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Impact of arbuscular mycorrhizal fungi and seaweed extract on growth parameters of coriander (*Coriandrum sativum* L.)

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Abstract : The present study was carried out during the two successive growing seasons of the 2019–2020 and 2020–2021 at the experimental farm of faculty of science, Mansoura University, Mansoura, Al-Dakahlia governorate, Egypt to investigate the effect of Arbuscular mycorrhiza1 fungi (AMF) and seaweed extracts, as well as, their interactions on vegetative growth of coriander (*C. sativum*). The experimental design was split plot design with four replicates, Chemical fertilizers (NPK) were assigned to the main plots, while AMF inoculation, seaweed extracts and their mixture concentrations were assigned to the subplots. The obtained results could be summarized as follows: (1) the addition of S.W + AMF yielded a notable increase in the coriander plants' vegetative growth and chemical composition in both seasons. (2) The addition of 50% of the recommended dose of NPK chemical along with seaweed extracts and arbuscular mycorrhizal fungi was the best combination with the highest mean values of root length, number of branches / plant., shoot fresh weight, shoot dry weight, shoot length, number of leaves/plant, and leaf area; photosynthesis pigments content (chromophyll a, b, a+b, and carotenoids content); total carbohydrates content; and the coriander plants' N, P, K, Mg, and Ca contents.

Keywords: Coriander, growth, biostimulants, Arbuscular Mycorrhizal Fungi, seaweed extracts.

Introduction

Coriander (*Coriandrum sativum* L.) is one of the umbelliferous family's it belonging to aromatic and medicinal plants, and it is widely cultivated in Egypt. The area under cultivation of green coriander in Egypt was 418 Fed. with a total production of 489 tons and an average yield of 0.855 tons Fed.$^{-1}$ in 2022 (Statistical report of agriculture and land reclamation ministry of Egypt, 2022). Fertilization is one of the most important factors affecting the growth of medicinal and aromatic crops (, 2). It is the crucial input that plays an important role in exploring the highest yield capacity of a crop. Nowadays, there is a strong trend to minimize or reduce the use of chemical fertilizers and replace them with biostimulants such as arbuscular mycorrhizal fungi and seaweed extracts, which are more cost-effective and environmentally friendly when growing medicinal and aromatic plants (3, 4, 5).

Arbuscular mycorrhizal fungi (AMF) are the most common and widespread group of Mycorrhizal fungi in soil. (6, 7) reported that more than 80% of mycorrhizal plants are associated with arbuscular mycorrhiza.

Aquatic photosynthetic creatures are known as macroalgae, or seaweeds (8). Seaweed extracts contain a variety of macronutrients (Ca, K, P) and micronutrients (Fe, Cu, Zn, B, Mn, Co) that are beneficial to plant growth, in addition to complex polysaccharides, amino acids, vitamins (A, E, C, B12, and B1), betaines, phytohormones (IAA, IBA, and cytokinins), and sterols (9, 10, 11).

Many studies reported that the application of arbuscular mycorrhizal fungi and seaweeds extracts improved the growth of many medicinal plants: (12) on sweet basil

(*Ocimum basilicum* L), (13), and (14) on parsley (*Petroselinum crispum* (Mill.) Fuss, (15) on sage (*Salvia officinalis* L.).

Therefore, the aim of the present study was to evaluate the effect of Arbuscular mycorrhizae fungi and seaweed extracts, as well as, their interactions on vegetative growth of coriander (*C. sativum*).

Material and methods

Experimental Site

This experiment was conducted at the faculty of science experimental farm at Mansoura University (latitude: 31.04°N, longitude: 31.35°E, and 4.96 m above the Mediterranean Sea level), Mansoura, Al-Dakahlia governorate, Egypt, during the two succeeding growing seasons of 2019–2020 and 2020–2021. Table (1) displays the results of the experimental soil's chemical analysis.

Table 1. Physical and chemical characteristics of the experimental soil (0–20 cm).

Properties	Values	Properties	Values
Sand (%)	27.6	E.C. (dSm^{-1})	1.3
Silt (%)	31.5	Total N mg/kg soil	99.7
Clay (%)	40.9	Ca^{2+} mg/kg soil	331.1
Texture class	Clay-loam	K^+ mg/kg soil	427.2
Organic matter %	1.0	Na^+ meq/l	13.7
$CaCO_3$	17.2	P mg/kg soil	3.3
pH(1:2.5 extract)	7.6	Fe mg/kg soil	24.4

Plant Materials

Coriander (*C. sativum* var. Balady) seeds were obtained from the Therapeutic and Perfumed Plants (MAP) Dept. of Mansoura Horticulture Research Station, Agricultural Research Center.

Arbuscular mycorrhizal inoculum

To prepare the inoculum of AMF, spores of arbuscular mycorrhizal fungi were obtained and isolated from the rhizosphere soil of bean plants in the Dakhalia region using the wet filtering and decanting technique (16) and propagated on roots of Sudan grass plants as stock plants.

Spores of AMF, including the following species: *Glomus mosseae*, *Glomus clarum*, *Glomus monosporium* and *Gigaspora margarita*; these fungi were developed and fully recognized by Prof. Dr. Gamal M. Abdel Fattah Department of Botany, Faculty of Science Mansoura University, Egypt.

Seaweed extracts preparation

A commercial seaweed extract (SW) prepared from *Sargassum sp.*; *Ascophythum nodosum* and *Luminaria sp.* was used. Chemical analysis of SW product is shown in Table 2. SW foliar explanation was equipped by liquefying 1.5 g in one liter of purified water.

The experimental design and treatments

The research was conducted in a split-plot design with four replicates in both seasons; Chemical fertilizers (NPK) were assigned to the main plots, while AMF inoculation, seaweed extracts and their mixture concentrations were assigned to the subplots. Each plot was 3.5 m^2 (2 × 1.75 m^2) and each unit contained 18 hills (25 cm apart) in three rows (50 cm apart).

The experiment included 7 treatments which are summarized as follows:

T1 = Control (chemical fertilizer 100 % NPK)

T2 = Seaweeds extract (S.W)

T3 = Arbuscular mycorrhizal fungi (AMF)

T4 = S.W + AMF

T5 = S.W + 50% NPK

T6 = AMF + 50% NPK

T7 = S.W + AMF + 50% NPK

C. sativum seeds were superficial disinfected in 7% sodium hypochlorite for 10 min and then cleaned with sterilized distilled water to remove the excess of the disinfectant (17). The sterilized seeds were sown on a hill in the open field in the last week of November (2–3 seeds per hill) and irrigated.

Regarding the mycorrhizal treatments, half of the plants received a mycorrhizal fungi inocula consisting of soil, spores and chopped roots of leek colonized by stock culture of the mycorrhizal fungi. Mycorrhizal inocula were placed 5 cm near by the root of *C. sativum* seedlings after digging out the root carefully. Non-mycorrhizal plants received equal amount of autoclaved inoculums to produce the same nutrients without mycorrhizal propagules. After AMF inocula plants were thinned, leaving only one plant per hill when seedlings grew to a height of at least 10 cm.

Seaweed extract was applied to the leaves three times after 30 days of sowing at an interval of 15 days in amounts of 1.5 g/L. All other agricultural practices were conducted according to the recommendation of the Egyptian Ministry of Agriculture.

Table (2): Physical and Chemical analysis of seaweed extracts.

Component	Value	Component	Value
pH	4.5	Methionine	1.39
Density(g/cm^3)	0.6	Aspartic	5.44
Maximum moisture (%)	6.5	Phenylalinine	2.82
Organic matter (%)	44-5	Proline	4.42
Ash (minerals) (%)	44-55	Glutamic acid	7.69
Macro elements (%)		Serine	0.1
Total nitrogen (N)	1.0-2.0	Glycine	3.1
Available phosphoric acid (P_2O_5)	2.0-4.0	Threonine	1.2
Soluble potash (K_2O)	18.0-22.0	Histidine	0.4
Sulfur (S)	1.0-2.0	Tyrosine	1.8
Magnesium(Mg)	0.2-0.5	Isoleucine	1.9
Calcium (Ca)	0.1-0.2	Valine	3.4
Manganese(Mn)	8-12	Leucine	4.8
Micro elements (ppm)		Vitamins (ppm)	
Boron (B)	75-150	Provit	40
Copper (Cu)	1-10	C	200-400
Iron (Fe)	75-250	B1	6.8
Zinc (Zn)		25-75	4
Amino acids (average g of amino acid/ 100 g of protein)		B2	6
Alanine	3.8	E	70
Lysine	1.3	B12	0.04
Arginine	0.2	Niacin	70

Data measurements and recorded

Random samples of ten plants were taken from every experimental treatment at three periods 80, 120 and 180 days from planting in both seasons to evaluate the vegetative growth parameters and the mineral composition (N, P, K, Ca and Mg).

The growth parameters of common coriander plants were measured as follow:

- Shoot length (cm)
- Number of leaves
- Shoot length, root length (cm)
- Number of branches/plant
- Shoot fresh weight
- Root fresh weight
- Shoot dry weight
- Root dry weight
- Leaf area (cm^2)
- Number of umbels/ plant
- Number of flowers/ plant

All the weights were expressed as grams; dry weights were calculated after drying at 110^{0}C for 24 hr in oven until constant weight.

The nitrogen content was assayed by the Kjeldahl method (18). Phosphorus content was measured by the vando-molybdo-phosphoric colorimetric method (19). Potassium percents were measured by flame photometer (20). Magnesium (Mg), Sodium (Na), and Calcium (Ca) content was determined according to (21).

Statistical analysis

Data obtained were subjected to a statistical analysis of variance (ANOVA) using SPSS 22.0 (SPSS, Inc., USA) package. Significant differences among treatment means were estimated on the Least Significance Difference test (LSD), at $p \leq 0.05$ (22).

Results

Growth parameters

According to Table 3 and Figure 1, the vegetative growth characteristics indicate that there are significant differences ($P < 0.05$) between the fertilization treatments. All growth metrics were improved in both seasons by AMF inoculation (Table 3 and Figure 1). When compared to the control treatment (100% NPK), all AMF treatments produced significantly ($P \leq 0.05$) best values for root length, number of branches/plant, shoot fresh weight, root fresh weight, shoot dry weight, shoot length, and number of leaves/plant and leaf area. The 50% NPK+SW+AMF treatment may be the best option among interaction treatments. Treatment containing (50% NPK+SW+AMF) gave the highest values for root length (23.13, 32.17 cm), number of branches/ plant (14.40, 23.66), shoot fresh weight (97.66, 179.48 g), root fresh weight (21.63, 25.92 g), shoot dry weight (15.33, 20.10 g), root dry weight (4.84, 6.94 g), shoot length (56.48, 149.67 cm), number of leaves/ plant (57.66, 85) and leaf area (734.24, 786.15cm^2) at vegetative stages during two seasons, respectively, while the treatment (0 NPK + S.W.) gave the lowest value for root length (11.5, 16.5 cm), number of branches/ plant (6.67, 7.66), shoot fresh weight (72.70 , 124.7 g), root fresh weight (11.40, 16.22 g), shoot dry weight (8.40, 12.41 g), root dry weight (1.52, 2.72 g), shoot length (39.13, 104.33cm), number of leaves/ plant (25, 39.33) and leaf area (303.76, 515.03 cm^2) at vegetative stages during the two seasons, respectively (Table 3, Figure 1)

Table (3). Effect of biofertilizers on growth parameters of coriander (*C. sativum* L.) plants during two seasons at vegetative stages.

Treatment		Parameter											
NPK (%)	Biological	Root length (cm)		Number of branches/ plant		Shoot fresh weight (g)		Root fresh weight (g)		Shoot dry weight (g)		Root dry weight (g)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
0	S.W	11.5g	12.5g	6.6f	6.9g	72.7g	75.2g	11.4e	11.7f	8.4b	8.6g	1.5f	1.8f
	AMF	17.3d	14.9f	7.2e	7.8f	77.0f	78.7f	13.9d	14.3e	9.7ab	10.0f	2.0e	2.4e
	SW+AMF	14.0f	17.1e	9.3d	9.4e	82.0e	83.6e	15.4c	15.8d	10.3ab	11.3e	2.7d	2.8d
50	S.W	18.5c	19.2c	11.0b	12.0c	89.7c	91.3c	17.8b	18.4c	11.0ab	13.3c	3.2c	3.9b
	AMF	20.9b	22.4b	11.4b	13.2b	92.9b	94.2b	18.6b	19.5b	12.9a	14.0b	3.8b	4.0b
	SW+AMF	23.1a	23.9a	12.3a	14.4a	96.0a	97.6a	20.8a	21.6a	13.7a	15.3a	4.2a	4.8a
100	0	16.2e	18.6d	10.0c	11.2d	88.0d	89.0d	17.2b	17.5c	10.7ab	12.1d	2.9d	3.1c
LSD at 5%		0.4	0.2	0.4	0.4	1.6	0.9	1.5	1.1	4.4	0.1	0.2	0.23

Values in each column for each harvest followed by the same letter are not significantly different (p=0.05) Duncan multiple range test.

Table (4): Effect of biofertilizers on N, P, K, Mg and Ca contents **of coriander** (*Coriandrum sativum* L.) plants during two seasons at vegetative stages.

Tr.	**Parameter**										
NPK	Biological	N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
0	S.W	2.3f	2.6d	0.3g	0.3c	2.6g	2.7g	1.0g	1.0g	0.4f	0.4b
	AMF	2.6e	2.8cd	0.3f	0.3bc	2.7f	2.9f	1.0f	1.0f	0.4e	0.4b
	SW+AMF	2.8d	2.9bcd	0.4e	0.4abc	3.1e	3.3e	1.1e	1.1e	0.4d	0.5ab
50	S.W	3.0c	3.2ab	0.4c	0.5abc	3.5c	3.6c	1.2c	1.2c	0.5b	0.5ab
	AMF	3.2b	3.3ab	0.4b	0.6ab	3.6b	3.7b	1.2b	1.2b	0.5a	0.5ab
	SW+AMF	3.4a	3.5a	0.4a	0.6a	3.7a	3.9a	1.3a	1.3a	0.5a	0.8a
100	0	3.0c	3.1abc	0.4d	0.4abc	3.2d	3.4d	1.2d	1.2d	0.5c	0.5ab
LSD at 5%		0.1	0.4	6.9	0.2	0.1	0.1	7.1	8.0	8.4	0.3

Values in each column for each harvest followed by the same letter are not significantly different (p=0.05) Duncan multiple range test.

N, P, K, Mg and Ca content

The findings demonstrated that the foliar application of seaweed extract (SWE) and arbuscular mycorrhizal fungus (AMF) inoculation had a substantial (P < 0.05) impact on the content of N, P, K, Ca, and Mg. Generally speaking, it is discovered that adding NPK fertilizer along with AMF and SWE caused the mean values to significantly rise of mineral contents (N, P, K, Ca and Mg) of *C. sativum* plants across the two seasons.

The most successful combination was that of arbuscular mycorrhizal fungus (AMF) and 50% of the suggested NPK dose, which was followed by the combination of AMF, SWE, and 50% of the recommended NPK dose (Table 4). Nonetheless, the coriander plants treated with SWE alone showed the lowest levels of N, P, K, Ca, and Mg in the shoot and root.

Photosynthesis pigments (chlorophyll a, chlorophyll b, chlorophyll a+b and cartenoid) contents

Figure (2) depicts the impact of the following applied treatments on the pigments (chlorophyll a, b, a+b, and cartenoid) contents of coriander plants at vegetative stages over the course of two seasons: chemical fertilizer levels (NPK), arbuscular mycorrhizal fungi (AMF), and seaweed extract (S.W.). Figure (2) shows significant changes (P≤0.05) in the contents of coriander in terms of chlorophyll a, chlorophyll b, chlorophyll a+b, and cartenoid. Generally, the presence of AMF significantly increased chlorophyll a, chlorophyll b, chlorophyll a+b, and

cartenoid) contents of coriander plants figure (2).

The combined treatments significantly increased (P≤ 0.05) the chlorophyll a, chlorophyll b, chlorophyll a+b, and cartenoid contents of coriander compared to the individual treatments.The indigenous AMF inoculation which was combined with the NPK fertilizers at 50% resulted in significant improvement in the chlorophyll a, chlorophyll b, chlorophyll a+b, and cartenoid when it is compared to the control (100% NPK).

Concerning the dual treatments, it was observed that the dual treatment (Arbuscular Mycorrhizal Fungi (AMF) + seaweed extract (S.W.) + 50% of the recommended dose of NPK) was more favorable than the other dual treatments and control (100% NPK) at the vegetative stages in both seasons, and the descending order was as follows: AMF+S.W. + 50% NPK > AMF + 50 % NPK).

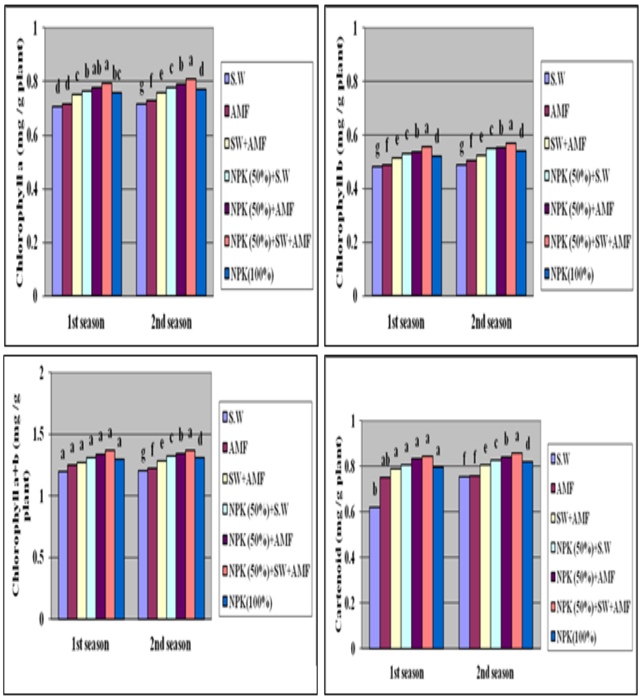

Figure 2. Effect of biofertilizers on pigments (chlorophyll a, chlorophyll b, chlorophyll a+b and cartenoid) of coriander (*C. sativum*) plants during two seasons at vegetative stages.

Carbohydrates content

Figure 3 shows the effects of the applied treatments—chemical fertilizer levels (NPK), arbuscular mycorrhizal fungi (AMF), and seaweed extract (S.W.) either separately or in combination on the amount of carbohydrates in coriander plants during their vegetative stages over the course of two seasons.

The various treatments had a substantial (P≤0.05) impact on the amount of carbohydrates in coriander plants during their vegetative phases over the course of two seasons.

The highest values of the carbohydrates content, which were tested over the course of two seasons, generally tended to be under the AMF + S.W. + 50% NPK treatment. The carbohydrate content values in this therapy were greater than those in the 100% NPK control group (Figure 3).

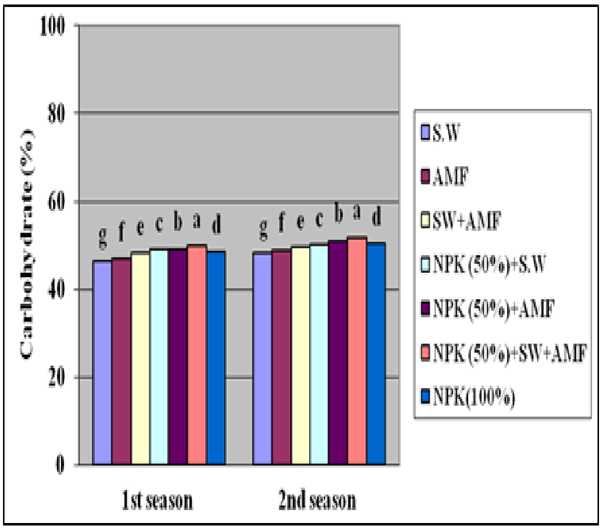

Figure 3. Effect of biofertilizers on carbohydrates content o**f coriander** (*C. sativum* L.) plants at vegetative stages during two seasons.

Table (5). Frequency of mycorrhizal colonization (F), intensity of mycorrhizal colonization (M) and arbuscular frequency (A) in root tissue of coriander plants (*C. sativum*) under different biofertilizers treatments.

Treatment		**Mycorrhizal colonization levels (%)**					
NPK	Biological	F		M		A	
		2019	2020	2019	2020	2019	2020
0	S.W	0.0	0.0	0.0	0.0	0.0	0.0
	AMF	70.0	70.2	45.3	46.0	25.1	25.5
	SW+AMF	75.5	75.8	55.0	54.6	33.2	34.0
50	S.W	0.0	0.0	0.0	0.0	0.0	0.0
	AMF	77.0	77.2	55.8	56.0	30.8	30.5
	SW+AMF	85.5	85.8	62.0	64.6	38.2	39.0
100	0	0.0	0.0	0.0	0.0	0.0	0.0

Values in each column for each harvest followed by the same letter are not significantly different (p=0.05) Duncan multiple range test

Mycorrhizal root colonization

Arbuscular mycorrhizal fungus (AMF) and 50% of the prescribed NPK dose proved to be the most effective combination. AMF, SWE, and 50% of the recommended NPK dose were the next most successful combinations (Table 4). However, the coriander plants treated with SWE alone had the lowest shoot and root levels of N, P, K, Ca, and Mg.

Discussion

Seaweed extract (SWE) and arbuscular mycorrhizal fungi (AMF) were used in tandem in this study to improve the growth parameters of the C. sativum plants, including root length, number of branches/plant, shoot fresh weight, shoot dry weight, shoot length, number of leaves/plant, and leaf area; photosynthesis pigments content (Chlorophyll a, b, a+b, and carotenoids content); total soluble carbohydrates content; and N, P, K, Mg, and Ca contents (Table 3 and figure 1).

Plant growth and biomass are enhanced by AMF inoculation, which may be related to an increase in internal hormone levels (23). Growth promotion is thought to change the plant's hormonal state, which greatly boosts nutrient uptake (24). (12) observed that the

use of AMF boosted the growth parameters of the sweet basil (Ocimum basilicum L) plant, which is consistent with our results. Additionally, (5) and (25) reported that through a symbiotic relationship and the development of an extensive hyphal system, the arbuscular mycorrhizal fungus enhanced growth parameters photosynthesis pigments content (Chlorophyll a, b, a + b, and carotenoids content), total soluble carbohydrates content, and nutrients content in tomato plants (Solanum lycopersicum L. cv. "Rio Fuego") and fennel plants (Foeniculum vulgare L) plants.

Additionally, since seaweed extract contains micro and macronutrients, vitamins, amino acids, and plant hormones (gibberellin and cytokinin), the improvement in the growth parameters of coriander plants after applying SWE can be attributed to the roots' absorption of nutrients and water as well as the action of plant hormones to promote growth. Additionally, SWE improves the growth characteristics of plants by increasing the turgor pressure and division of meristem cells.

In agreement with our findings, (13) reported that the foliar utilization of SWE had a considerable impact on the plant height (cm) and number of branches/plant of *C. sativum*. Also, (14) reported that plant fresh and dry weights/fed and photosynthetic pigments (chlorophyll a, b and carotenoids) of parsley (*Petroselinum crispum* (Mill.) were increased by using SWE.

The co-application of AMF and SWE in the current experiment resulted in a significant increase in the growth parameters (plant height, leaf number and dry weight), photosynthesis pigments content (chlorophyll a, b, a + b, and carotenoids content), total soluble carbohydrates content, and NPK contents.In accordance with our findings, (5) reported that the co-application of AMF and SWE improved the fennel (F. vulgare) plants' growth parameters (plant height, leaf number, and leaf dry weight), as well as the content of photosynthesis pigments (chlorophyll a, b, a+b, and carotenoids), total soluble carbohydrates, and NPK through a symbiotic relationship and the development of an extensive hyphal system. Furthermore, it was observed by (25) that the combination of AMF and SWE had a substantial impact on the growth metrics and physiological parameters of photosynthesis in tomato plants (S. lycopersicum).

Conclusions

The best results with regard to the growth parameters (root length, number of branches/plant, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight shoot length, number of leaves/plant and leaf area), photosynthesis pigments content (Chlorophyll a, b, a + b, and carotenoids content), total soluble carbohydrates content as well as the N, P, K, Mg and Ca content of coriander were obtained as a result of the use of 50% of the recommended dose of NPK chemical fertilizers in combination with arbuscular mycorrhizal fungi and seaweed extracts.

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