

A tool for simplified evaluation of salt and heavy metal stress – Example of salt stressed basil plants (*Ocimum basilicum*) amended with castor cake

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ABSTRACT

A tool is proposed for integrating the results from studies on alleviation of heavy metal or salt stress in plants. The results of such studies are usually presented as separate variables for each treatment, but their interpretation is not intuitive, and sometimes what is assumed as stress alleviation is simply the balance between the detrimental effect of the stress and the beneficial effect of the alleviating treatment. The synergy index integrates the results from the stressing, alleviating, and combined treatments in just one value that objectively indicates if the plant stress was alleviated. The tool is demonstrated in a study on basil (*Ocimum basilicum*) exposed to salinity and amended with castor cake for stress alleviation. The concept is also illustrated on the results extracted from several research papers on plant stress alleviation.

1. Introduction

Accumulation of salts and toxic metals is a major challenge for agriculture worldwide. These toxic elements are detrimental for plant growth and productivity, and they accumulate in the soil for many reasons, such as industrial activity, mining, irrigation, or for natural reasons (Chen et al., 2025; Corwin, 2021; Hao et al., 2025; Mukhopadhyay et al., 2021). In the coming decades, there is a risk that more cultivated land will accumulate salts and metals to toxic levels because of climate change and increased mining and industrial activity. Considering the importance of these abiotic stresses, this article proposes a tool designed to simplify the analysis of experiments on alleviation of salt and heavy metal toxicity in plants. The problem to be addressed is that the results of such experiments are presented as multiple opposing variables, in which the interpretation is not trivial (Danish et al., 2024; Dooz et al., 2024; Guo et al., 2025; Oliveira et al., 2025).

Treatments applied for alleviation of salt or heavy metal stress

include organic amendments, biochar-based products, hormones, osmotically active substances, microorganisms, among others. Predominantly, the experiments evaluate if a given factor is effective to alleviate a specific plant stress or toxicity. The results are presented as separate variables regarding (1) a control, in which the plant is exposed neither to the stressing factor nor to the alleviating treatment, (2) only the stressing treatment, (3) only the alleviating treatment, and (4) the combination of the stressing factor with the alleviating treatment. As a rule, the stress causes a detrimental effect on plant growth or production, while the alleviating treatment causes a beneficial effect. The scientific concern that this tool aims to clarify is on the interpretation of those four variables because it is frequent that the beneficial effect is mistakenly assumed as an alleviation effect. When the results are presented as four separate variables, it is not intuitive if the observation made in the treatment combining stress and alleviating factor is simply the sum of the detrimental effect of the stress and the beneficial effect of the alleviation treatment or if the alleviation treatment is really relieving the stress.

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It is plausible that, in some cases, plants respond to the stress and to the alleviating factors as independent effects, and the observed result is just their sum. However, in other cases, there are complex interactions that deviates the result from the sum of the independent effects. The effective stress alleviation requires a positive balance between those single effects, but such balance is not commonly presented.

Integrative indexes are broadly adopted in scientific studies, particularly when there are many variables of interest, and an objective result is required for making a decision. For instance, plant breeding developed several integrative indexes such as stress tolerance index, yield stability index, and mean productivity index as tools for easier selection of genotypes under complex influences, and they were proven effective for the selection of superior genotypes for stressful conditions (Gaikwad et al., 2025; Lamba et al., 2023; Porch, 2006).

This article proposes an easy tool for integrating the four variables usually presented in studies of stress alleviation in plants. It consists in the balance between the effects of the stressing and the alleviating factors. It provides for an easier interpretation because the four variables are integrated in just one meaningful variable, which is called “synergy index”.

The synergy index is demonstrated in a study with basil plants (*Ocimum basilicum*) stressed by irrigation with saline water and amended with castor cake as the alleviating treatment. Basil is an herb cultivated all over the world as edible vegetable (spice) or for production of essential oils and other metabolites with aromatic and pharmacologic properties (Farouk et al., 2020; Tolay, 2021). Castor cake is the main by-product of castor oil extraction, and it is predominantly used as organic amendment with nematicide properties (Cardoso et al., 2020; Galbieri et al., 2024; Mello et al., 2018; Parecido et al., 2024; Pedroso et al., 2018, 2020; Rocha et al., 2022). The synergy index is also illustrated using the results from several research articles recently published on alleviation of heavy metal toxicity and salt stress.

The objective of this article is to present synergy index as a tool to integrate the variables commonly measured in studies on plant stress alleviation and demonstrate it in an experiment with basil plants and on several research papers found in the literature.

2. Material and methods

2.1. Calculation of the synergy index on the alleviation of plant stress

The isolated effects of the stressing treatment, the alleviating treatment, and the combined treatments were calculated for each variable as the difference to the control treatment. The synergy index was calculated as the effect of the combined treatments minus the effect of the two isolated treatments. For each variable of growth, yield or leaf and soil chemical composition, the isolated effects and the synergy index were calculated as follow:

$$(i) \vec{S} = X_S - X_C$$

$$(i) \vec{A} = X_A - X_C$$

$$(i) \vec{A/C} = X_{A/C} - X_C$$

$$(i) \vec{Synergy} = \vec{A/C} - \vec{S} - \vec{A}$$

in which:

\vec{S} is the isolated effect of the stressing treatment

\vec{A} is the isolated effect of the alleviating treatment

$\vec{A/C}$ is the isolated effect of the alleviating and stressing combined treatment

$\vec{Synergy}$ is the indicator of plant stress alleviation

X_S is the value observed in the stressing treatment

X_C is the value observed in the control treatment

X_A is the value observed in the alleviating treatment

$X_{A/C}$ is the value observed in the combined alleviating and stressing treatment

2.2. Experiment of salt stressed basil plants amended with castor cake

This experiment was carried out in *Universidade Federal da Paraíba* (Areia, PB, Brazil) from September to December/2020. Basil seeds cv. Basilicão alfavaca (ISLA®) were sown (four seeds per cell) in plastic trays filled with the substrate Bioplant® (composed by a mix of coconut fiber, pine bark, manure, sawdust, vermiculite, rice husk, ash, agricultural gypsum, and calcium carbonate). At 10 days after sowing (DAS), the plants were thinned to one plant per cell. At 25 DAS, the plants had three pairs of fully expanded leaves and were transplanted to 8 L pots filled with loam sandy soil (collected in the layer 0–20 cm).

All the chemical and physical analysis on soil were made according to Teixeira et al. (2017). The initial chemical characteristics of the soil were as follow: pH 5.5, 5 g/kg of organic matter, 0.47 mg/kg of phosphorus, 33.0 mg/kg of potassium, 0.65 cmol_c/kg of calcium, 0.25 cmol_c/kg of magnesium, 0.03 cmol_c/kg of sodium, 1.75 cmol_c/kg of H+Al³⁺, 0.10 cmol_c/kg of aluminum, 0.67 dS/m of electric conductivity, and 1.34 of sodium adsorption ratio. The physical characteristics of the soil were 836 g/kg of sand, 88 g/kg of silt, 76 g/kg of clay, degree of flocculation of 1000 kg/dm³, 1.44 g/cm³ of density, 2.71 g/cm³ of particle density, and 0.47 m³/m³ of total porosity.

Four treatments were assigned to a Randomized Blocks Design with four replications. The treatments were (i) a control irrigated with tap water with electrical conductivity of 0.5 dS/m; (ii) amended with castor cake in the dose of 17 g/plant; (iii) irrigated with saline water (3 dS/m), and (iv) a combination of the treatments with organic amendment and irrigation with saline water.

The organic amendment was previously mixed to the substrate, one week before transplanting, and irrigated with tap water. The pots were placed in a greenhouse with temperature of 29 ± 3 °C and relative humidity 59 ± 10 % (daily measurements made with a digital thermo-hygrometer). Castor cake was supplied by a local castor oil extraction industry (Natal, RN, Brazil), and it had the following chemical characteristics: 49.4 g/kg of nitrogen, 13.2 g/kg of phosphorus, 9.7 g/kg of potassium, 3.3 g/kg of sulfur, 22.9 g/kg of calcium, 0.87 g/kg of magnesium, 27 mg/kg of boron, 21 mg/kg of copper, 222.2 mg/kg of iron, 293 mg/kg of manganese, and 126 mg/kg of zinc.

The saline water was prepared mixing tap water (0.5 dS/m) with sodium chloride (NaCl), calcium chloride (CaCl₂·2H₂O), and magnesium chloride (MgCl₂·6H₂O), in the respective proportion of 7:2:1 (w:w:w). The quantity of salts added to the water followed the approximation of 1 mmol_c/L = electric conductivity x 10 aiming to reach 3.0 dS/m, assuming the relation of electric conductivity x 10 = concentration of salts (mmol_c L⁻¹) (Rhoades et al., 1992). The ratio Na:Ca:Mg approaches the predominant composition of water used for irrigation in the semiarid region of Brazil. The saline solutions were stored in 60 L plastic buckets until they were used for irrigation. The chemical composition of the saline water was measured (Table 1), and the electrical conductivity was checked every week with a portable conductivity meter (model CD 680, Instrutherm, USA). The salinity was stable along the experiment period.

The lysimeter method was employed to control the amount of irrigation water (Bernardo et al., 2009). Prior to the experiment, the maximum water retention capacity (field capacity) was estimated as the weight of the pot when the substrate was saturated with water. The basil plants were transplanted, and each pot was daily weighed and irrigated with saline or tap water in a volume to reach 80 % of the weight at field capacity. As this was a short-duration experiment, the pots were not irrigated with water enough to drain. The salts did not accumulate to a critical level.

Table 1

Chemical characteristics of the irrigation water used in the control and salinity treatments.

Treatment	pH	SO ₄ ²⁻	Mg ²⁺	Na ⁺	K ⁺	Ca ²⁺	CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻
		mmol/L							
Control	7.0	0.10	0.93	2.28	0.26	1.08	0.0	2.67	4.17
Salinity	7.4	0.32	5.20	18.09	0.28	9.90	0.50	1.70	39.70

2.3. Measurement of growth and essential oil content

The basil plants were harvested at 85 DAS (after 60 days exposed to the treatments). Leaf area was estimated with the method of leaf discs. At harvest, 25 leaf discs (0.79 cm²) were collected on the third leaf from the top of each plant, oven dried (65 °C, 24 h), and weighed. The plants were separated in four components: leaves, branches, inflorescences, and roots. Leaves were washed in distilled water for the measurements of nutrients content (Section 2.4). Each component was oven dried (65 °C, 72 h) and weighed. Leaf area was calculated with the dry weights of leaf discs and leaves. The shoot dry weight was the sum of leaves, branches, and inflorescences dry weight.

The content of essential oil was measured in 50 g samples of leaves and inflorescences. The samples were crushed, and the essential oil was extracted with steam distillation for 90 min. Anhydrous sodium sulfate was added to the hydrolate for 30 min to remove water, the salt was filtered, and the essential oil was weighed (Machado et al., 2022).

2.4. Nutrient content in the leaves

Oven-dried leaf samples (as described in the Section 2.3) were ground in a Wiley mill. Nitrogen was measured with the Kjeldahl method. The samples were weighed, mixed with sulfuric acid and a catalyst, digested for 2 h at 420 °C, mixed with NaOH, and distilled to release the ammonium (nitrogen), which was trapped in a solution of boric acid. The solution was titrated with NaOH for determining the total nitrogen. For measuring the content of the other nutrients, the samples were weighed, mixed with HNO₃, digested at 115 °C for 30 min, cooled, mixed with HClO₄, and digested at 170 °C for 2 h (Miyazawa et al., 2009). The content of P was measured with UV–VIS spectrophotometry at 660 nm after mixing the extract with molybdate (MoO₄) (Miyazawa et al., 2009). The contents of K and Na were measured by flame spectroscopy. The content of Ca and Mg were measured by atomic absorption spectrophotometry (Shimadzu AA-6300, Kyoto, Japan). The content of S was measured by turbidimetry (Miyazawa et al., 2009). The ratios of the three main cations in relation to sodium (K:Na, Ca:Na, and Mg:Na) were calculated according to the content of each element in the leaves.

2.5. Soil chemical composition

One sample of soil was collected in each pot in the same day that the basil plants were harvested. The electrical conductivity was measured in the saturation extract (Richards, 1954). Soil organic matter content was determined using the method of chromic acid wet oxidation (Walkley and Black, 1934). The available phosphorus (P), exchangeable potassium (K⁺), and sodium (Na⁺) contents were determined in the Mehlich extract. The exchangeable calcium (Ca²⁺) and magnesium (Mg²⁺) were measured in KCl extracts (Teixeira et al., 2017). The ratios of the three main cations in relation to sodium (K:Na, Ca:Na, and Mg:Na) were calculated according to the content of each ion in the substrate.

2.6. Calculations, statistical procedures, and presentation of results

Statistical analysis were performed on the software R (v. 3.6.3) with the package easyanova. The data were subjected to analysis of residual normality using the Shapiro-Wilk test, homogeneity of variances using the Bartlett test, followed by analysis of variance using the F test

($p < 0.05$) and Tukey test ($p < 0.05$). The results were presented in tables with indication of the significant differences between means. This is the traditional method of presenting the results from studies on plant stress alleviation.

The synergy index was calculated for each variable related to plant growth, essential oil yield, leaf composition, and soil chemical characteristics. The synergy index was calculated for each replicate ($n = 4$), and the replicated effects were subjected to t test to measure if the mean was significantly different from zero ($p < 0.05$).

Aiming to illustrate the presentation of the synergy index in two different styles, the variables related to plant growth and leaf composition were presented in tables and the variables related to soil chemical characteristics were presented in a chart.

2.7. Synergy index applied on research papers on plant stress

A search was made in March/2025 on the Scopus database using the key words [“salt stress” OR salinity OR “heavy metals”] AND [“plant growth” OR productivity OR yield] AND [alleviati*], published after 2014.

Seven research papers published in 2024 or 2025 were selected for illustrating how the synergy index could be measured, including one case of the alleviating treatment applied in doses (in which regression analysis could be applied). The values for one variable were selected from each research paper, and the calculations were made for the isolated effects and for the synergy index. Statistical analysis were not performed on the data obtained from the research papers because only the means were considered (not the raw data with replications).

3. Results and discussion

3.1. Plant growth, leaf composition, and substrate chemical characteristics

As the main objective of this article is to present and discuss the synergy index, this section just briefly presents the effect of the treatments on plant growth, leaf composition, and substrate chemical characteristics. The mean values observed in the control, stressing (saline water), alleviating (castor cake), and combined stressing and alleviating treatments were presented with means comparison (Tables 2 and 3).

The organic amendment favored the growth of basil plants, while the irrigation with saline water was detrimental for biomass accumulation and essential oil yield (Table 2). The treatments also influenced some chemical characteristics of the substrate. The treatments had remarkable effect on the leaf area and consequently on the essential oil yield. All the other characteristics related to plant growth and leaf composition were significantly influenced, except the potassium content on the leaf. The treatments significantly influenced all variables related to chemical characteristics of the substrate, except the ratio of calcium and magnesium to sodium (Table 3).

3.2. Synergy index - integration of variables for easier interpretation

The synergy index alone would be enough for evaluating the stress alleviation. Anyway, the isolated effects of each treatment were presented for easier understanding of its calculation (Table 4). The interpretation of the synergy index can be made as follows.

When basil plants were exposed to saline water, the leaf area was

Table 2

Growth and nutrient leaf content of macronutrients and sodium of basil plants treated with combinations of castor cake amendment and irrigation with saline water.

Growth and leaf nutrients	Castor cake	Saline water	Castor cake + saline water	Control
Leaf area (cm ² /pl)	937 ^b	547 ^c	1409 ^a	812 ^{bc}
Shoot weight (g/pl)	37.8 ^a	20.3 ^b	35.5 ^a	25.0 ^b
Root weight (g/pl)	1.43 ^a	0.25 ^c	0.68 ^b	0.45 ^{bc}
Essential oil yield (g/pl)	0.42 ^b	0.17 ^c	0.59 ^a	0.32 ^b
Leaf N (g/kg)	14.98 ^a	8.93 ^b	17.13 ^a	6.25 ^b
Leaf P (g/kg)	3.03 ^a	0.83 ^c	2.75 ^a	1.83 ^b
Leaf K (g/kg)	31.1 ^a	28.3 ^a	31.6 ^a	25.8 ^a
Leaf Ca (g/kg)	17.4 ^c	24.1 ^b	30.6 ^a	13.8 ^c
Leaf Mg (g/kg)	3.60 ^c	5.95 ^b	6.78 ^a	2.23 ^d
Leaf S (g/kg)	3.36 ^{ab}	2.90 ^b	4.30 ^a	3.03 ^b
Leaf Na (g/kg)	0.16 ^b	0.24 ^a	0.14 ^b	0.18 ^b
Leaf K/Na ratio	200.1 ^{ab}	119.8 ^b	225.9 ^a	147.7 ^b
Leaf Ca/Na ratio	112.9 ^b	100.9 ^b	216.3 ^a	78.3 ^b
Leaf Mg/Na ratio	23.2 ^{bc}	24.9 ^b	48.3 ^a	12.8 ^c

The means followed by the same letter in the row are not significantly different by the test of Tukey ($p < 0.05$).

Table 3

Substrate chemical composition at the end of the experiment with basil plants growing under treatment with combinations of castor cake amendment and irrigation with saline water.

Characteristic	Castor cake	Saline water	Castor cake + saline water	Control
Organic matter (g/kg)	6.45 ^a	5.63 ^{ab}	6.70 ^a	4.35 ^b
Electric conductivity (dS/m)	3.45 ^c	10.35 ^a	6.10 ^b	2.65 ^c
Substrate P (mg/dm ⁻³)	15.08 ^a	8.63 ^b	14.85 ^a	11.35 ^{ab}
Substrate K (mg/dm ⁻³)	116.6 ^b	97.0 ^c	136.4 ^a	59.1 ^d
Substrate Ca (cmol _c /dm ⁻³)	1.93 ^b	4.48 ^a	4.65 ^a	1.38 ^b
Substrate Mg (cmol _c /dm ⁻³)	0.80 ^b	1.88 ^a	1.95 ^a	0.75 ^b
Substrate Na (cmol _c /dm ⁻³)	1.08 ^b	3.03 ^a	2.40 ^a	0.80 ^b
Substrate K/Na ratio	120.7 ^a	32.6 ^b	60.7 ^{ab}	84.1 ^{ab}
Substrate Ca/Na ratio	1.98 ^a	1.50 ^a	2.12 ^a	1.97 ^a
Substrate Mg/Na ratio	0.91 ^a	0.63 ^a	0.89 ^a	1.08 ^a

The means followed by the same letter in the row are not significantly different by the test of Tukey ($p < 0.05$).

reduced by 265 cm²/plant in comparison with the control treatment, and when the plants were amended with castor cake, the leaf area increased by 125 cm²/plant (Table 4). If the plants responded to the opposing treatments as independent effects, the leaf area in the combined treatment should be a reduction on leaf area by 140 cm²/plant (i. e., 125 – 265 cm²/plant); however, the result was a rise in leaf area by 597 cm²/plant. The synergy index of 737 cm²/plant means that the leaf area grew on that amount in excess of what was expected by the isolated effects of the salinity stress and the organic amendment. As this value is positive and statistically significant (different from zero), the researcher may affirm that castor cake alleviated the effect of salt stress on the leaf area of basil plants.

The synergy index was not significant on shoot dry weight. When considered the four variables separately (as traditionally reported), both the amendment with castor cake and the combined treatment were significant in comparison with the control treatment, and they could be mistakenly interpreted as significant stress alleviation (Table 2); however, the shoot dry weight observed in the combined treatment did not exceed the sum of the isolated effects of the saline water and organic amendment, and the synergy index was statistically equal to zero. In this

Table 4

Isolated effects of the stressing treatment, alleviating treatment, combined stressing + alleviating treatment, and synergy index on plant growth, essential oil yield, and leaf composition of salt-stressed basil plants amended with castor cake as alleviating treatment.

Isolated effect	Synergy index
<u>Leaf area (cm²/plant)</u>	
Stressing treatment	–265
Alleviating treatment	125
Stress + alleviation	597
<u>Shoot dry weight (g/plant)</u>	
Stressing treatment	–4.75
Alleviating treatment	12.75
Stress + alleviation	10.50
<u>Root dry weight (g/plant)</u>	
Stressing treatment	–0.20
Alleviating treatment	0.98
Stress + alleviation	0.23
<u>Essential oil yield (g/plant)</u>	
Stressing treatment	–0.15
Alleviating treatment	0.10
Stress + alleviation	0.27
<u>Leaf nitrogen content (g/kg)</u>	
Stressing treatment	2.68
Alleviating treatment	8.73
Stress + alleviation	10.88
<u>Leaf phosphorus content (g/kg)</u>	
Stressing treatment	–1.00
Alleviating treatment	1.20
Stress + alleviation	0.93
<u>Leaf potassium content (g/kg)</u>	
Stressing treatment	2.50
Alleviating treatment	5.30
Stress + alleviation	5.80

case, the researcher would conclude that castor cake did not alleviate the salt stress on the shoot dry weight of basil plants.

The synergy index can assume negative and significant values, as observed on the root dry weight (Table 4). In this case, the researcher should interpret accordingly if that is a beneficial or detrimental outcome. For instance, someone can interpret that the shoot is the harvestable product of basil plants; therefore, a reduced root biomass is not detrimental for the farmer. Others can interpret that a reduced root system can compromise the plant growth in the long run. The synergy index is just the integration of variables, and it is open for subjective interpretation. A negative synergy index could also be called “antagonism” whenever it expresses with more precision the researcher’s interpretation.

The substrate chemical characteristics were presented in a figure aiming to demonstrate this possibility (Fig. 1). For each variable, the figure displays brown-open arrows, which represent the isolated effect of the alleviating effect (castor cake), blue-closed arrows, which represent the isolated effect of the stressing treatment (salt stress), and black thin arrows, which represent the synergy index. The arrows are pointing to the right when the effect is positive and to the left when negative. The arrows length is proportional to the intensity of the effect. The isolated effects were presented only for the stressing and alleviating treatments, while the statistical significance was displayed only for the synergy index. The synergy index is intended to simplify the analysis and presentation of results. Therefore, Fig. 1 could be simplified to present only the synergy index, which is the most relevant information; however, the detailed effects were presented here aiming to provide a clear explanation of the tool for calculating and interpreting the synergy index.

3.3. Synergy index on experiments with quantitative variables

The synergy index can be calculated for experiments testing doses of either alleviating or stressing treatments. The example presented (Fig. 2) considered the doses of methyl jasmonate as the alleviating treatment of narcissus plants exposed to saline water with electrical conductivity of 8

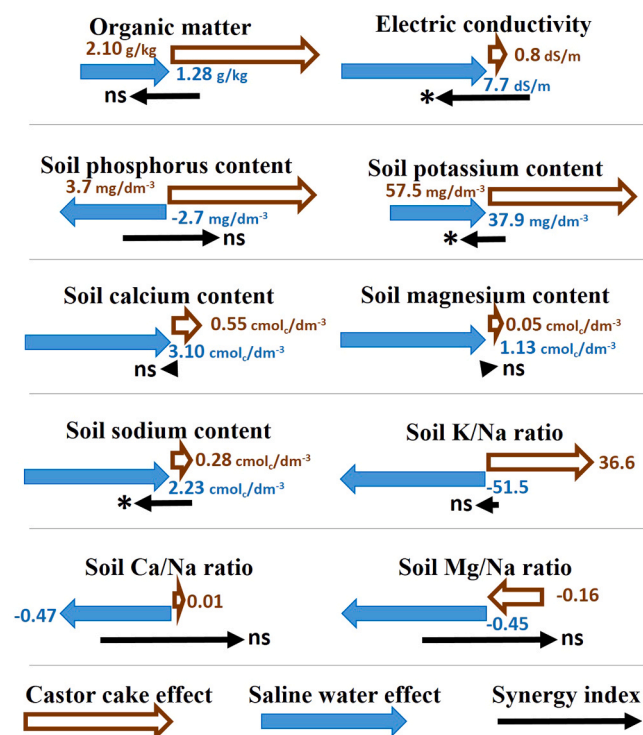


Fig. 1. Isolated effects and synergy index of the castor cake (organic amendment) and the saline water used for irrigation on the chemical characteristics of the substrate cultivated with basil plants. The effect of the organic amendment (brown) or the saline water (blue) are presented besides the arrow. The arrow points to the right when the effect is positive and to the left when negative. The synergy index was significant ($p < 0.05$) or insignificant if the black arrow is followed by * or ns, respectively.

dS/m (NaCl) in the experiment reported by Dooz et al. (2024). The synergy index could also be presented for increasing doses of the stressing factor exposed to a constant dose of the alleviating treatment. It is also possible to build a surface of response with the synergy index calculated for two alleviating treatments. The data on this example was not subjected to regression analysis, but there is no restriction for applying that statistical procedure for the synergy index.

This experiment with narcissus flower illustrates how conclusions can be questionable if based on intuitive interpretation of the results. The dose of 100 μ M of methyl jasmonate promoted higher plant growth than the dose of 200 μ M, and the authors concluded that the best alleviation of salt stress was obtained on that intermediate dose (Dooz et al., 2024). Nevertheless, that dose did not alleviate the stress, but it was just the optimal dose for promoting plant growth, and the synergy index at the dose of 100 μ M of methyl jasmonate was in fact negative (Fig. 2). The real stress alleviation was observed in the dose of 200 μ M, which was not adequate for plant growth, but resulted in a positive synergy index in the plants exposed to the stress.

3.4. How the scientific literature reports the alleviation of plant stress

The search made on Scopus for reports on alleviation of salt and heavy metal stress in plants in the last 10 years reached 1878 hits. Many of those reports were not research articles on the specific subject of the present article, as they had other approaches such as genes regulation, microbial communities, other abiotic stresses, and review articles. Some articles were not considered because they have not all the treatments necessary (usually missing the alleviation treatment in the absence of the stressing factor). Six recent articles were selected, and the results were extracted for calculating the isolated effects and synergy index (Table 5). As a rule, experiments in which the stress was artificially

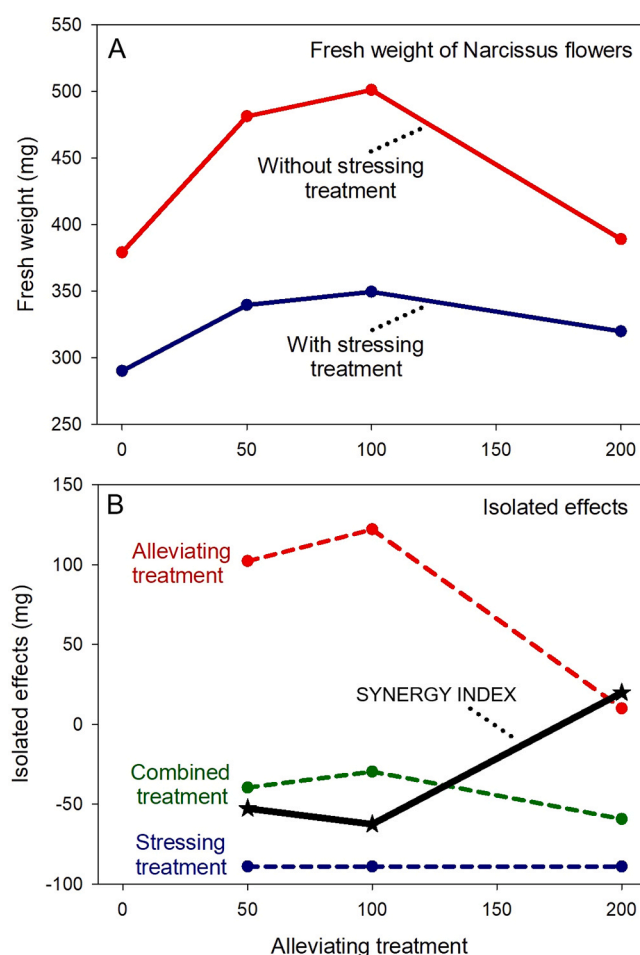


Fig. 2. A: Isolated effects on the fresh weight of narcissus flowers exposed to doses of methyl jasmonate varying from 0 to 200 μ M (alleviating treatment) with and without a stressing treatment (NaCl, 8 dS/m) (source: Dooz et al., 2024). B: synergy index and isolated effects of the alleviating, stressing, and combined treatments.

created (e.g., irrigation with saline water or heavy metals added to the substrate compared with a control treatment) present the data that could be used to calculate the synergy index, although an equivalent integration variable was not found (e.g., Danish et al., 2024; Guo et al., 2025; Oliveira et al., 2025). However, the synergy index could not be estimated (as proposed in this article) in experiments lacking a control treatment, such as experiments performed directly in a contaminated or salinized soil (e.g., Farid et al., 2025; Xiao et al., 2025). For allowing statistical analysis, the experimental design should preferentially be in blocks, in which repeated and independent estimations of the synergy index can be made.

The synergy index calculated on some other studies found in the literature, in which organic amendments or other soil conditioners were used to alleviate salt stress, demonstrated that most frequently the outcome was not positive for the plant growth or seed yield (Ding et al., 2020; Farsaraei et al., 2020; Naveed et al., 2020; Rezaie et al., 2019; Sikder et al., 2020; Tolay, 2021). Although there were also examples of beneficial synergy index, such as on the biomass of dill plants (*Anethum graveolens*) growing in biochar-based nanocomposites (Rahimzadeh, Ghassemi-Golezani, 2022) or in the leaf area of common bean (*Phaseolus vulgaris*) amended with biochar (Farhangi-Abri and Torabian, 2018).

The scientific articles on this subject are not providing an integrating variable as herein proposed, but the results are commonly presented as separate variables, what makes the interpretation difficult and sometimes questionable. To the best of our knowledge, no research paper was

Table 5

Studies reporting treatments for alleviation of salt stress in plants, the growth characteristic selected for analysis, the individual effects, and the synergy index.

Stressing and alleviating treatment	Selected characteristic	Individual effects and synergy index	Reference
Salt stress alleviated by melatonin	Shoot dry weight of canola plants (g)	Control treatment 4.4 Stress treatment 2.8 Alleviation treatment 4.9 Stress + alleviation 3.1 Synergy index: -0.2	Ali et al. (2025)
Cadmium toxicity alleviated by arbuscular mycorrhizal inoculation	Shoot dry weight of coriander plants (g)	Control treatment 0.59 Stress treatment 0.40 Alleviation treatment 0.92 Stress + alleviation 0.88 Synergy index: 0.15	Chen et al. (2025)
Salt stress alleviated by seed priming with NaCl	Dry weight of rapeseed seedlings (g)	Control treatment 5.5 Stress treatment 1.0 Alleviation treatment 5.6 Stress + alleviation 2.5 Synergy index: 1.3	Damalas and Koutroubas (2025)
Arsenic toxicity alleviated by pork bone biochar	Fresh biomass of rice plants (g)	Control treatment 31.0 Stress treatment 18.9 Alleviation treatment 36.0 Stress + alleviation 32.0 Synergy index: 8.1	Hao et al. (2025)
Cadmium and nickel toxicity alleviated by endophytic fungus (<i>Fusarium proliferatum</i>)	Shoot and root fresh weight of tomato plants (g)	Control treatment 15.1 Stress treatment 8.7 Alleviation treatment 17.3 Stress + alleviation 12.1 Synergy index: 1.2	Khan et al. (2025)
Salt stress alleviated by plant growth promoting bacteria (<i>Pseudomonas geniculata</i>)	Pod yield of peanut plants (g/plant)	Control treatment 8.3 Stress treatment 5.9 Alleviation treatment 10.6 Stress + alleviation 9.2 Synergy index: 1.0	Trung and Tri (2025)

found proposing an integrating variable for alleviation of plant stress, but as just a limited number of papers were reviewed, it is possible that some other analysis were made with similar objective.

As illustrated along this article, it was frequently found that research papers conclude that a given treatment alleviated the stress, when the alleviating treatment just promoted some plant growth despite the stress; however, the combined treatment did not exceed what was expected from the sum of isolated effects. The synergy index is proposed aiming to improve the reliability of such conclusions.

3.5. Synergy arises from complex interactions

Synergy occurs because plants are able to adjust to the stress/alleviation condition with different responses than the exposure to those treatments separately. Plants have cognitive abilities to optimize its growth considering complex variables sensed on the environment (Bonato et al., 2024; Severino, 2021). If plants only responded to each environmental stimuli in the same predictable way, the output would be always the sum of each individual factor. The search for stress-alleviating treatments requires special attention to the unpredictable and complex interactions that may arise from the opposing effects.

The positive synergy index detected in some variables of the experiment with basil plants illustrates how plants are able to elaborate different strategies when salinity and organic amendments occurred concomitantly. Organic amendments are traditionally used in agriculture, and in general, they are beneficial because they provide nutrients and improve soil properties (capacity for water retention, increased porosity, and higher biodiversity of macro and microorganisms). When toxic salts accumulate in the soil, the increased osmotic potential hinders water uptake and the salts are absorbed, causing several physiological disorders. Basil plants exposed to both conditions reduced the biomass allocation to root growth and shifted the biomass to grow leaves. Considering that plants optimize decisions regarding growth according to the resources abundance or restriction (Severino, 2021),

and nutrients and water were available in this experiment, it was hypothesized that basil plants increased the leaf area to dilute or compartmentalize the excessive sodium, while it reduced root biomass because less roots were required to uptake the amount of nutrients needed for its growth. In other studies with basil plants, promoting factors like vermicompost or nitrogen leaf fertilization caused similar effect, favoring the plant to adjust pigments and carbohydrates content and biomass allocation among compartments as a strategy to cope with salt stress (Reyes-Pérez et al., 2024; Silva et al., 2024).

Sodium plays the major role on the salinity effect because it is abundant in arid regions, it accumulates to toxic levels in the plant, and it reduces the assimilation of other nutrients such as K, Ca, and Mg (Silva et al., 2024; Zamljen and Slatnar, 2024). The positive synergy index arising from the combination of castor cake and salinity reflected the plant's adjustment regarding less sodium accumulation in the leaves and less electrical conductivity rise in the soil of the combined treatment than it would be expected from the sum of the isolated effects (Fig. 2).

The castor cake's capacity to retain sodium would not be beneficial when sodium content was low, but it played a role when high sodium levels were present. A similar effect was also observed in faba bean (*Vicia faba*) amended with biochar (Rezaie et al., 2019). Alleviation of saline effect was also observed in basil plants in a substrate amended with superabsorbent polymers for water retention (Farsaraei et al., 2020) and on canola plants (*Brassica napus*) in a soil treated with manure enriched with calcium (Naveed et al., 2020).

Potassium is the main nutrient with capacity to counterbalance the deleterious effect of sodium because both ions have similar chemical and physiological properties (Tammam et al., 2023). Significant synergy index was detected in potassium content in the substrate, as the K content did not increase as expected from the sum of individual effects. The nutrient was probably uptaken by the plant, but it was not translocated to the leaves (Table 2). It was likely compartmentalized in roots or stems after playing its role as sodium-antagonist.

Organic amendments also change the soil structure and provide an additional factor to counterbalance the effect of saline water. Besides the

toxic effect of salts accumulated in the soil, the poor soil structure (reduced soil aggregates stability) limits the oxygen availability to the roots and reduces percolation of water and nutrients. Castor cake, as a source of organic matter, favors the formation of soil aggregates (Bello et al., 2021), which improve many soil characteristics, such as porosity, aeration, hydraulic conductivity, and water retention. When the soil structure is favorable, less roots are needed for the function of water and nutrients uptake, as was observed in the present study that demonstrated negative synergy index for root biomass.

The complex interactions between organic amendments and salt stress in other studies with basil plants caused adjustments not only in the characteristics directly related to cations content and plant growth, but also in the content of pigments (chlorophyll), phenolics (flavonoids, anthocyanins, terpenes), proteins, antioxidant enzymes (ascorbate peroxidase, catalase, malondialdehyde, superoxide dismutase), and osmoprotectants (proline) (Cabot et al., 2014; Farsaraei et al., 2020; Naveed et al., 2020; Reyes-Pérez et al., 2024; Silva et al., 2024; Zamljen and Slatnar, 2024). The plants also adjust the uptake of nutrients according to the strategy and relative content among them, searching for homeostasis and ionic balance (Severino et al., 2014). The increased activity of beneficial microbes in soil promoted by castor cake amendment can also be considered as an additional factor counterbalancing the salt stress (Bello et al., 2021; Cabot et al., 2014).

4. Conclusions

The synergy index was proposed as a tool to integrate the results that are traditionally presented as four separate variables in studies of alleviation of salt and heavy metal stress. It was demonstrated and discussed in depth for one experiment of salt stressed basil plants alleviated with castor cake applied as organic amendment. The concept was also illustrated on several studies compiled from the scientific literature on plant stress alleviation.

CRediT authorship contribution statement

Lauriane Almeida dos Anjos Soares: Methodology, Investigation. **Diego Silva Batista:** Methodology, Investigation. **Adriano Salviano Lopes:** Methodology, Investigation. **Jéssica Aline Linné:** Methodology, Investigation, Conceptualization. **Juliane Maciel Henschel:** Methodology, Investigation, Conceptualization. **Francisco Hélio Alves de Andrade:** Methodology, Investigation, Formal analysis. **Liv S. Severino:** Writing – review & editing, Writing – original draft, Formal analysis. **Thiago Jardelino Dias:** Methodology, Investigation, Formal analysis. **Valéria Fernandes de Oliveira Sousa:** Methodology, Investigation, Data curation, Conceptualization. **Geovani Soares de Lima:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2025.121802](https://doi.org/10.1016/j.indcrop.2025.121802).

Data availability

Research data is available as supplementary material

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