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Phytochemical Responses of White Savory (Satureja mutica Fisch and C.A.Mey.) to Foliar Application of Seaweed Extract and Ecormon Fertilizer

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ABSTRACT

BACKGROUND: Sustainable agriculture is a great solution to reduce chemical pollutants to overcome environmental pollution.

OBJECTIVES: Considering the importance of the savory and the consequences of fertilizers application, current study was carried out to determine the optimum concentration of seaweed extract and Ecormon fertilizer -as an organic matter and growth promoting stimulus- on some biochemical characteristics of white savory.

METHODS: The present study was conducted as a factorial experiment in a randomized complete blocks design including three concentrations of seaweed extract (0, 1.5, and 3/1000) and three concentrations of Ecormon fertilizer (0, 0.5 and 1/1000) in three replications to study the biochemical properties of white savory.

RESULT: According to the results, the highest chlorophyll a, b, total chlorophyll and carotenoid levels were observed in the treatment of Ecormon1 (2.65, 2.31, 4.95 and 2.88 $mg.g^{-1}$ respectively), whereas Seaweed 1.5 had the lowest pigments amounts. The highest amount of phenolic compounds (804.00 $mg.g^{-1}$) was obtained from seaweed 3 plus Ecormon 1, while the lowest amounts were belonging to seaweed 1.5 plus Ecormon1 and seaweed 3 plus Ecormon 0.5 (247.40 and 248.30 $mg.g^{-1}$), respectively. The highest amounts of antioxidant activity were observed in treatments of seaweed 3 plus Ecormon1 and Ecormon 0.5 (46.25 and 45.26 %, respectively) and the lowest value (25.87 %) was observed in control treatment.

CONCLUSION: In general, application of Ecormon and seaweed extract showed a positive effect on photosynthetic pigments, phenolic compounds and antioxidant activity of white savory. So, it can be recommended to farmers as efficient bio-elicitors that improve the biochemical properties of the plant through induction of the immune system.

KEYWORDS: *Antioxidant activity, Biofertilizers, Carotenoids, Phenolic compounds.*

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1. BACKGROUND

White savory or forest savory is a perennial and woody plant at the base with 30 to 50 cm height and many flowering branches (Salehi-Arjmand *et al*., 2014). White savory belonging to the Lamiaceae family and is highly aromatic species. This plant is covered with short gray lints, which cover the entire surface of the stems and branches evenly (Ghorbanpour *et al*., 2016). The amount of effective compounds of the plant will vary according to the climatic conditions of growth region, plant nutrition and environmental stresses (Tozlu *et al*., 2011). Chemical fertilizers have been widely used in agricultural production since the mid-20th century and have increased the yield of horticultural crops greatly. The application of fertilizers will definitely offset the nutrient deficiencies in the soil, but over-use of fertilizers around the world has damaged the texture of crop soils and has caused environmental pollution and problems for human and other living things health (Dixon, 2018). Sustainable agriculture with the aim of reducing fertilizers is a desirable solution to overcome these problems (Calabi-Floody *et al*., 2017). In sustainable farming systems, any improvement in agricultural systems should lead to increased production and reduced environmental detrimental effect, which ultimately increases the sustainability of agricultural systems. One of these methods is the use of biological stimuli and biofertilizers that can increase the effect of mineral fertilizers (Ji *et al*., 2019). Application of algae extract can increase plant growth, stimulate root growth, delay aging and improve resistance to environmental stresses such as drought, salinity and temperature (Thambiraj *et al*., 2012). Microalgae are the most important organic matter producers in aquatic environments because of their chlorophyll content which enables them to photosynthesize and they are considered as the first ring of food chain in aquatic ecosystems. Phototrophic microalgae are organisms which produce food and energy through photosynthesis (Cook *et al*., 2018). Microalgae flow at all levels of the food chain and food web. For this reason, they form an important part of the aquatic organism's food chain in aquatic ecosystems. In addition, they play an important role as biological water purifiers and environmental pH adjusters (Zamani *et al*., 2013). Selvam and Sivakumar (2013) stated that application of algae liquid fertilizer increased growth parameters, chlorophyll a, b and total chlorophyll, protein, sugars and leaf stomata of black gram (*Vigna mango* L.). Ecormon fertilizer is also an active promoter of vegetative and reproductive growth of plants. It stimulates flower induction and increases flower production in plants. It is used via spraying on blossom and leaves, direct use in irrigation water or nutrient solution of hydroponic system. Ecormon contains amino acids, hormones, molybdenum, phosphorus and nitrogen. It can be combined with VOLKE oil. Ecormon will be absorbed through the flower producing buds (El-Nemr *et al*., 2012; Niknejad and Pirdashti, 2012).

2. OBJECTIVES

Considering the importance of the savory and the consequences of fertilizers application, current study was carried out to determine the optimum concentration of seaweed extract and Ecormon fertilizer -as an organic matter and growth promoting stimulus- on some biochemical characteristics of white savory.

3. MATERIALS AND METHODS

3.1. Greenhouse and Treatments Information

The present study was carried out in the research farm of Islamic Azad University of Isfahan (Khorasgan) located in Isfahan East (32° 40' N, 51° 48' E) with an elevation of 1555 m. The study was conducted as a factorial experiment in a randomized complete blocks design including three concentrations of seaweed extract (0, 1.5, and 3/1000) and three concentrations of Ecormon fertilizer (0, 0.5 and 1/1000) in three replications. The number of plots was 27 plots.

3.2. Greenhouse Management

Seeds were first sown in PittMoss containing trays in the second half of March. Seedlings were placed in a greenhouse with day temperature of 28°C and night temperature of 18°C. In the 4-6 leaf stage, seedlings were transplanted in experimental plots in the research farm. Seedlings were planted on furrows with 60 cm distance. The distance between plants was 40 cm. plots had 2×3 meters dimensions. One month after plants establishment, different Concentrations of Seaweed and Ecormon were sprayed in four steps at 15 day intervals in the cool morning hours. Due to weather conditions irrigation was carried out twice a week in early cultivation and then three times a week (in summer) until plant sampling. Finally, plants were harvested at flowering stage and transferred to laboratory for evaluating phytochemical properties.

3.3. Measured Traits

Total chlorophyll content was determined using a spectrophotometer (Model D-6320) at 663 and 645 nm wavelengths. Also equations (1-4) were used to calculate total chlorophyll content (in mg/g fresh leaf tissue). In these equations, Abs 645 and Abs 663 were absorbance read at 645 and 663 nm wavelengths, V was acetone volume in milliliters and W was fresh leaf weight in gram (Li *et al.*, 2009).

Equ.1: Chl.a $(mg.g^{-1}) = [(12.7\times A_{663}) - (2.6\times A_{645})] \times V/W \times 1000$

Equ.2: Chl.b $(mg.g^{-1}) = [(22.9\times A_{645}) - (4.68\times A_{663})] \times V/W \times 1000$

Equ.3: Chl. total $(mg.g^{-1})$ = Chl. a + Chl. b

Equ.4: Car. = (1000 A470 - 1.82 Chl. a - 85.02 Chl. b) /198

The total amount of phenolic compounds in the plant extract was evaluated according to the method of Slinkard and Singleton (1977). Absorbance of samples was compared with standard curves and total phenol content of each extract was calculated in mg/gr of dry extract. To evaluate the antioxidant capacity of the extract, a free radical source is needed. For this purpose, the DPPH or 2 2-Diphenyl-1-picrylhydrazyl was used. Finally, the optical absorption of the samples was read at 517 nm by a Uv-Vis Shimadz Spectrophotometer. The ability of scavenge DPPH radical was calculated as percentage according following equation (Pisoschi and Negulescu, 2011). **Equ.5**: % inhibition= $\{(Abs_{control} - Abs_{sample})\}/(Abs_{control}) \times 100$.

3.4. Statistical Analysis

Obtained data were analyzed using SAS statistical program and means were compared using LSD at 5 % probability level.

4. RESULT

4.1. Photosynthetic pigments

The results of analysis of variance showed that the effects of Ecormon, seaweed and their interaction effects on chlorophyll a, b and total were significant at 1 % level (Table 1). The highest levels of chlorophyll a, b, total chlorophyll and carotenoids (2.65, 2.31, 4.95 and 2.88 mg.g^{-1}, respectively) were observed in ecormon 1, whereas seaweed 1.5 had the lowest amounts of these traits (Table 2).

Table 1. Analysis of Ecormon fertilizer

Compounds	W/W	W/V
Amino acids	6	7.5
Molybdenum	4	5
Phosphor	5	6
Nitrogen	3.5	4.5

4.2. Phenolic compounds

Variance analysis results showed that effects of Ecormon, seaweed and their interaction effects on chlorophyll a, b and total were significant at 1 % level (Table 3). The highest amount of phenolic compounds (804.00 mg.g^{-1}) was observed in seaweed 3 plus Ecormon 1. The lowest values were obtained from seaweed 1.5 plus Ecormon 1 and seaweed 3 plus Ecormon 0.5 (247.40 and 248.30 mg.g^{-1}, respectively) (Table 4).

4.3. Antioxidant activity

Variance analysis results showed that the effects of Ecormon, seaweed and their interaction effects on chlorophyll a, b and total chlorophyll were significant at 1 % probability level (Table 3). The highest antioxidant activity was observed in seaweed3 with the Ecormon1 and 0.5 (46.25 and 45.26 %, respectively). The lowest activity (25.87 %) were obtained from the control treatment (Table 4).

Table 2. Analysis of seaweed extract

Compounds	Amount	Compounds	Amount
Organic matter	4.95	Total nitrogen (%)	0.10
Organic carbohydrate (mg.lit^{-1})	1.87	Available phosphate (%)	0.10
Organic acids (%)	0.71	Soluble potash (%)	1.5
Organic carbon (mg.lit^{-1})	52	Boron (mg.lit^{-1})	11
Amino acids (g.lit^{-1})	107	Copper (mg.lit^{-1})	30
Auxin activity (mg.lit^{-1})	15	Iron (mg.lit^{-1})	150
Gibberellins activity (mg.lit^{-1})	24	Manganese (mg.lit^{-1})	80
Cytokinin activity (mg.lit^{-1})	11	Zinc (mg.lit^{-1})	50

5. DISCUSSION

Concepts such as sustainable and bio-based farming are in focus and costs of producing organic fertilizers are also high. We need to look for a system that maintains the yield and can be sustainable in the long run (Hendawy *et al*., 2010). The results showed that seaweed extract and Ecormon had significant effects on measured traits. The increase in the amount of chlorophyll and carotenoid pigments by Ecormon1 may be due to the stimulating effect of this substance on leaves and more production and transport of photosynthetic materials. Ecormon also increase the viability of photosynthetic tissues and increases leaf chlorophyll through positive physiological effects, including plant cell metabolism (Asghari *et al*., 2014). Ecormon fertilizer performed well by activating and increasing the expression of genes in the biosynthetic pathway of chlorophyll production and increased chlorophyll content (Khajeh and Naderi, 2014). Ecormon performed well by activating and increasing the expression of genes in the biosynthetic pathway of chlorophyll production which led to increased chlorophyll content (Khajeh and Naderi, 2014). In addition, the presence of magnesium and iron in this fertilizer has probably had a positive effect on the synthesis of chlorophyll and carotenoids and consequently increased photosynthesis (Pise and Sabale, 2010). The presence of auxin and gibberellic acid in this fertilizer are other probable reasons of Ecormon positive effects on increasing chlorophyll content (Shahbazi *et al*., 2015). Ecormon fertilizer causes systemic resistance in plants through various mechanisms such as production of antibiotics and also through production of phytohormones such as auxins and gibberellins and can influence pigments production (Santos *et al*., 2014). Also, increased chlorophyll content in treated plants can be due to improvement of water status of plant and more absorption of minerals (Delshadi *et al*., 2017). The results of this study showed positive effect of seaweed extract and Ecormon fertilizer on antioxidant activity and phenol content of savory. Phenolic compounds are secondary metabolites that are synthesized in many plants. The amount of these+ compounds is increased as a result of plant nutritional conditions (Zancan *et al*., 2008). There is a correlation between phenolic compounds and antioxidant activity in seaweeds and most of plants. Solvents as well as algae species are effective in antioxidant activity (Horincar *et al*., 2011). Algae species probably have different components and can be used in antioxidant activities of enzymes and reduce their risk. The relationship between chemical compounds and antioxidant activity of algae extracts needs further investigation (Amoudi *et al*., 2009). However, most studies have reported a positive relationship between phenolic content and antioxidant activity. This means that by increasing phenol content, antioxidant activity increases and by reducing it, antioxidant potential decreases as well. Seaweed extract seems to have the potential to kill free radicals as a biological elicitor (Yen *et al*., 2008).

Table 3. Variance analysis results of some plant characteristics

S.O.V	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Phenolic compounds	Antioxidant activity
Blocks	2	0.012**	0.003	0.009	0.004	1.60	2.44
Ecormon	2	1.42**	0.73**	3.93**	1.98**	84038**	66.69**
Seaweed	2	1.14**	1.06**	4.30**	1.35**	203207**	455.85**
Ecormon × Seaweed	4	0.95**	0.66**	3.02**	1.07**	241661**	6.30**
Error	16	0.001	0.003	0.003	0.002	118.7	0.84
CV (%)	-	2.06	4.58	2.10	2.27	4.82	2.43

* and **: Significant at 5 and 1% probability levels, respectively.

Many studies have also found a positive relationship between the phenolic and flavonoid compounds of the seaweed and their antioxidant activity (Horincar *et al*., 2011). It can be inferred that the seaweed extracts may activate new genes that trigger different enzymes and ultimately biosynthetic pathways to form phenolic compounds (Zhang *et al*., 2006). These compounds appear to be involved in the induction of secondary metabolites. These elicitors lead to the formation and regulation of secondary metabolites by determining the biosynthetic pathways and they are effective in stimulating the production of many secondary metabolites including phenolic compounds (Ionkova, 2007).

Table 4. Mean comparison results of plant characteristics

Seaweed (1/1000)	Ecormon (1/1000)	Chlorophyll a (mg.g^{-1})	Chlorophyll b (mg.g^{-1})	Total chlorophyll (mg.g^{-1})
0	**0**	1.08^{*f}	0.73^{e}	1.81^{g}
	0.5	2.26^{b}	1.44^{b}	3.70^{b}
	1	2.65^{a}	2.31^{a}	4.95^{a}
1.5	**0**	0.72^{g}	0.59^{f}	1.31^{h}
	0.5	1.99^{c}	1.05^{cd}	3.05^{c}
	1	1.14^{f}	0.81^{e}	1.95^{f}
3	**0**	1.83^{d}	1.12^{c}	2.95^{c}
	0.5	1.56^{e}	0.98^{d}	2.53^{e}
	1	1.76^{d}	1.03^{d}	2.79^{d}

Continue Table 4.

Seaweed (1/1000)	Ecormon (1/1000)	Carotenoids (mg.g^{-1})	Phenolic compounds (mg.g^{-1})	Antioxidant activity (%)
0	**0**	1.37^{g}	62.20^{g}	25.87^{g}
	0.5	2.78^{b}	357.30^{c}	30.41^{f}
	1	2.88^{a}	598.10^{c}	34.36^{e}
1.5	**0**	0.92^{h}	340.20^{e}	36.46^{d}
	0.5	2.48^{c}	510.70^{d}	39.01^{c}
	1	1.48^{f}	247.40^{f}	39.44^{c}
3	**0**	2.34^{d}	783.30^{b}	41.68^{b}
	0.5	2.04^{e}	248.30^{f}	45.26^{a}
	1	2.34^{d}	804.00^{a}	46.25^{a}

The onset of defense responses in plant induces a network of signal transducers that begins with recognition of elicitor molecules by acceptors. The increase in phenolic compounds appears to be due to stimulation of biosynthetic enzymes from the previous phenylalanine ammoniacase and polyphenol chalcone synthase (Heng *et al*., 2012).

5. CONCLUSION

Application of Ecormon and seaweed extract showed a positive effect on photosynthetic pigments, phenolic compounds and antioxidant activity of savory. So they can be recommended to farmers as efficient bio-elicitors that improve biochemical properties of plant through induction of immune system.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

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