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Effects of Disodium Octaborate Tetrahydrate (DOT) on Seed Germination and Development in Rocket (Eruca sativa Mill.) and Cress (Lepidium sativum L.)

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Abstract: Rocket (Eruca sativa Mill.) is cultivated throughout the year. Thanks to the rich metabolites of its leaves, it has a wide usage area in pharmacy. Cress (Lepidium sativum) is in the group of annual vegetables and has a herbaceous structure. Due to its fragrant and slightly spicy structure, it is a vegetable that is used as an appetizer. Its seeds and green parts are very beneficial for health. Rocket is a short-day plant whose leaves are considered to be rich in many minerals and vitamins. The present research was conducted to investigate the effects of doses of Disodium octaborate tetrahydrate (DOT) (0 (control), 15, 30, 45, 60 mg L⁻¹ on the germination and development of seeds of rocket and cress plants grown in pot experiments. In order to determine the effect of DOT on the development and yield of rocket and cress plants, the percentage of germinated seeds, cotyledon length and dry matter amounts were determined. ANOVA test was used to analyze the data obtained in the present study. Tukey test was used to determine which groups were in significant differences between the groups. Overall, it was seen that there were significant growth differences between the doses used statistically, the particularly 45 mg L^{-1} application DOT positively affected the germination and dry matter content of rocket and cress seeds.

Keywords: Rocket, Cress, Disodium octaborate tetrahydrate (DOT), seed germination.

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1. INTRODUCTION

In recent years, vegetable consumption has increased considerably with its beneficial effects on human health and nutritive contents (Chang et al. 2013). Rocket (Eruca sativa Mill.) and cress (Lepidium sativum L.) both cruciferous plants have rich sulforaphane ingredients that provide to induce NRF2-HO-1 antioxidant pathway (Bell et al. 2015). In usual, it is beneficial to eat brassicaceous greens that reduce cancer, cardiovascular issues and diabetes (Podsedek 2007; Tounsi et al. 2019).

Rocket is classified into Brassicaceae family. In general, rocket are harvested from nature as well as cultivated edible plants. Leaves are highly nutritive in terms of vitamins and minerals (Moussa 2006). Rocket is a plant rich in nutrients and has been reported to contain 5.13% K, 4.32% N, 0.25% P, 0.58% Mg, 2.95% Ca, 799.88 mg kg⁻¹ Na, 64.86 mg kg⁻ ¹ Zn, 5.36 mg kg⁻¹ Cu, 350 mg kg⁻¹ Fe, and 40.58 mg kg⁻¹ ¹ Mn (Barlas et al. 2011). Rocket has health promoting beneficial properties (Guijarro-Real et al. 2020). It is known that rocket cultivation dates back to ancient times as a source of food, oilseeds crop and medicinal plants

(Padulosi and Pignone 1996; Hall et al. 2012). Rocket is used as a salad vegetable because of low-calorie content, it is as well beneficial for health-promoting nutraceutical and anticancer properties (Higdon et al. 2007; Bell and Wagstaff 2014). Rocket essential oil is an important precursor for triazoles synthesis which creates aglucosidase inhibitors. Therefore could be an important oppress for postprandial hyperglycemia in diabetic patients (Hichri et al. 2019). Seeds of rocket have been used in folk medicine since ancient times for their diuretic, antimicrobial, lactagogue, aphrodisiac, and many other effects (Hussain et al. 2020). It was determined that rocket was used as a garden crop and spice in studies dating back to the middle ages, and special attention was paid to its biological diversity (Yaniv et al. 1998). Cruciferous plants have some phytochemicals protective against DNA damage such as sulforaphane erusine and erysoline. Rocket diet could increase hepatic ABC transporters' expression which reduces the risk of toxic compounds (Roma et al. 2019). Rocket is widely spread all over the world (Barillari et al. 2005).

Cress belongs to the Brassicaceae family has been cultivated in Europe, the US and India. The seeds are edible and beneficial with medicinal properties (Gokavi et al. 2004; Mali et al. 2007; Karazhiyan et al. 2009; Diwakar et al. 2010). Cress is a fast-growing herb with a tangy flavour and aroma (Manohar et al. 2012). Cress could grow up to 50 cm as annual plants and rich in some minerals also vitamins A and C (Ajdanian et al. 2019). Besides, it is included an important amount of folic acid, iron and calcium. (Sharma and Agarwal, 2011). Previous studies have shown that cress seeds are used for nutritional food or dietary purposes (Gokavi et al. 2004; Karazhiyan et al. 2009). In addition, seeds were traditionally used for diet breastfeeding woman's milk secretion (Diwakar et al. 2008; Datta et al. 2011). In addition, leaves have antibacterial properties, also helpful in cure hepatopathy and scurvy (Karazhiyan et al. 2011a). The performed studies on cress hepatoprotective effect were indicated that CCl4 (carbon tetrachloride) liver damage avoided in rats (Wadhwa et al. 2012). Cress seed has the potential to be used as a thickener in the food industry due to its hydrocolloid property (Karazhiyan et al. 2009; Karazhiyan et al. 2011 b; Behrouzian et al. 2014). Another study conducted by Naji et al. (2012) was stated that cress seed gum stands higher thermal treatments increase viscosity as desirable. In oppositely refrigeration conditions, cress seed gum was also functional with increased viscosity (Naji and Razavi, 2014). Leaves are generally used for salad, sandwiches, garnish and animal forage (Mali et al. 2007; Karazhiyan et al. 2009). Nehdi et al. (2012) was stated that cress seed oil has potential for biodiesel production due to be atomized readily and finer droplets.

Plants are important to drug research even though desired substances can be obtained as synthetic molecules (Eddouks et al. 2005). One of the medicinal plants is cress. Cress seed mucilage was used as a natural ingredient in pharmaceuticals (Behrouzian et al. 2014). Studies conducted by Paranjape and Mehta (2006) signify that cress was highly effective against bronchial asthma, 4 weeks treatment cress seed powder to 30 patients without any other cure medication. There were important beneficial improve clinical symptoms and asthmatic attacks. Another study performed by Maghrani et al. (2005) stated that aqueous extract of cress demonstrated antihypertensive and diuretic activities. Furthermore, cress seed oil was beneficial in albino rats due to the dietary source of alphalinolenic acid (Diwakar et al. 2008). Cress leaves and seeds were suggested to rheumatism and muscular pain. Moreover, it has aperient, aphrodisiac properties (Sharma and Agarwal 2011; Doke and Guha 2014; Hadi and Hameed 2017). Aqueous extract of cress seed stimulates apoptosis and necrosis in human breast cancer cell line MCF-7 (Mahassni and Al-Reemi 2013).

The main fatty acids in cress oil were linoleic and oleic acid. In addition to its high viscosity, cress oil has excellent lubricating properties (Moser et al. 2009). It was stated that cress seed oil is highly steady at cold temperatures. Cress seed oil is contained carotenoids and tocopherol as natural antioxidants. Therefore it maintains oil quality; cress seed oil is very helpful for the treatment of skin disease, leprosy, lumbago, dysentery (Kirthikar 1952; Diwakar et al. 2010).

Investigation performed by Kasabe et al. (2012) expressed that cress seed has medicinal and nutritional properties also antioxidant activities. Attia et al. (2019) reported that cress methanol extract was anti-diabetic and anti-oxidant properties. It was stated that cress has fracture healing properties in rabbits (bin Abdullah 2007). Chemo protective effects of cress investigation were performed by Kassie et al. (2002) signify that cress provides an important decrease to DNA damage in colon and liver cells. Conducted investigation along 14 weeks by Datta et al. (2011) emphasizes that cress seeds were none-toxic and trustworthy for feeding to rats in both genders. Cress seed has plenty of rich protein content and fat (Sharma and Agarwal 2011; Doke and Guha 2014).

Macro and micronutrients are very important in the agricultural ecosystem, but the insufficient knowledge of the use of micronutrients in agricultural lands and their limited availability in the soil cause microelement deficiency in agricultural plants. Boron is a micronutrient element that is rapidly depleted in the soil (Tahir et al. 2009). Boron is an essential element required for optimum growth and development in advanced plants (Marschner, 1995). It is known that boron plays an important role in nutrient transport by plant membranes (Tanada, 1983). Studies show that boron can affect the accumulation and utilization of other plant nutrients as a regulator or inhibitor (Alvarez- Tinaut et al., 1979). In plant cell walls, boron is an important component to crosslink pectic polysaccharides rhamnogalacturonan-II (RG-II) complex (Onuh and Miwa 2021).

We have done extensive research on the benefits and different uses purpose of both herbs. Cress and rocket are used in the treatment of various diseases. It is also rich in vitamins and minerals and especially consumed as a salad. Therefore it is important to rapid growth to meet the demand. In this research, we investigated germination percentage (%), cotyledon length (mm), Dry weight per plant (mg) under boron fertilizer in the 4th, 8th, 12th days.

2. MATERIAL AND METHODS

2.1. Materials and Treatments

Standard seeds of rocket (Rota variety) and cress (Helen variety) were used as plant material in the study. Pot soil with properties of pH 5.5-6.8, EC (µs cm⁻¹) 220, organic matter 54-60%, humidity 53.43%, water holding capacity 575.03, and purity 95% was used as the growing medium. Seeds were planted in 1.5 L (15x15 cm) pots with 40 seeds each. The study was set up according to a randomized plot trial pattern with three replicates and 3 pots for each repeat. The seeds were grown in pots at 22±1°C in a growth chamber under controlled conditions, 12 hours dark and 12 hours light (400 μ mol m⁻² s⁻¹) photo-cycle. The seeds in the pot were irrigated with different doses of sodium borohydride dissolved in distilled water at regular intervals during application. Sodium borohvdride the (Na₂B₈O₁₃.4H₂O disodium octaborate tetrahydrate) (DOT) (Brand name: ETIDOT-67, water soluble boron 20.8%) administration doses were determined as 0 (control), 15, 30, 45, 60 mg L⁻¹. The harvest time was determined according

to the preliminary studies we carried out before. Accordingly, the cotyledon samples were harvested on the 4th, 8th, and 12th days. Generally, after the seeds of arugula and cress are planted, they begin to absorb water into their bodies. On the fourth day after planting, the cotyledon grows upward while its roots move through the soil. These properties were effective in the selection of the first harvest time.

2.1. Methods

Determination of the germination percentage

While determining the germination percentage; The seeds germinated on the 4th, 8th, and 12th days of pots in each application were counted and calculated according to the formula below.

Germination (%)= (Number of seeds that germinated / Number of seeds on the pot) x 100

Determination of average cotyledon length

In determining the average cotyledon length; On days 4, 8, and 12, the above-soil part of five plants randomly selected from each pot was measured with a digital caliper and their average was calculated in mm.

Determination of cotyledons dry weight per plant

On the harvest days, the parts above the root node of 5 plants randomly selected from each pot were dried in the oven at 45 $^{\circ}$ C until they reached constant weight. It was then weighed on an analytical balance and divided by the number of plants and expressed in mg.

Data analysis

Experiments were conducted with 3 replicates per analysis. The importance of the implementation effect was determined at the 5 % prospect level by utilizing the Tukey test of one-way ANOVA with the assistance of SPSS 15 (Statistical Package for Social Sciences, SPSS Inc., IL, USA).

3. RESULTS AND DISCUSSION

The increasing polycyclic aromatic hydrocarbons were decreased to *L. sativum* germination rate in polluted

artificial soil (Maila and Cloete 2002). Pavel et al. (2013) stated that metal ions were inhibiting to seed germination of L. sativum. Another investigation indicated that Myrigalone A was impeded seed germination of L. sativum. (Oracz et al. 2012). The imidazolium ionic liquids were decreased to seed and root germination (Studzińska and Buszewski 2009). Research clarifies that volatile organic compounds (VOC) affected seed germination. It was expressed that high-VOC biochar causes total inhibition (Buss and Masek 2014). Another study revealed that microplastics can cause a delay of germination and root growth to L. sativum (Bosker et al. 2019). Therefore it is important to understand negative factors to prevent seed germination. As a result of our research, it has been determined that DOT promotes seed germination of both two plants (Table 1). We applied plants to DOT and harvested to 4th, 8th,12th days of growth.

Results indicated that treatments of $0 \text{ mg } L^{-1}$, 15 mg L^{-1} , 30 mg L⁻¹, 45 mg L⁻¹, 60 mg L⁻¹ DOT affect seed germination in cress. It is seen that the germination percentage is quite low in control plants that have never been applied DOT by examined in table 1. Control (0 mg L⁻¹) plants germination percentage 4th days 30%, 8th days 42.5%, and 43.3% in the end of 12th days of growth in cress (Table 1). The 45 mg L⁻ ¹ DOT treatments have the best results in seed germination. In the cress, treatments of 45 mg L⁻¹ sodium borahydride demonstrated germination rate in a row 4th days 50%, 8th days 75% and 76.66% at the end of 12th day of growth. According to other concentrations, 45 mg L⁻¹ DOT treatments are showed the fastest germination rate at the end of the 8th and 12th day in cress (Table 1). In similar to our research, the effect of 0, 0.1, 0.2, 0.4 and 0.8 kg da-1 DOT doses in vetch (Vicia ervilia (l.) willd), Kılıç (2019) was found that 0.4 kg da-1 DOT application increased seed yield compared to all applications.

Rocket is normally a fast-growing plant in nature. However, many factors can affect germination and growth (Garg and Sharma 2014; Shariatinia et al. 2021). Rocket germination results are similar to cress plants, control plants (0 mg L⁻¹) have minimum germination 70.83 %, the best germination rate 92.5 % in 45 mg L⁻¹ DOT treatments. Germination rate in both cress and rocket, 60 mg L⁻¹ DOT treatment shows similarly less germination than 45 mg L⁻¹. Therefore we suggest 45 mg L⁻¹ DOT fertilizer in both cress and rocket (Table 1).

Days (cress seed germination percentage)		4. day	8. day	12. day
	0 mg L ⁻¹	30.00 ± 1.44^{d}	42.50±1.44°	43.33±0.83 ^d
Treatmonta	15 mg L ⁻¹	37.50±1.44°	45.00 ± 0.00^{bc}	45.00 ± 1.44^{d}
Treatments (DOT mg L ⁻¹)	30 mg L ⁻¹	37.50±0.00°	50.00±1.44 ^b	55.00±1.44°
	45 mg L ⁻¹	50.00 ± 0.00^{b}	75.00 ± 1.44^{a}	76.66 ± 0.83^{a}
	60 mg L ⁻¹	62.50±2.88ª	68.33±2.20 ^a	68.33 ± 2.20^{b}
Days (rocket seed germination percentage)		4. day	8. day	12. day
	0 mg L ⁻¹	62.50±1.44°	70.00±2.88°	70.83±2.20 ^d
Treatments	15 mg L ⁻¹	74.16±0.83 ^b	78.33±1.66bc	78.33±1.66 ^{cd}
$(DOT mg L^{-1})$	30 mg L ⁻¹	82.50 ± 1.44^{a}	82.50 ± 1.44^{b}	82.50±1.44 ^{bc}
	$45 \text{ mg } \text{L}^{-1}$	87.33±2.33ª	92.50±1.44 ^a	92.50 ± 1.44^{a}
	45 mg L	01.33±2.33	J2.J0±1.++	72.30±1.44

Table 1. Cress and rocket germination percentage (%)

^{a, b, c, d} The values designated by different letters on the same column are significantly different on 5 % significance level.

Note: (***) means 99.9% confidence level, (**) means 99% confidence level, (*) means 95% confidence level (ns) is statistically insignificant and (a, b, c, d, e) means homogeneous groups

Pandey (2012) investigated the effects of different concentrations of boron (0, 0,33, 3.3, 33, 330 mM) applications on flaxseed (Linum usitatissimum L. var R552) on seed germination and cotyledon length. It was stated that low doses of boron applications affected the cotyledon length positively, but with the increase of boron concentration, seed germination and vitality index decreased and percent phytotoxicity increased. Cress and rocket DOT treatment is importantly effective to cotyledon length (Table 2). On the one hand, cress control treatments

maximum cotyledon length 20.05 mm, on the other hand, 45 mg L⁻¹ boron fertilizer were positively increased to 50.42 mm at the end of the 12th growth. Rocket control plants cotyledon length 26.07 mm in 12th growth. However, this results up to 49.64 mm when the 45 mg L⁻¹ boron fertilizer was applied to plants (Table 2). Both two plants growing regularly, according to our observation 45 mg L⁻¹ boron fertilizer quite effective over cotyledon length.

Table 2 Cress and rocket cotyledon length (mm	Table 2 C	ress and	rocket	cotyledon	length	(mm
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Days (Cress cotyledon length mm)		4.day	8.day	12.day
	0 mg L ⁻¹	6.54 ± 0.55^{b}	8.86±0.22°	20.05 ± 1.12^{d}
Tuestusente	15 mg L ⁻¹	7.46±0.91 ^b	9.80±0.224°	30.41±0.97°
Treatments (DOT mg L ⁻¹)	30 mg L ⁻¹	29.73±0.21 ^a	36.30±1.47 ^b	39.91±1.89 ^b
	45 mg L ⁻¹	30.40 ± 0.34^{a}	40.19 ± 1.07^{a}	50.42±0.83 ^a
	60 mg L ⁻¹	28.15±0.94 ^a	38.68±0.17 ^{ab}	40.07 ± 2.03^{b}
Days (Rocket cotyledon length mm)		4.day	8.day	12.day
	0 mg L ⁻¹	5.29 ± 0.28^{d}	21.94±1.30 ^b	26.07±1.75°
Treatments	15 mg L ⁻¹	20.19±0.72°	38.12±0.81 ^a	40.31±0.61 ^b
$(DOT mg L^{-1})$	30 mg L ⁻¹	24.43±0.76 ^b	38.04 ± 0.54^{a}	40.80±1.17 ^b
_	45 mg L ⁻¹	29.90±0.31ª	39.97±1.81ª	49.64 ± 1.02^{a}
	60 mg L ⁻¹	26.24 ± 0.18^{b}	38.11±0.53 ^a	42.47 ± 0.24^{b}

^{a, b, c, d} The values designated by different letters on the same column are significantly different on 5 % significance level.

Although boron is generally considered immobile, plays an important role in the transport of sugars. It also helps carbohydrate, RNA (ribonucleic acid) and IAA (indoleacetic acid) metabolisms (Kacar et al. 2020).

Cress and Rocket dry weight per plant were analyzed in presence of various concentrations of boron fertilizer (Table 3). There were no negative effects of DOT fertilizers. All other concentrations were higher than control treatments. In the parallel of our investigation, Besheit et al. (1992) applied 40 ppm doses of B, Zn, Mn and Fe separately to sugar beet seeds before planting. In the same study, they reported that the dry weight was increased by 40 ppm Boron application compared to the control and other applications. Cress and Rocket's dry weight per plant (mg) were more raised at 45 mg L^{-1} DOT fertilizers (Table 3). In the 4th day of growth dry weight per plant was 2.23 fold and 1.42 fold more at cress and rocket respectively in the 45 mg L^{-1} DOT treatments when compared to control plants.

In the parallel of our investigation, Besheit et al. (1992) applied 40 ppm doses of B, Zn, Mn and Fe separately to sugar beet seeds before planting. In the same study, they reported that the dry weight was increased by 40 ppm Boron application compared to the control and other applications.

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Days (Cress Dry weight per plant mg)		4.day	8.day	12.day
	0 mg L ⁻¹	0.59±0.04°	0.78±0.07°	0.99 ± 0.08^{b}
Treatments	15 mg L ⁻¹	$0.92{\pm}0.04^{b}$	1.08 ± 0.05^{b}	1.16 ± 0.04^{b}
$(DOT mg L^{-1})$	30 mg L ⁻¹	1.21 ± 0.03^{a}	1.45 ± 0.03^{a}	1.48 ± 0.05^{a}
(DOT ling L)	45 mg L ⁻¹	$1.32{\pm}0.06^{a}$	$1.58{\pm}0.03^{a}$	1.67 ± 0.04^{a}
	60 mg L ⁻¹	1.28±0.02ª	1.48 ± 0.04^{a}	$1.54{\pm}0.05^{a}$
Days (Rocket Dry weight per plant mg)		4.day	8.day	12.day
Days (Rocket Dry weight per plant mg)	0 mg L ⁻¹	4.day	8.day	12.day
Days (Rocket Dry weight per plant mg)	0 mg L ⁻¹ 15 mg L ⁻¹		•	-
Days (Rocket Dry weight per plant mg) Treatments	U I	1.01±0.01 ^b	1.16±0.02 ^b	1.20±0.01 ^d
	15 mg L ⁻¹	1.01±0.01 ^b 1.13±0.00 ^b	1.16±0.02 ^b 1.60±0.03 ^a	1.20±0.01 ^d 1.64±0.02 ^c

Table 3 Cress and rocket dry weight per plant (mg)

^{a, b, c, d} The values designated by different letters on the same column are significantly different on 5 % significance level.

4. CONCLUSION

It is important that DOT fertilizer has not been studied before to stimulate the germination of cress and rocket seeds and subsequent growth. Cress, at the end of the 12th day 45 mg L⁻¹ DOT treatments 1.76 fold more germination rate than control plants. Rocket, at the end of the 12th day 45 mg L⁻¹ DOT treatments 1.30 fold more germination rate than control plants. Cress and rocket, boron fertilizer was increased to cotyledon length according to control treatments. In both two plants, 45 mg L⁻¹ boron fertilizer was the highest cotyledon length. When comparing control plants to 45 mg L⁻¹ boron fertilizer, cress 2.51 fold rocket 1.90 fold have more cotyledon length. Cress and rocket dry weight per plant 45 mg L⁻¹ boron fertilizer were 1.68 fold and 1.60 fold more than control plants respectively. Overall, we proposed that 45 mg L^{-1} DOT treatments have a quite good improvement at germination %, cotyledon length, dry weight per plant both cress and rocket. Therefore it shortens the period to deliver products from field to market. We think that the determination of the appropriate dose with our research will shed light on future studies and guide the growers in terms of product yield.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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