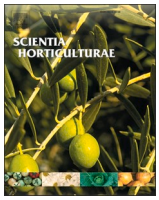
Scientia Horticulturae 328 (2024) 112955

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Research Paper

Corn Steep Liquor application on pepper plants (*Capsicum annum* L.) stimulates growth under nitrogen-deficient growing conditions

Iván Navarro-Morillo [a], Begoña Blasco [b], José M. Cámara-Zapata [c,*], Julia Muñoz-Acero [c], Silvia Simón-Grao [d], Marina Alfosea-Simón [d], Felix Plasencia [e], Francisco García-Sanchez [d]

[a] *R + D Department of Atlántica Agrícola, 03400 Villena, Spain*
[b] *Departamento de Fisiología Vegetal, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*
[c] *Instituto de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Universidad Miguel Hernández, 03312 Orihuela, Spain*
[d] *Centro de Edafología y Biología Aplicada del Segura, Consejo Superior de Investigaciones Científicas, 30100 Murcia, Spain*
[e] *Escuela EFA El Campico. El Campico s/n. 10 Jacarilla, Alicante, Spain*

ARTICLE INFO

Keywords:
Biostimulants
Efficient use of nutrients
Excessive fertilization
Nutritional requirements

ABSTRACT

Excessive nitrogen fertilization in crops is causing serious environmental problems related to eutrophication of natural resources such as seas, aquifers, coastal lagoons like the Mar Menor (Spain), etc. Therefore, there is a need to design agronomic strategies that allow plants to be grown below their nutritional requirements, primarily nitrogen. One of these strategies is the use of biostimulants products made from botanical extracts, as they contain active compounds that stimulate certain metabolic processes in plants favoring nitrogen absorption and assimilation. In this experiment, foliar and root application of Corn Steep Liquor (CSL) extract was evaluated at four stages of pepper plant (*Capsicum annuum* L.) cultivation under different amounts of nitrogen, ranging from 100 % to 25% N. At the end of the experiment parameters of vegetative growth, nitrate, ammonium, total nitrogen concentration in the leaves, amino acid concentration, soluble proteins, and the enzymatic activities of glutamine synthetase and nitrate reductase were measured. The results showed that plants reduced their vegetative growth as nitrogen concentration in the nutrient solution decreased. However, this reduction was less pronounced in plants treated with exogenous CSL in the leaves, improving the efficient use of nitrogen (NUtE and NUE in particular) by increasing nitrogen assimilation (NR and GS enzymatic activities) and the concentration of amino acids and proteins.

1. Introduction

One of the challenges of 21st century agriculture is increasing the production and quality of crops to compensate for the progressive growth of the world population, which is estimated to reach 9.6 billion people in 2050. Currently, to enhance food production, it is necessary to increase the amount of nitrogen fertilizers applied to crops. Thus, in 2018, the global demand for synthetic N fertilizers exceeded 200 Mtn (FAO, 2023). However, the disproportionate use of these fertilizers is having a negative impact on the environment in both agricultural and non-agricultural terrestrial and aquatic ecosystems (Kocira et al., 2020; Hamedani et al., 2020). The most common impact resulting from the improper use of nitrogen fertilizers is nitrate (NO_3^-) leaching into groundwater, leading to eutrophication of freshwater and marine ecosystems. Additionally, gaseous N oxides are generated, which can reach the troposphere and react with ozone, resulting in toxic emissions of ammonium. Conventional nitrogen fertilizers have relatively low efficiency, ranging from 70 to 80 % at best (Dimkpa et al., 2020). In this context, it is necessary to develop agronomic strategies that allow for proper and sustainable management of nitrogen fertilization, including the selection of suitable chemical forms, dosage, and application timing. Additionally, the use of agronomic techniques that contribute to increased and improved nitrogen use efficiency (NUE) by plants is essential. NUE is defined as the biomass production per unit of available nitrogen. This can be divided into two fundamental processes: (i) plant's capacity to absorb nitrogen from the soil (Nitrogen Uptake Efficiency - NUpE) and (ii) the efficient use of the absorbed nitrogen, which is defined as the plant's ability to transfer and utilize this element in biomass production of different plant organs (Nitrogen Utilization Efficiency - NUtE) (Dimkpa et al., 2020; Xu et al., 2012). Improved NUE

* Corresponding author.
E-mail address: jm.camara@umh.es (J.M. Cámara-Zapata).

https://doi.org/10.1016/j.scienta.2024.112955
Received 13 September 2023; Received in revised form 5 October 2023; Accepted 29 January 2024
Available online 3 February 2024

could reduce economic costs and decrease environmental degradation caused by nitrogen fertilizer application. In recent years, the focus on enhancing NUE has involved the implementation of biotechnology and plant breeding strategies, as well as the use of biostimulant agrochemical products.

According to the European Regulation on Fertilizer Products, biostimulants are defined as "product whose function is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; (d) availability of confined nutrients in the soil or rhizosphere." In general, biostimulants can be formulated with the following active compounds (Conesa et al., 2020; Zarzecka et al., 2020; Dalal et al., 2019; Cozzolino et al., 2020; Rouphael et al., 2018; Campobenedetto et al., 2020). Organic substances based on humic and fulvic acids; animal or plant hydrolyzed proteins; compounds or amino acids containing N, poly- and oligosaccharides; vitamins, chitins and chitosan; extracts from seaweed (macro and microalgae); inorganic substances such as silicon; plant extracts (seeds, leaves, roots, and fruits); and microorganisms such as fungi and bacteria. The mode/mechanism of action of "biostimulants" is highly diverse and can include N metabolism activation, phosphorus (P) release from soils, microbial generic activity stimulation of soil, root growth stimulation, physiological processes enhancement such as germination, photosynthesis, and nutrient absorption from the soil, etc. Biostimulants can also mitigate the negative effects of abiotic stress factors in plants, such as drought, heat, salinity, frost, boron toxicity, etc. (Du Jardin, 2015).

Currently, one of the biostimulant types that is receiving significant attention is plant-derived biostimulants (PDBs). So far, the most evaluated PDBs include extracts from licorice root, hydrolyzed proteins derived from legumes, lemongrass extracts, garlic extract, and particularly, moringa leaf extract (Rouphael et al., 2018; Hussein et al., 2014; Kurepin et al., 2014; Elzaawely et al., 2017; Hayat et al., 2018; Younis et al., 2018; Rady et al., 2019; Zulfiqar et al., 2020). One type of PDB that has not been widely evaluated yet is the so-called Corn Steep Liquor (CSL). CSL is an organic liquid residue obtained from the water used in the wet milling of corn, which still contains a high proportion of organic and mineral substances (Zhu et al., 2019). In bean plants, the application of CSL to the irrigation solution at a concentration of 1 % improved germination, plant growth and flowering, and increased N, P, potassium (K), manganese (Mn) and copper (Cu) foliar concentrations (Xi et al., 2013). This article evaluated the CSL foliar and root application to pepper plants grown under different levels of nitrogen in the nutrient solution. The aim was to determine if the application of this extract could stimulate plant growth in a way that would allow for a reduced nitrogen concentration on crops compared to the current levels used nowadays. The hypothesis was based on the presence of certain substances in CSL, such as free amino acids, mineral nutrients, and phenolic compounds including vanillic, ferulic, coumaric, sinapic, and caffeic acids (Niwa et al., 2001; Xi et al., 2013; Joshi et al., 2018), that stimulate plant growth by enhancing physiological and metabolic processes. To test this hypothesis, a detailed chemical analysis of CSL was conducted, and various parameters related to nitrogen metabolism were analyzed.

2. Materials and methods

2.1. Chemical analysis of corn steep liquor product

In this experiment, the product called CSL-B in Navarro-Morillo et al. (2023) was used. The identification of non-volatile metabolites was carried out using a sample of 1 g homogenized with Milli-Q water in a 1:100 ratio, in an ultrasonic bath for 10 min. Subsequently, the sample was filtered with a 0.22 μm filter and injected into an Ultra-High Performance Liquid Chromatography (UHPLC) system (LC 1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) coupled with a Time-of-Flight Mass Spectrometer (LC/Q-TOF Agilent 6546, Santa Clara, CA, USA), operating in positive and negative ionization mode. The chromatographic separation was achieved using an Agilent Zorbax RRHD SB-C18, 2.1 × 50 mm, 1.8 μm column. The mobile phase A was composed of Milli-Q water, and the mobile phase B was prepared using acetonitrile with gradient elution: 0 min, B at 2 %; 22 min, B at 95 %; 25 min, B at 5 %. Both phases were acidified with 0.1 % formic acid. The column was equilibrated for 3 min before each analysis. The flow rate used was 0.4 mL min^{-1}, and 5 μL of the sample was injected for analysis. The conditions of the dual AJS ESI source were as follows: 325 °C gas temperature; 10 L min^{-1} gas flow; 275.8 kPa nebulizer pressure; 295 °C curtain gas temperature; 12 L min^{-1} curtain gas flow; 4000 V capillary voltage; 500 V nozzle voltage; 120 V fragmentor voltage; 70 V skimmer voltage; 50–1500 Da product ion scan range; 5 spectra/s MS scan rate; 3 spectra/s MS/MS scan rate; 2 maximum precursors per cycle; 10, 20, 40 eV collision energy. The analysis of metabolites was performed in triplicate. The integration, data processing, and metabolite identification were managed using MassHunter qualitative analysis software version 10.0 and the PCDL Manager B.08.00 library. The library utilized contains over 200 bioactive compounds described during fermentation processes with microorganisms.

The quantification of metabolites was carried out using a calibration curve with a phenyllactic acid standard at a concentration range between 0.08–100 mg L^{-1}.

The analysis of volatile organic compounds (VOCs) was carried out following the method described by Xu et al. (2012). For the headspace extraction, 5 g of the product were homogenized with 10 mL of Milli-Q water and transferred to a 20 mL vial. The VOCs were extracted using a DVB/C-WR/PDMS-coated SPME fiber (80 μm x 10 mm) for 45 min at 50 °C in a water bath with constant agitation.

The analysis of VOCs was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 7000A triple quadrupole mass spectrometer equipped with an Electron Impact (EI) ionization source. The chromatographic separation was achieved using a ZB-624plus column (30 m x 0.25 mm, 1.40 μm). The temperature ramp was programmed as follows: 40 °C for 2 min with increased at a rate of 15 °C min^{-1} up to 220 °C, below increase at a rate of 10 °C min^{-1} up to 250 °C and held at this temperature for 5 min. Helium (99.99 %) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The detection of compounds was performed in Full Scan mode, scanning a mass range from m/z 50 to 450 amu.

The data collected with MassHunter G7000C software was analyzed using MassHunter qualitative Analysis B.07.00 software, and the NIST20 spectral library was used to identify the detected components. The analysis was performed in triplicate.

2.2. Vegetal material and growth conditions

Pepper plants (*Capsicum annuum* cv. Alycum) were used for this experiment. The seeds of these plants were germinated and grown for 45 days in a tray with cells (cell size: 3 cm x 3 cm x 10 cm). Subsequently, the seedlings were transferred to a growth chamber under controlled conditions with a relative humidity of 60–80 %, temperature of 29 °C / 20 °C (day/night), and a 16/8 hour photoperiod with a photosynthetic photon-flux density (PPFD) of 450 μmol photons $m^{-2}s^{-1}$ (measured using an SB Quantum 190 sensor, LI-COR Inc., Lincoln, NE, USA). Under these conditions, the plants were grown in individual pots (13 cm top diameter, 10 cm bottom diameter, 12.5 cm height, and a volume of 2 L) filled with a perlite:peat moss mix (75:25). Fertilization was carried out using a Hoagland-type nutrient solution composed of the following compounds: 8 mM $NaNO_3$, 3 mM KCl, 4 mM $CaCl_2$, 1.5 mM $MgCl_2$, 2 mM $MgSO_4$, 2 mM KH_2PO_4, 1 mM NaH_2PO_4, 2 μM $MnCl_2$, 1 μM $ZnSO_4$, 0.25 μM $CuSO_4$, 0.1 μM Na_2MoO_4, 125 μM Fe-EDDHA, and 50 μM H_3BO_3, with a pH of 5.8. The plants were watered once every 3–5 days with approximately 50–100 mL of water, depending on their vegetative growth stage.

2.3. Experimental design and plant sampling

After 20 days of germination, the treatments described in Table 1 were applied. The volume used in both root application and foliar treatment was 50 mL.

The reduction of N in the treatments from T4 to T9 was achieved by decreasing the supply of $NaNO_3$. The treatments were applied four times, with a seven-day interval between each application. Finally, the experimental design consisted of a randomized block with eight plants per treatment, arranged in individual pots, with the treatments randomly distributed in the growth chamber.

After 7 days following the last application of the treatments, all plants were immediately processed for subsequent analysis. The vegetal material was washed and then dried on filter paper to obtain fresh mass (FM). Samples were harvested and kept fresh or frozen at −40 °C for the analysis of the following parameters: leaf area, activity of nitrogen metabolism enzymes (nitrate reductase and glutamine synthetase), concentration of amino acids and soluble proteins. Additionally, samples were harvested and dried in a forced air oven to determine dry mass (DM) as well as the concentration of different forms of N (total N, organic N, and nitrates) for calculating nitrogen use efficiency (NUE).

2.4. Analysis of vegetal material

2.4.1. Leaf and aboveground biomass

At the end of the experiment, the biomass of the aboveground plant parts was measured in terms of dry weight. Leaf area was measured using an optical reader, LI-COR brand, model LI-3000A (IRGA: LI-COR Inc., Nebraska, USA).

2.4.2. Enzymatic activities of nitrogen metabolism

The nitrate reductase (NR) activity was determined following the procedure described by Navarro-León et al. (2016). 0.2 g of fresh plant material was macerated in a mortar with 1 mL of extraction buffer containing 2 mM EDTA-Na, 2 mM DTT, 1 % (w/v) PVPP in 100 mM KH_2PO_4 (pH 7.5). The suspension was centrifuged for 20 min at 20,600 g at 4 °C (Heraeus Sepatech 132 Biofuge 17RS, Hanau, Germany). The obtained supernatant was added to the reaction mixture containing: 100 mM KNO_3, 2 mM NADH, 10 mM cysteine, and 10 mM $MgCl_2$ in 100 mM KH_2PO_4 buffer (pH 7.5), and the mixture was incubated at 30 °C for 30 min. Subsequently, 1 mM zinc acetate was used as a stop reagent, and 1 % sulfanilamide in 1.5 M HCl and 0.02 % (w/v) NNEDA in 0.2 M HCl were used for the detection of the formed NO_2^-. Finally, the NR activity was determined by measuring the absorbance at 540 nm of the produced NO_2^-.

The glutamine synthetase (GS) activity was determined using an adaptation of the hydroxamate synthetase assay published by Navarro-León et al. (2016). 0.1 g of leaf tissue was macerated in a mortar with 1 mL of extraction buffer containing: 100 mM sucrose, 2 % (v/v) β-mercaptoethanol, and 20 % (v/v) ethylene glycol in 100 mM maleic acid-KOH (pH 6.8). The suspension was centrifuged for 20 min at 20,600 g at 4 °C (Heraeus Sepatech 132 Biofuge 17RS, Hanau, Germany). The resulting extract was used to measure GS activity. The reaction mixture used consisted of 150 mM sodium glutamate, 30 mM hydroxylamine, and 10 mM ATP as substrates, along with 45 mM $MgSO_4 \cdot 7\ H_2O$ and 4 mM EDTA-Na, all dissolved in 150 mM imidazole-HCl buffer (pH 7.8). After incubation at 28 °C for 30 min, the formation of glutamylhydroxamate was determined by measuring its absorbance at 540 nm after its complexation with acidified ferric chloride. Six measurements of each parameter per treatment were performed.

Table 1
Treatments of nitrogen and CSL applied in the trial.

Treatment	Description	Compounds
T1	N-100 %	8 mM N
T2	N-100 % + CSL Leaf	8 mM *N* + 5 mL CSL L^{-1}
T3	N-100 % + CSL Root	8 mM *N* + 5 mL CSL L^{-1}
T4	N-50 %	4 mM N
T5	N-50 % + CSL Leaf	4 mM *N* + 5 mL CSL L^{-1}
T6	N-50 % + CSL Root	4 mM *N* + 5 mL CSL L^{-1}
T7	N-25 %	2 mM N
T8	N-25 % + CSL Leaf	2 mM *N* + 5 mL CSL L^{-1}
T9	N-25 % + CSL Root	2 mM *N* + 5 mL CSL L^{-1}

2.4.3. Amino acids and soluble proteins concentration

For the determination of soluble amino acids and proteins, approximately 0.5 g of vegetal material was weighed and homogenized with 5 mL of 50 mM pH 7.0 phosphate buffer. The homogenate was filtered with 4 layers of gauze and then centrifuged at 12,360 g (Heraeus Sepatech 132 Biofuge 17RS, Hanau, Germany) for 15 min. The supernatant was used for quantifying soluble amino acids and proteins. The concentration of soluble amino acids was quantified using the ninhydrin method (Yemm and Cocking, 1955). As for soluble proteins, 0.1 mL of the supernatant was mixed with 0.9 mL of 50 mM pH 7.0 phosphate buffer and 5 mL of coomassie blue. After 20 min, the samples were measured at a wavelength of 595 nm, using a standard curve of albumin (Navarro-León et al., 2016). Six measurements of each parameter per treatment were performed.

2.4.4. Total nitrogen, organic nitrogen and nitrates concentration, and nitrogen use efficiency use

For the determination of total nitrogen, 0.2 g of dried leaves were ground and mineralized with 98 % H_2SO_4 and 30 % H_2O_2 at a temperature of 300 °C, and the resulting mineralized solution was used for nitrogen analysis. The concentration of total nitrogen was determined using colorimetry based on the Berthelot reaction, following the method described by Krom (1980). To determine the concentration of soluble NO_3^-, an aqueous extraction was performed following (Cataldo et al., 1975). The determination of NO_3^- was based on the colorimetric reaction formed by the combination of NO_3^- with salicylic acid in a basic medium (Cataldo et al., 1975). The organic nitrogen content was obtained by subtracting the soluble nitrate content from the total nitrogen (Wolf, 1982). Six measurements of each parameter per treatment were performed.

For the calculation of Nitrogen Utilization Efficiency (NUtE), Nitrogen Uptake Efficiency (NUpE) and Nitrogen Use Efficiency (NUE) the equations described by Xu et al. (2012) were used. NUtE is defined as the ratio of biomass production to the amount of N in the plant; NUpE is defined as the ratio of N content in the plant to the amount of N applied in fertilization; NUE is obtained as the product of NUtE and NUpE.

2.5. Statistical analysis

Results were statistically evaluated using an analysis of variance, simple ANOVA, with a 95 % confidence interval. Differences between the means of the treatments were compared using Fisher's Least Significant Difference (LSD) test at a 95 % probability level. The levels of significance were expressed as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS non-significant.

3. Results

3.1. Chemical composition of corn steep liquor extract

The analysis of Corn Steep Liquor (CSL) using UHPLC-MS-TOF has allowed the identification of a total of 91 bioactive compounds. Table 2 shows the most abundant ones.

- 2-Ethyl-2-hydroxybutyric acid: classified within the group of hydroxy fatty acids (also known as HFAs)

Table 2
Quantification of the major metabolites of the CSL product.

Metabolites	Concentration (g kg^{-1})
2-Ethyl-2-hydroxybutyric acid	4.2 ± 0.2
DL-3-Phenyllactic acid	2.2 ± 0.1
Mannitol	0.56 ± 0.06
Erythritol	0.54 ± 0.04
GABA	0.26 ± 0.07
Isoleucine	0.56 ± 0.06
Proline	0.83 ± 0.08

- DL-3-Phenyllactic acid: a phenolic acid primarily produced through lactic acid bacteria fermentation. Literature suggests studies related to its activity as a promoter of root growth.
- Mannitol and erythritol: polyols with bio-stimulation effects in crops.
- GABA, isoleucine, and proline: amino acids with effects on crop nutrition as well as improvement of plant stress response.

It is a highly diverse composition of non-volatile bioactive metabolites, comprising a wide range of compounds belonging to the groups of polyols, amino acids, fatty acids, organic acids, phenolic acids, and phytohormones. Many of the identified compounds have been described as promoters of plant growth, such as amino acids, phenylacetic acid, and indoleacetic acid.

Fig. 1 displays the profile of volatile organic compounds (VOCs) in CSL. The group of volatile compounds including alkanes, alcohols, aldehydes, and ketones were found to be the most abundant in the product. VOCs are substances with the ability to diffuse rapidly through the soil and can be distributed within the plant's tissue. Some VOCs are utilized by plants as nutrients and may exhibit a biostimulatory effect on plant growth.

3.2. Vegetative development of pepper plants (*Capsicum annum L.*)

The effects of different nitrogen doses in the nutrient solution and the application of CSL product, both foliar and root, on plant growth can be seen in Table 3 and Fig. 2. The growth of plants cultivated with 100% N nutrient solution (8 mM) was not affected by the application of CSL product in either foliar or root application modes. The reduction of 50 %

Table 3
Aerial biomass production and leaf area per plant in pepper plants (*Capsicum annum* L.) with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively).

Treatments	**Dry mass aboveground part** (g)	**Leaf area** (cm^2)
T1	4.7 ± 0.8	900 ± 90
T2	4.9 ± 0.6	940 ± 90
T3	4.9 ± 0.3	930 ± 30
p-valor	NS	NS
T4	2.0 ± 0.2c	640 ± 60c
T5	2.5 ± 0.1bc	700 ± 80bc
T6	2.6 ± 0.1b	760 ± 30b
p-valor	***	***
T7	1.7 ± 0.2c	400 ± 40c
T8	2.1 ± 0.2bc	440 ± 40c
T9	2.3 ± 0.1b	580 ± 30b
p-valor	***	***

The values indicate the means ± standard deviations ($n = 8$). The differences between treatments were analyzed with Fisher's least significant difference test (LSD; $p = 0.05$); distinct letters in the second and third groups (treatments from T4 to T9) and in the same column show significant differences when comparing the results between treatments and with the control (T1) at $p < 0.05$. In the ANOVA, the significance level is shown by *** ($p < 0.001$), NS non-significant.

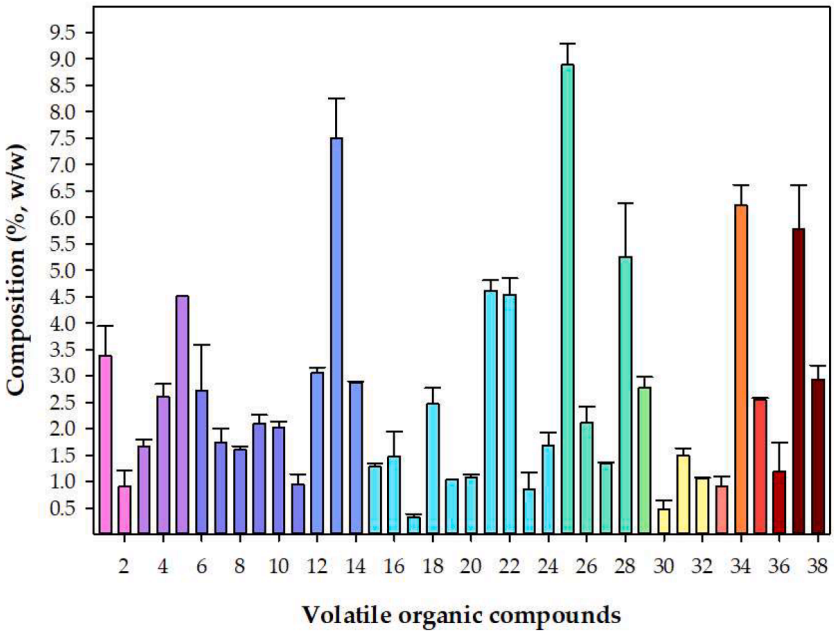


Fig. 1. Composition of volatile organic compounds in the CSL product. Dicarboxylic acids (1: 2-Oxopentanedioic acid; 2: 1,2-Propanediol, diacetate); Fatty acids (3: Butanoic acid, 3-methyl-; 4: Hexanoic acid; 5: Nonanoic acid); Alkanes (6: Toluene; 7: d-Limonene: 8: Naphthalene, 1-methyl-7-(1-methylethyl)-; 9: Cyclohept[*f*] indene, 1,2,3,5,6,7,8,9-octahydro-; 10: Chamazulene; 11: 4,4′-Dimethylbiphenyl); Alcohols (12: 2,3-Butanediol, [R-(R*,R*)]-; 13: 1-Decanol; 14: 2-Naphthalenethiol); Aldehydes (15: Butanal, 3-methyl-; 16: Butanal, 2-methyl-; 17: 2-Butenal, 2-ethyl-; 18: 1,3-Dioxolane, 4-methyl-2-propyl-; 19: Furfural; 20: 2-Thiophenecarboxaldehyde; 21: Nonanal; 22: 2-Isopropyl-5-methylhex-2-enal; 23: 1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-; 24: Benzeneacetaldehyde, .alpha.-ethylidene-); Ketones (25: Ethanone, 1-(2-furanyl)-; 26: Ethanone, 1-(2,5-dihydroxyphenyl)-; 27: Resorcinol, 2-acetyl-; 28: 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-3-en-2-one); Cyclohexanones (29: 2,6,6-Trimethyl-2-cyclohexene-1,4-dione); Esters (30: Ethyl Acetate; 31: 3-(Methylthio)propyl acetate; 32: Acetic acid, decyl ester); Phenols (33: 2,4-Di-tert-butylphenol); Indanones (34: 1H-Inden-1-one, 2,3-dihydro-); Methoxybenzenes (35: Benzene, 1-methoxy-4-methyl-); Monoterpenoid (36: 2,4,6-Cycloheptatrien-1-one, 2-hydroxy-4-(1-methylethyl)-); Pyrazines (37: Pyrazine, 2,6-dimethyl-; 38: 2,3,5-Trimethyl-6-ethylpyrazine).

Fig. 2. Appearance of pepper plants (*Capsicum annum* L.) subjected to treatments with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively).

and 25 % of N in the nutrient solution resulted, as expected, in a decrease in the dry weight of the aboveground part and leaf area. However, this reduction was mitigated by the root application of CSL product at both N levels, as indicated by the fact that for the T6 and T9 (N-50 % + CSL Root and N-25 % + CSL Root) treatments, the vegetative growth of the plants was significantly higher than for the T4 and T7 (N-50 % and N-25 %) treatments, respectively.

3.3. Characterization of nitrogen in pepper plants (Capsicum annum L.)

The concentration of total nitrogen, organic nitrogen, and nitrates in the plants of the N-100 % treatment was 62, 61, and 0.78 mg g^{-1} dry weight, respectively, with no significant differences observed with foliar or root application of CSL. The concentration of these three forms of nitrogen progressively decreased as the nitrogen concentration in the nutrient solution was reduced (N-50 % and N-25 %) in all treatments. However, foliar application of CSL in both N-50 % and N-25 % resulted in a higher concentration of total and organic nitrogen compared to plants that received root application of CSL. In the latter, no significant differences were observed compared to the non-application of CSL. Regarding nitrate concentration, there were no differences with root or foliar application of CSL at the N-50 % level. However, at the N-25 % level, the concentration significantly decreased in the order of T8 (N-25 % + CSL Leaf) > T7 (N-25 %) > T9 (N-25 % + CSL Root) (Table 4).

Fig. 3 represents the total nitrogen content of the aboveground part of the pepper plants for each treatment. The plants in the control treatment (N-100 %) had significantly higher values than the N-50 % plants, and the values for N-50 % were higher than those for N-25 %, regardless of CSL application. For each nitrogen level, it is evident that the application of CSL increased the nitrogen concentration compared to plants without CSL application. The foliar CSL treatment had higher values than the root treatment, although these differences were not significant.

Table 4
Foliar concentration of different forms of N in pepper plants (*Capsicum annum* L.) with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively).

Treatments	Total N (mg g^{-1} DM)	Organic N (mg g^{-1} DM)	Nitrates (mg g^{-1} DM)
T1	62 ± 2	61 ± 4	0.78 ± 0.07a
T2	67 ± 5	66 ± 4	0.78 ± 0.03a
T3	66 ± 4	65 ± 5	0.63 ± 0.06b
p-valor	NS	NS	***
T4	41 ± 4c	40 ± 3c	0.34 ± 0.02b
T5	50 ± 2b	50 ± 1b	0.33 ± 0.02b
T6	42 ± 2c	42 ± 1c	0.38 ± 0.04b
p-valor	***	***	***
T7	32 ± 2c	32 ± 2c	0.09 ± 0.01d
T8	35 ± 1b	35 ± 1b	0.38 ± 0.05b
T9	31 ± 1c	31 ± 1c	0.18 ± 0.01c
p-valor	***	***	***

The values indicate the means ± standard deviations ($n = 8$). The differences between treatments were analyzed with Fisher's least significant difference test (LSD; $p = 0.05$); distinct letters in the second and third groups (treatments from T4 to T9) and in the same column show significant differences when comparing the results between treatments and with the control (T1) at $p < 0.05$. In the ANOVA, the significance level is shown by *** ($p < 0.001$), NS non-significant.

3.4. Nitrogen metabolisms in pepper plants (Capsicum annum L.)

In the biochemical study of nitrogen assimilation, amino acid concentration, and protein content, it can be observed that when plants grow in an optimal nitrogen medium (N-100 %), the root application of CSL increases GS activity and protein concentration, while foliar application only increases the latter parameter. The reduction of nitrogen in the N-50 % and N-25 % levels result in a significant decrease in each of these parameters, with a greater reduction observed in the N-25 % level. The application of CSL, whether root or foliar, mitigated this reduction in such a way that in the N-50 % and N-25 % levels for both application modes, NR, amino acid concentration, proteins, and GS activity significantly increased (Table 5).

3.5. Efficient use of nitrogen in pepper plants (Capsicum annum L.)

Using the leaf nitrogen data and plant vegetative growth, the Nitrogen Utilization efficiency (NUtE), Nitrogen Uptake Efficiency (NUpE) and Nitrogen Use Efficiency (NUE) were calculated. In plants cultivated with the optimal nitrogen level (N-100 %), these parameters were not affected by the application of CSL (root or foliar). The N-50 % and N-25 % nitrogen levels without CSL application resulted in a decrease in NUE, NUtE, and NUpE. The application of CSL at both nitrogen levels, whether foliar or root, led to certain changes in the values: In the N-50 % level, there was an increase in NUE and NUpE with foliar and root CSL application, and an increase in NUtE with foliar application, compared to the treatment without CSL application; In the N-25 % level, both root and foliar CSL application increased all the parameters (NUE, NUtE, and NUpE) compared to the treatment without CSL application (Table 6).

4. Discussion

One of the challenges facing 21st-century agriculture is the excessive use of nitrogen fertilizers, which, when they reach the environment, cause eutrophication problems in aquifers, coastal lagoons, seas, and oceans. To prevent agriculture from harming nature, it is necessary to develop agronomic strategies that allow plants to be grown below their nutritional needs. In this experiment, the application of CSL product has been tested as a potential biostimulant to reduce the use of conventional nitrogen fertilizers. Since nitrogen concentration directly influences growth parameters (Lea and Azevedo, 2006; Iqbal et al., 2020; Emamverdian et al., 2023), it has been chosen as a bioindicator to assess the bio-stimulating effect on the parameter of dry biomass of the aerial part

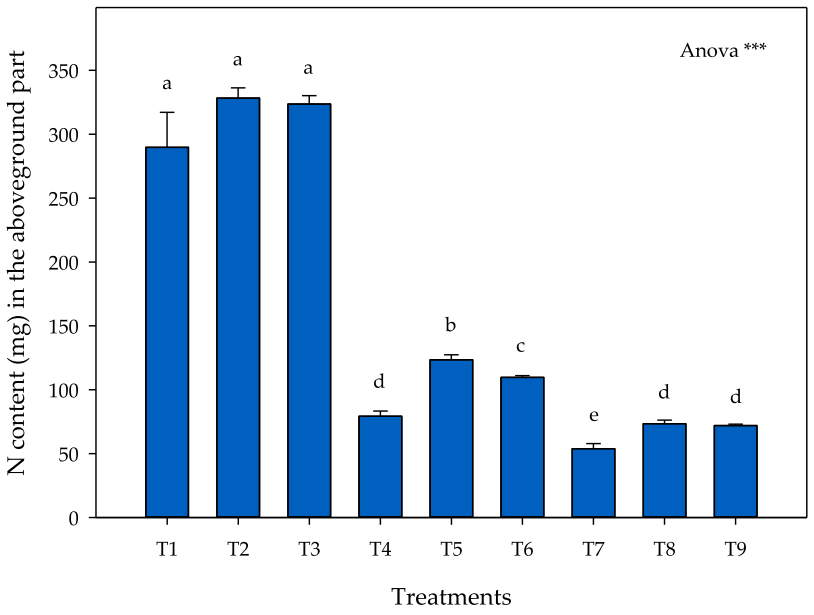


Fig. 3. Total nitrogen content in the aboveground part in pepper plants (*Capsicum annum* L.) with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively). In the ANOVA, the significance level is represented by $p < 0.001$ (***). The different lowercase letters indicate significant differences ($p < 0.05$) between the means, as established by Fisher's least test (LSD). The values indicate the means ± standard deviations (SDs) ($n = 8$).

Table 5
Nitrogen assimilation parameters in pepper plants (*Capsicum annum* L.) with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively).

Treatments	NR (μM NO_2^- mg $prot^{-1}$ min^{-1})	GS (mM γ-GH mg $prot^{-1}$ min^{-1})	Amino acids (mg g^{-1} FM)	Proteins (mg g^{-1} FM)
T1	0.52 ± 0.04a	1.9 ± 0.2b	1.0 ± 0.1a	8.8 ± 0.9b
T2	0.49 ± 0.03a	2.0 ± 0.1b	0.9 ± 0.1a	11.0 ± 1.3a
T3	0.49 ± 0.02a	2.9 ± 0.1a	0.9 ± 0.1a	10.5 ± 1.0a
p-valor	NS	***	NS	**
T4	0.12 ± 0.01c	0.8 ± 0.1c	0.7 ± 0.1b	6.9 ± 1.1c
T5	0.14 ± 0.01b	0.9 ± 0.1c	0.9 ± 0.1a	10.5 ± 1.1a
T6	0.15 ± 0.02b	1.4 ± 0.2b	0.9 ± 0.1a	10.0 ± 0.9a
p-valor	***	***	**	**
T7	0.04 ± 0.01d	0.6 ± 0.1c	0.6 ± 0.1c	3.8 ± 0.7c
T8	0.06 ± 0.01c	0.6 ± 0.1c	0.8 ± 0.1b	7.3 ± 0.9b
T9	0.09 ± 0.01b	1.0 ± 0.1b	0.8 ± 0.1b	7.0 ± 0.8b
p-valor	***	***	***	***

The values indicate the means ± standard deviations ($n = 8$). The differences between treatments were analyzed with Fisher's least significant difference test (LSD; $p = 0.05$); distinct letters in the same column show significant differences between treatments at $p < 0.05$. In the ANOVA, the significance level is shown by *** ($p < 0.001$), ** ($p < 0.01$), NS non-significant.

Table 6
NUE, NUtE, and NUpE in pepper plants (*Capsicum annum* L.) with 8 mM N (N-100 %, control) and with CSL applications in leaves and roots (T1, T2 and T3, respectively); 50 % of the N dose (4 mM N) and applications of CSL in leaf and root (T4, T5 and T6, respectively); and 25 % of the N dose (2 mM N) and applications of CSL in leaf and root (T7, T8 and T9, respectively).

Treatments	NUE (mg^{-1})	NutE (mg DM mg^{-1} N)	NUpE (mg N mg^{-1} N)
T1	113 ± 6	75 ± 4	1.51 ± 0.03
T2	105 ± 9	73 ± 3	1.57 ± 0.04
T3	107 ± 8	75 ± 3	1.55 ± 0.05
p-valor	NS	NS	NS
T4	40 ± 2c	48 ± 1c	0.83 ± 0.01d
T5	64 ± 4b	50 ± 2c	1.29 ± 0.04b
T6	70 ± 5b	61 ± 3b	1.14 ± 0.07c
p-valor	***	***	***
T7	58 ± 3c	52 ± 1c	1.12 ± 0.02b
T8	92 ± 5b	60 ± 4b	1.53 ± 0.05a
T9	111 ± 6a	74 ± 4a	1.50 ± 0.05a
p-valor	***	***	*

The values indicate the means ± standard deviations ($n = 8$). The differences between treatments were analyzed with Fisher's least significant difference test (LSD; $p = 0.05$); distinct letters in the same column show significant differences between treatments at $p < 0.05$. In the ANOVA, the significance level is shown by *** ($p < 0.001$), * ($p < 0.05$), NS non-significant.

of plants and leaf area. The data from the vegetative growth study indicate that deficient levels of N, N-50 %, and N-25 % reduced the dry biomass of the aerial part by 61 % and 64 % respectively, compared to the optimal level of N-100 %. This highlights the importance of maintaining the nutrient solution for irrigation at an optimal level of 8 mM N. Nitrogen is an essential element for plants, so such drastic reductions of 50 % or 25 % are challenging to manage in agriculture.

Regarding the application of the CSL product, root application partially restored the growth loss caused by the reduction in nitrogen levels in both N-50 % and N-25 % treatments (Table 4, Fig. 2). However, despite this recovery, the plants did not reach the same level of growth as the N-100 % treatment plants. On the other hand, foliar application of CSL failed to stimulate plant growth at either of the two deficient levels, N-50 % and N-25 %. Therefore, the CSL positive effect was observed in root applications for both nitrogen levels, although the plants did not reach optimal growth values like those in the N-100 % treatment. Although this result is not entirely satisfactory, it can be considered promising since CSL managed to stimulate plant growth. It is necessary to conduct further experiments with nitrogen reduction levels above 4 mM to precisely assess the potential of this agrochemical product at nitrogen reduction values that are acceptable for plants.

The decrease in vegetative growth parameters in plants grown under deficient nitrogen levels, N-50 % and N-25 %, was associated with a reduction in nitrogen concentration in the plant in its various forms (total, organic, and nitrates), as well as a decrease in the total nitrogen

content in the aerial part and in the enzymatic activities such as NR, GS, amino acids, and proteins. This indicates that a reduction exceeding 50 % of nitrogen in the nutrient solution disrupts nitrogen distribution in the plant and all processes related to nitrogen metabolism, absorption, and assimilation. In pepper plants, like in most plants, NO_3^- is the preferred nitrogen source. It is absorbed by the roots and transported to the leaves, where it is converted into assimilation products such as amino acids and proteins, which are necessary for biomass production. The first step in NO_3^- assimilation is its reduction to NH_4^+ in two reactions: first, NO_3^- is converted to NO_2^- by nitrate reductase (NR), and subsequently, nitrite reductase converts it to NH_4^+. This process requires reducing power in the form of NADH and reduced ferredoxin for the reaction to occur. The NH_4^+ produced is assimilated in an organic form by two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT), which produce glutamine (Gln) and glutamate (Glu), respectively. These compounds serve as precursors for the synthesis of other amino acids, proteins, nucleic acids, polyamines, chlorophylls, and hormones (Iqbal et al., 2020; The et al., 2021; Wang et al., 2022). In pepper plants grown under nitrogen deficiency, the processes of nitrogen absorption and assimilation are altered, as indicated by the data obtained in this experiment.

The beneficial effects of root application of CSL could be related to the improvement in nitrogen absorption by the roots and/or its assimilation within the plant. Although the nitrogen concentration in the leaves was similar between the N-50 % and N-50 % root-applied CSL treatments, as well as between the N-25 % and N-25 % root-applied CSL treatments, the plants treated with CSL had a higher total nitrogen content in the aerial part (Fig. 2). The increased vegetative development of these plants led to a dilution effect, resulting in the same nitrogen concentration for both treatments. Therefore, for the same nitrate concentration in the nutrient solution, the application of CSL increases the amount of nitrogen accumulated in the aerial part of the plants. Do not rule out the hypothesis that CSL could be stimulating the physiological and metabolic processes in plants that lead to increased vegetative growth, thereby enhancing the efficient use of nitrogen even at the same concentration in the nutrient solution or in the leaf. In fact, the results of amino acid and protein concentrations show that they are higher in plants treated with CSL. Both amino acids and soluble proteins play essential roles in plant growth, contributing to primary metabolism functions such as chlorophyll synthesis, increased photosynthetic activity, maintenance of water relations in the plant, synthesis of growth hormones like auxin, precursors for other amino acids, and protein synthesis. Additionally, these nitrogenous compounds also participate in the induction of secondary metabolism, generating defense compounds such as phenols, alkaloids, etc. (Iqbal et al., 2020; The et al., 2021; Cheng et al., 2022).

The chemical composition of the applied CSL product indicates that it contains higher amounts of metabolites such as 2-ethyl-2-hydroxybutyric acid, erythritol, mannitol, GABA, isoleucine, proline, and DL-3-phenyllactic acid. These metabolites are known to be associated with stimulating effects in plants and providing protection against environmental stresses. Some research has shown that the application of biostimulants based on the presence of amino acids can improve plant growth and productivity by stimulating the assimilation processes of certain essential nutrients like nitrogen. This is achieved through the induction of enzymes such as NR and GS, which are key regulators of this physiological process, as well as the synthesis of nitrogenous organic compounds like amino acids and soluble proteins (Di Mola et al., 2020; Wang et al., 2023).

In conclusion, these results indicate that foliar application of the CSL product under nitrogen-deficient conditions (N-50 % and N-25 %) not only induces increased synthesis of amino acids and soluble proteins (Tables 5 and 6) but also improves the nitrogen nutritional status by stimulating higher concentrations of total nitrogen and organic nitrogen in the leaves. Possibly, and although further research is needed, these results can be explained by the potential stimulation of CSL when applied via the roots, enhancing nitrogen absorption processes under limited nitrogen conditions in the growing medium (N-50 % and N-25 %). This hypothesis is supported by the results of nitrogen use efficiency. Under nitrogen-limited conditions in the growing medium (N-50 % and N-25 %), the application of the CSL product significantly improved various parameters that define nitrogen use efficiency (NUE) (Table 6). Specifically, the application of CSL significantly stimulated nitrogen uptake efficiency (NUpE), especially under N-50 % deficient conditions (Table 6). On the other hand, root application of CSL induced a significant increase in nitrogen utilization efficiency (NUtE) and NUE under N-50 % and N-25 % deficient conditions (Table 6). It is worth noting that both root and foliar applications of the CSL product improved all NUE parameters under nitrogen-limited conditions in the growing medium. This explains the biostimulating effect of this compound, leading to an increase in biomass production in the aerial part, particularly when CSL is applied via the roots (Table 3).

5. Conclusions

In this experiment, it can be concluded that pepper plants are highly dependent on the nitrogen concentration in the nutrient solution, and the application of Corn Steep Liquor (CSL), primarily through root application, can alleviate nitrogen deficiency in the growing medium. Under nitrogen-limited conditions (N-50 % and N-25 %), the use of CSL via root application is beneficial for pepper cultivation, as it substantially improves plant growth. The mechanisms of action include enhanced nitrogen assimilation (NR and GS enzymatic activities) and increased synthesis and accumulation of amino acids and proteins. There is also a significant increase in nitrogen use efficiency, NUtE and NUE, particularly. Therefore, the utilization of CSL, especially when applied via root application, can be a useful technique for developing crops in nitrogen-limited areas. Under nitrogen-limited conditions (N-50 % and N-25 %), foliar application of CSL also provides benefits, although to a lesser extent than root application. Future experiments should aim to determine the range in which nitrogen fertilization can be reduced with the application of CSL without compromising vegetative development. Additionally, it is important to study the agronomic performance of plants when this extract is applied and delve into the metabolic processes activated by CSL components.

CRediT authorship contribution statement

Iván Navarro-Morillo: Software, Formal analysis, Investigation, Resources, Data curation, Visualization. **Begoña Blasco:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **José M. Cámara-Zapata:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Julia Muñoz-Acero:** Software, Investigation, Visualization. **Silvia Simón-Grao:** Validation, Formal analysis, Writing – original draft. **Marina Alfosea-Simón:** Validation, Writing – original draft. **Felix Plasencia:** Validation, Data curation, Visualization. **Francisco García-Sanchez:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

References

Campobenedetto, C., Grange, E., Mannino, G., van Arkel, J., Beekwilder, J., Karlova, R., Garabello, C., Contartese, V., Bertea, C.M., 2020. A biostimulant seed treatment improved heat stress tolerance during cucumber seed germination by acting on the antioxidant system and glyoxylate cycle. Front. Plant Sci. 11, 836.

Cataldo, D.A., Maroon, M., Schrader, L.E., Youngs, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6, 71–80.

Cheng, M., Cui, Y., Yan, X., Zhang, R., Wang, J., Wang, X., 2022. Effect of dual-modified cassava starches on intelligent packaging films containing red cabbage extracts. Food Hydrocolloid 124, 107225.

Conesa, M.R., Espinosa, P.J., Pallarés, D., Pérez-Pastor, A., 2020. Influence of plant biostimulant as technique to harden citrus nursery plants before transplanting to the field. Sustainability 12, 6190.

Cozzolino, E., Giordano, M., Fiorentino, N., El-Nakhel, C., Pannico, A., Di Mola, I., Mori, M., Kyriacou, M.C., Colla, G., Rouphael, Y., 2020. Appraisal of biodegradable mulching films and vegetal-derived biostimulant application as eco-sustainable practices for enhancing lettuce crop performance and nutritive value. Agronomy 10, 427.

Dalal, A., Bourstein, R., Haish, N., Shenhar, I., Wallach, R., Moshelion, M., 2019. Dynamic physiological phenotyping of drought-stressed pepper plants treated with "productivity-enhancing" and "survivability-enhancing" biostimulants. Front. Plant Sci. 10, 905.

Di Mola, I., Cozzolino, E., Ottaiano, L., Nocerino, S., Rouphael, Y., Colla, G., El-Nakhel, C., Mori, M., 2020. Nitrogen use and uptake eficiency and crop performance of baby spinach (Spinacia oleracea L.) and lamb's lettuce (Valerianella locusta L.) grown under variable sub-optimal N regimes combined with plant-based biostimulant application. Agronomy 10, 278.

Dimkpa, C.O., Fugice, J., Singh, U., Lewis, T.D., 2020. Development of fertilizers for enhanced nitrogen use efficiency – Trends and perspectives. Sci. Total Env. 731, 139113.

Du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hort. 196, 3–14.

Elzaawely, A.A., Ahmed, M.E., Maswada, H.F., Xuan, T.D., 2017. Enhancing growth, yield, biochemical, and hormonal contents of snap bean (Phaseolus vulgaris L.) sprayed with moringa leaf extract. Arch. Agron. Soil Sci. 63, 687–699.

Emamverdian, A., Ghorbani, A., Li, Y., Pehlivan, N., Barker, J., Ding, Y., Liu, G., Zargar, M., 2023. Responsible mechanisms for the restriction of heavy metal toxicity in plants via the co-foliar spraying of nanoparticles. Agronomy 13, 1748.

FAO, 2023. Available online: https://www.fao.org/news/story/es/item/277654/icode/(20/07/2023).

Hamedani, S.R., Rouphael, Y., Colla, G., Colantoni, A., Cardarelli, M., 2020. Biostimulants as a tool for improving environmental sustainability of greenhouse vegetable crops. Sustainability 12, 5101.

Hayat, S., Ahmad, H., Ali, M., Hayat, K., Khan, M., Cheng, Z., 2018. Aqueous garlic extract as a plant biostimulant enhances physiology, improves crop quality and metabolite abundance, and primes the defense responses of receiver plants. Appl. Sci. 8, 1505.

Hussein, N.M., Hussein, M.I., Gadel Hak, S.H., Hammad, M.A., 2014. Effect of two plant extracts and four aromatic oils on tuta absoluta population and productivity of tomato cultivar gold Stone. Nat. Sci. 12, 108–118.

Iqbal, A., Qiang, D., Alamzeb, M., Xiangru, W., Huiping, G., Hengheng, Z., Nianchang, P., Xiling, Z., Meizhen, S., 2020. Untangling the molecular mechanisms and functions of nitrate to improve nitrogen use efficiency. J. Sci. Food Agric. 100, 904–914.

Joshi, S., Goyal, S., Reddy, M.S., 2018. Corn steep liquor as a nutritional source for biocementation and its impact on concrete structural properties. J. Ind. Microbiol. Biot. 45, 657–667.

Kocira, S., Szparaga, A., Hara, P., Treder, K., Findura, P., Bartoš, P., Filip, M., 2020. Biochemical and economical effect of application biostimulants containing seaweed extracts and amino acids as an element of agroecological management of bean cultivation. Sci. Rep. 10 (1), 1–16.

Krom, M.D., 1980. Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. Analysis 105, 305–316.

Kurepin, L.V., Zaman, M., Pharis, R.P., 2014. Phytohormonal basis for the plant growth promoting action of naturally occurring biostimulators. J. Sci. Food Agric. 94, 1715–1722.

Lea, P.J., Azevedo, R.A., 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. Ann. App. Biol. 149, 243–247.

Navarro-León, E., Barrameda-Medina, Y., Lentini, M., Esposito, S., Ruiz, J.M., Blasco, B., 2016. Comparative study of Zn deficiency in L. sativa and B. oleracea plants: NH4+ assimilation and nitrogen derived protective compounds. Plant Sci. 248, 8–16.

Navarro-Morillo, I., Navarro-Perez, V., Perez-Millan, R., Navarro-León, E., Blasco, B., Cámara-Zapata, J.M., Garcia-Sanchez, F., 2023. Effects of Root and Foliar Application of Corn Steep Liquor on Pepper Plants: a Physiological, Nutritional, and Morphological Study. Horticulturae 9, 221.

Niwa, T., Doi, U., Kato, Y., Osawa, T., 2001. Antioxidative properties of phenolic antioxidants isolated from corn steep liquor. J Agric. Food Chem. 49, 177–182.

Rady, M.M., Desoky, E.S., Elrys, A.S., Boghdady, M.S., 2019. Can licorice root extract be used as an effective natural biostimulant for salt-stressed common bean plants? S. Afr. J. Bot. 121, 294–305.

Rouphael, Y., Giordano, M., Cardarelli, M., Cozzolino, E., Mori, M., Kyriacou, M., Bonini, P., Colla, G., 2018. Plant-and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. Agronomy 8, 126.

The, S.V., Snyder, R., Tegeder, M., 2021. Targeting nitrogen metabolism and transport processes to improve plant nitrogen use efficiency. Front. Plant Sci. 11, 628366.

Wang, L., Li, X., Gao, F., Liu, Y., Lang, S., Wang, C., Zhang, D., 2023. Effect of ultrasound combined with exogenous GABA treatment on polyphenolic metabolites and antioxidant activity of mung bean during germination. Ultrason Sonochem. 94, 106311.

Wang, Y., Liu, S., Yang, X., Zhang, J., Zhang, Y., Liu, X., Zhang, H., Wang, H., 2022. Effect of germination on nutritional properties and quality attributes of glutinous rice flour and dumplings. J. Food Compos. Anal. 108, 104440.

Wolf, B., 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Anal. 13, 1035–1059.

Xi, Y.L., Chen, K.Q., Dai, W.Y., Ma, J.F., Zhang, M., Jiang, M., Wei, P., Ouyang, P.K., 2013. Succinic acid production by Actinobacillus succinogenes NJ113 using corn steepliquor powder as nitrogen source. Bioresour. Technol. 136, 775–779.

Xu, G., Fan, X., Miller, A.J., 2012. Plant nitrogen assimilation and use efficiency. Annu. Rev. Plant Biol. 63, 153–182.

Yemm, E.W., Cocking, E.C., 1955. The determination of amino-acids with ninhydrin. Analyst 80, 209–213.

Younis, A., Akhtar, M.S., Riaz, A., Zulfiqar, F., Qasim, M., Farooq, A., Tariq, U., Ahsan, M., Bhatti, Z.M., 2018. Improved cut flower and corm production by exogenous moringa leaf extract application on gladiolus cultivars. Acta Sci. Pol. 17, 25–38.

Zarzecka, K., Gugala, M., Mystkowska, I., Sikorska, A., 2020. Total and true protein content in potato tubers depending on herbicides and biostimulants. Agronomy 10, 1106.

Zhu, M.-M., Liu, E.-Q., Bao, Y., Duan, S.-L., She, J., Liu, H., Wu, T.-T., Cao, X.-Q., Zhang, J., Li, B., Sheng, Y.-T., Liang, M.-X., Zhang, H.-X., Guo, X.-T., 2019. Low concentration of corn steep liquor promotes seed germination, plant growth, biomass production and flowering in soybean. Plant Growth Regul. 87, 29–37.

Zulfiqar, F., Casadesús, A., Brockman, H., Munné-Bosch, S., 2020. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Sci. 295, 110194.