observations about biomass showed that increasing $CdCl_2$ (2, 6, 10 ppm) and $PbCl_2$ (50, 100, 500 ppm) concentrations had no significant effect on biomass increase of *S. candidissima* subsp. *occidentalis* seedlings*,* but increasing concentrations of $CuCl_2$ (20, 60, 150 ppm) had a significant inhibitory effect on biomass increase of *S. candidissima* subsp. *occidentalis* seedlings. We can claim that *S. candidissima* subsp. *occidentalis* could tolerate effect of $CdCl_2$ (up to 10 ppm) and $PbCl_2$ (up to 500 ppm) on biomass increase during seedling stage.

Petrescu *et al.* (2014) observed that cadmium solutions applied to *S. officinalis* seeds increased radicle development at concentrations of 10 ppm, but decreased at concentrations of 50 ppm and 100 ppm. Although our findings about seedling growth showed that increasing $CdCl_2$ (2, 6, 10 ppm) concentrations had no distinct impact on plant development of *S. candidissima* subsp. *occidentalis* seedlings*,* but increasing concentrations of $PbCl_2$ (50, 100, 500 ppm) and $CuCl_2$ (20, 60, 150 ppm) had significant inhibitory effects on seedling growth of *S. candidissima* subsp. *occidentalis* seedlings*.* There is an interesting result about effect of $CdCl_2$ concentrations on root growth. Different $CdCl_2$ concentrations had significant inhibitory effect on root growth of *S. candidissima* subsp. *occidentalis* seedlings.

Bini *et al.* (2012) reported that *Taraxacum officinale* is a bioindicator plant and also has ethnobotanical usage for liver disease and cooking, but its heavy metal accumulation ability is harmful for human health. *S. candidissima* subsp. *occidentalis* is also tolerant to both cadmium and lead (at a certain level) and is consumed as herbal tea by the local people, according to the information transmitted orally during field studies. therefore we can assert negative effects with *T. officinale* is valid for *S. candidissima* subsp. *occidentalis.* However, Carman Sosa *et al.* (2016) revealed that *Tagetes minuta* plants growing in areas polluted with lead, while lead was not found in essential oil and some ingredients were generated more than control group.

Novo *et al.* (2013) reported concerning the capacity of *Salvia verbenaca* to bear trace metals and actively replied to the following oxidative stress. *S. candidissima* subsp. *occidentalis* Hedge is also like *S. verbanaca* in terms of tolerance to heavy metal stress.

Chand *et al.* (2016) stated that when the essential oil is extruded through hydrodistillation, the heavy metals in the plant do not migrate to the essential oil, so the *Pelargonium graveolens* plant is suitable for the volatile oil even if it is grown in places exposed to heavy metal pollution. *S. candidissima* subsp. *occidentalis* has also valuable volatile oil content, its tolerance to Pb and Cd makes this plant appropriate for grown in polluted areas for volatile oil production.

Similarly, to findings of this study, Torun (2019) observed that *Salvia officinalis* was showed no significant difference comparatively water ingredient and chlorophyll fluorescence under salt and cobalt stress, but relative growth rate was raised after salt+cobalt application and it can be said that the mixture of salt and cobalt is suitable for improving toleration of *S. officinalis* for stress (Torun, 2019). Heavy metals can stimulate plant growth performance at a certain level (Arif et al., 2016).

Duka *et al.* (2015) stated that *Salvia officinalis* plant should be checked for heavy metal ingredients before being processed for human consumption. Similar to the findings of Duka *et al.* (2015), we can say that it is necessary to be careful in consuming *Salvia candidissima* subsp. *occidentalis* as it is tolerant to heavy metals such as cadmium and lead, if the gathering areas of the plant in question are exposed to cadmium and lead pollution, we can say that the plant can be harmful to people consuming it due to heavy metal accumulation.

5. CONCLUSION

S. candidissima subsp. *occidentalis* is a perennial plant that can be used in park and garden landscaping because of its white and interesting flowers. In addition, it can be grown for medical purposes owing to its chemical substances such as diterpenoids. According to the results, it can be said that *S. candidissima* subsp. *occidentalis* can be grown in habitats contaminated with both cadmium and lead due to its tolerance ability against heavy metals. It has been revealed that ecophysiological parameters such as% Germination, Hypocotyl and radicle lengths, biomass, SVI and MTI are the values that must be taken into consideration when examining the effects of heavy metals on the plant species in question.

Declaration of Conflicting Interests and Ethics

The author declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

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