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Agronomic evaluation of hydrogels containing antimicrobial agents applied on *Lactuca sativa* L. seeds and plantlets

Marika De Angelis [a], Pasquale Napoletano [a,b], Martina Cofelice [a,c], Erika Di Iorio [a], Claudio Colombo [a], Michele Baglioni [c,d], Claudio Rossi [c,d], Elena Sorrentino [a], Arturo Alvino [a], Francesco Lopez [a,c], Stefano Marino [a,c,*]

[a] Dept. of Agricultural, Environmental and Food Sciences (DiAAA), University of Molise, Via De Sanctis, I-86100, Campobasso, Italy
[b] Dept. of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine 18, 50144, Florence, Italy
[c] Centre for Colloid and Surface Science (CSGI), University of Florence, Via della Lastruccia 3, Sesto Fiorentino, 50019, Firenze, Italy
[d] Dept. of Biotechnology, Chemistry and Pharmacy, University of Siena, Via A. Moro 2, Siena, 53100, Italy

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ABSTRACT

Lettuce (*Lactuca sativa* L.) is a leafy vegetable commonly consumed in human diets. Greenhouse cultivation of lettuce often involves intensive agricultural practices, using compounds that negatively impact agroecosystems. In light of this, it would be useful to consider the potential drawbacks of using such compounds in lettuce cultivation. With the aim of replacing the traditional soil fumigation products, hydrogels containing slow-release biocidal compounds represent an innovative and sustainable solution. In this research, the ecotoxicological impact and agronomic performance of cross-linked hydrogels containing natural antimicrobial agents on lettuce seeds and plants were examined. For this purpose, six hydrogels, based on carboxymethyl cellulose (CMC) and alginate (AL) empty or loaded with aqueous microemulsions including ethyl isothiocyanate (Eth) or phenyl isothiocyanate (Phe), at different concentrations, were tested on seed germination and seedling growth. The influence of hydrogel treatments on lettuce growth parameters, including fresh weight, number of leaves, leaf surface, and susceptibility to moulds, was highlighted through seedling tests. In the present investigation we are able to report that during the detection time 72 h , significant differences were observed concerning parameters assessing the germination such the root elongation values and the percentage of germinated seeds (GI). Hydrogels containing combinations of CMC with Eth or Phe exhibited high levels of phytotoxicity (GI % = 26–42 %), indicating an inhibitory effect on seed germination and root elongation. Furthermore, a gradual dissipation of these inhibitory effects was observed over time. After 216 h all hydrogels demonstrated a phytotoxicity-free profile, with variable levels of biostimulant potential, particularly CMC with Eth and Phe (GI % = 105–112 %). Alginate hydrogels revealed encouraging results in promoting plant growth: however, hydrogels with Eth and Phe showed a decrease of fresh weight ranging from 5 to 10 % compared to AL itself.

1. Introduction

Society's changing lifestyles have led to a growing need for sustainable and ethical food choices [1]. At the same time, there has been a shift in consumer preference towards food to be readily available (ready-to-eat products). Fresh-cut vegetables are important examples of such products due to their time-saving benefits and ability to minimise the loss of vital nutrients and biologically active compounds [2–4]. Lettuce (*Lactuca sativa* L.), a primary greenhouse-grown vegetable, exemplifies the agricultural landscape's reliance on intensive practices [5, 6]. However, this intensification has led to a decline in agroecosystem services, impacting substantially food production, and fostering soil degradation, soil depletion, weeds, disease and pests. Remedial measures are necessary to address the escalating pathogen burden on soil degradation within greenhouse environments. Chemical soil fumigation has been a longstanding agronomic strategy to address these issues, but it has adverse ecological and health ramifications [7,8]. Due to the high impact of traditional soil fumigation methods, there has been an increasing interest in exploring alternative sustainable pest control natural strategies. Brassica species are rich in glucosinolates, which

* Corresponding author. Dept. of Agricultural, Environmental and Food Sciences (DiAAA), University of Molise, Via De Sanctis, I-86100, Campobasso, Italy.
E-mail address: stefanomarino@unimol.it (S. Marino).

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Table 1
Main physical and chemical characteristics of soil (means of three replicates ± standard deviation) used for germination tests: Sand, Silt, Clay, pH, Water Holding Capacity (WHC), Total Carbonates ($CaCO_3$), Total Organic Carbon (TOC), Total Nitrogen [5] and C/N ratio following the methods of MIPAFF (2000) [38].

	Sand (%)	Silt (%)	Clay (%)	pH	WHC (%)	$CaCO_3$ (%)	SOM (g kg^{-1})	TN (g kg^{-1})	C/N
Soil	34 ± 1	32 ± 1	34 ± 1	7.5 ± 0.1	25.3 ± 2.9	2 ± 0.5	25.3 ± 0.4	4.0 ± 1.5	3.6 ± 0.2

make them a promising option for biofumigation as they can produce biologically active molecules [9,10]. Among these, isothiocyanates (ITCs) have broad-spectrum activity against soil-borne pathogens, insects, nematodes, and weeds, making them potential environmentally sustainable pest management agents [10–16]. ITCs have been shown to inhibit seed germination and growth in both monocotyledonous and dicotyledonous plants [10,17–20]. As for these targets the role of emulsions and microemulsions has received a noticeable attention I the field of delivery systems. Emulsions are heterogeneous systems composed of at least two immiscible liquids, water and oil, one of which is usually uniformly dispersed as fine droplets throughout the other liquid phase by a mechanical agitation process. microemulsions are transparent, optically isotropic, thermodynamically stable, dispersions composed of water, oil, surfactant, and a cosurfactant. Microemulsions can be a reasonably noticeable viable solution for the delivery of water insoluble active chemicals. [21–23]. Isothiocyanate-Based Microemulsions were used, for the disinfection of agricultural soils, in view of the biocidal effect [24]. Therefore, hydrogel technology received attention for its potential ability to release natural fumigant agents and improve soil quality for agricultural use. Hydrogels have been used for this purpose since the 1980s [25,26]. These materials consist of natural polymers cross-linked with metal cations to form a three-dimensional network that can absorb significant amounts of water and nutrients [27,28]. Some hydrogels can release water or specific compounds over an extended period without physical breakage or degradation [29]. The rate of compound release is variable and can be adjusted to suit agricultural management and the requirements of specific plant species [30]. Hydrogels with slow-release compounds, such as biocidal components linked to biopolymers, can combine their capabilities with a crop protection product, making them useful for environmentally friendly weed or pathogen control [31]. Polysaccharides, such as alginate and cellulose derivatives, are preferred to produce hydrogels due to their biocompatibility and biodegradability [32,33]. Alginate beads have been shown to optimize biostimulant characteristics and improve lettuce growth [34], while cellulose-based hydrogels have demonstrated to enhance seed germination rates [35]. However, before the widespread application of biocompounds, it is essential to assess their potential toxicity to crops [36].

Biocompatible and biodegradable polymers, based on sodium carboxymethylcellulose (CMC) and sodium alginate (AL), which are employed to encapsulate ethyl-isothiocyanate (Eth) and phenyl-isothiocyanate (Phe) to develop hydrogel beads as disinfectants for agricultural soils were tested.

The research aimed to investigate the agronomic performance and ecotoxicological impact of hydrogels containing antimicrobial agents on lettuce. Germination and seedling tests were conducted to assess the response of key crop traits in *Lactuca sativa* (L.) seeds and plantlets to six hydrogel compounds.

2. Materials and methods

2.1. Materials

Commercial polymers i.e., sodium alginate (AL) and carboxymethyl cellulose (CMC) were from Sigma-Aldrich (Milan, Italy) and Roeper GmbH (Hamburg, Germany). $CaCl_2$, $FeCl_3$ and Tween 80 were purchased from Merck (Milan, Italy).

Both the germination and seedling tests were carried out on lettuce (*Lactuca sativa* L. seed cv Lunaverde) on a soil collected at the depth of 0–10 cm in Battipaglia (Salerno, Italy) and classified as Calcaric Cambisol [37]. The soil was a clay loam texture, medium calcareous, had sub-alkaline pH, low water retention and moderate soil organic matter (SOM) content (Table 1).

2.2. Hydrogels preparation

The tested hydrogels have been prepared starting from 1 % w/v aqueous AL and CMC solutions. In the case of unloaded gels, polymer solutions were added dropwise into salt solutions of, respectively, 0.3 M $CaCl_2$ (for AL) and $FeCl_3$ (for CMC) at room temperature. The forming hydrogel beads (5–8 mm diameter) were then magnetically stirred for 15 min and then taken out from crosslinking solutions, washed with distilled water to remove any unreacted metal ions on the surface, and stored in polyethylene containers. They were then loaded with the previously formulated isothiocyanate-based oil in water (o/w) microemulsions simply by immersing them in an adequate amount of the nanostructured fluid. Isothiocyanate-Based Microemulsions were prepared as reported in literature [24].

All the hydrogel formulations and acronyms used in the present study are reported in Table 2.

2.3. Germination test

The germination test was carried out according to the methods reported by USEPA [39] and APAT [40], to assess the impact and to establish any potential toxic effects of the investigated hydrogels on lettuce as reference plant. The experimental protocol was designed to replicate open-field conditions, using a substrate commonly employed for lettuce cultivation. Therefore, 10 seeds of *Lactuca sativa* L. cv Lunaverde were distributed on filter paper of a Petri dish (Ø 9 cm) containing 10 g of soil. Three different amounts (0.1, 0.2 and 0.4 g of beads) of each of the six hydrogels (AL, AL Eth, AL Phe, CMC, CMC Eth and CMC Phe) were tested (Fig. 1) in comparison with control Petri dishes without hydrogel. An amount of 4.3 ml of distilled water was

Table 2
Codes and formulation of hydrogels containing antimicrobial agents used in this study.

Sample	Description of hydrogels formulations
AL	beads of alginate hydrogels cross-linked with Ca^{2+}
AL Eth	beads of alginate hydrogel cross-linked with Ca^{2+} and loaded with an o/w microemulsion based on Tween 80 and ethyl isothiocyanate (3 % w/w)
AL Phe	beads of alginate hydrogel cross-linked with Ca^{2+} and loaded with an o/w microemulsion based on Tween 80 and phenyl isothiocyanate (1 % w/w)
CMC	hydrogel beads of carboxymethyl cellulose cross-linked with Fe^{3+}
CMC Eth	beads of carboxymethyl cellulose hydrogel cross-linked with Fe^{3+} and loaded with an o/w microemulsion based on Tween 80 and ethyl isothiocyanate (3 %w/w)
CMC Phe	beads of carboxymethyl cellulose hydrogel cross-linked with Fe^{3+} and loaded with an o/w microemulsion based on Tween 80 and phenyl isothiocyanate (1 % w/w)

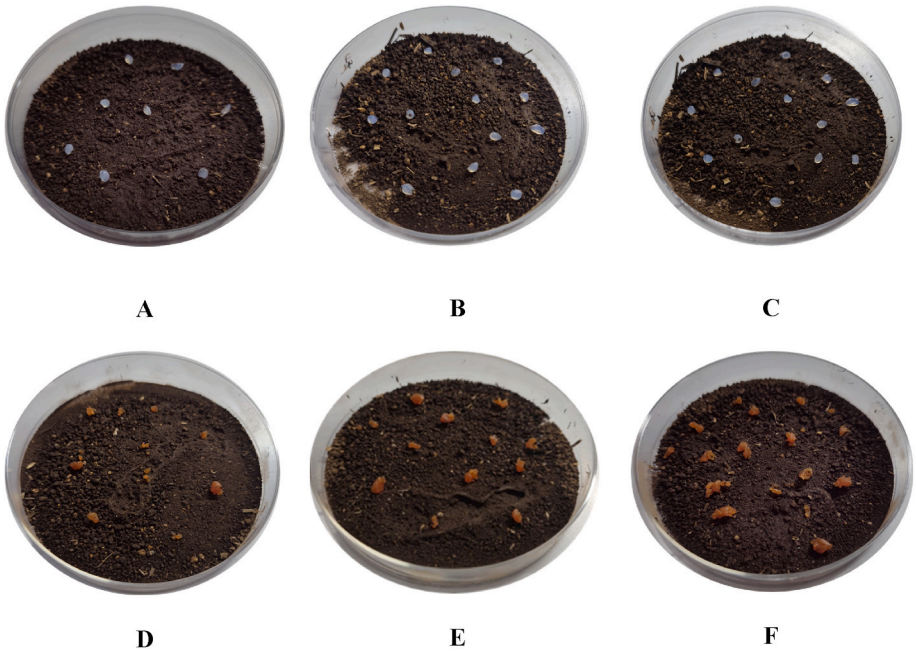

Fig. 1. Germination test: Petri dish with three different concentrations of alginate (AL) hydrogels: 0.1g (A), 0.2g (B) and 0.4 g (C), and carboxymethylcellulose (CMC) hydrogel: 0.1 g (D), 0.2g (E) and 0.4g (F).

added to each Petri dish to reach the calculated soil water holding capacity.

Germination test involved a sowing phase at 0 h and a germination test at 72 h after the application of the hydrogels, according to the USEPA [39]. Furthermore, to evaluate the hydrogels-controlled release ecotoxicological effect, the germination test was also assessed at 144 and 216 h. The Petri dishes were incubated at 25 ± 2 °C in the dark for 72, 144 and 216 h. The experimental design included 6 treatments, and negative control, with 4 replicates (each Petri dish is considered one replicate containing 10 seeds). The mean root elongation (mRE) in cm was evaluated for all the treatments and concentrations. Moreover, seed germination percentage (SG %), was measured following equation (1) according to Luo et al. [36] and Alongi et al. [41]:

$$SG\ (\%) : \frac{Number\ of\ germinated\ seeds}{Number\ of\ total\ seeds} x\ 100 \quad (1)$$

The germination index, expressed as percentage (GI %), was calculated following the method outlined by USEPA [39] and APAT [40], as detailed in equation (2):

$$GI\ (\%) = \left[\frac{(G_T \times L_T)}{(G_c \times L_c)}\right] x\ 100 \quad (2)$$

where G_T and G_C represent the mean values of germinated seeds for the sample and the control respectively, while L_T and L_C correspond to the mean root lengths for the sample and the control, respectively. The germination index values were categorized into distinct classes based on the criteria established by Zucconi et al. [42]: GI < 50 % = high phytotoxicity, 50–80 % = moderate phytotoxicity, >80 % = no phytotoxicity, >100 % = potential phytonutrient or biostimulant effects.

2.4. Seedling test

The seedling test was assessed to investigate the potential phytotoxicity of recommended doses, equivalent to 2 g of hydrogel per plant. The test was carried out on 9 seedlings per treatment (AL, AL Eth, AL Phe, CMC, CMC Eth and CMC Phe). The pots, having a hole inside, were prepared with 4 g of perlite and 50 ml of soil also containing the hydrogels. Additional water was given when necessary. The trial was conducted within a growth chamber whose parameters were set as reported in Table 3. The light duration was set at 16 h, while the dark period lasted 8 h.

Table 3
Growth chamber and environmental parameters. Minimum, maximum, mean and standard deviation of daily humidity, temperature and CO_2 concentration.

	Humidity (%RH)	Temperature (°C)	CO_2 concentration (ppm)
min	32.8	24.0	384
max	47.0	31.7	524
mean	39.0	27.0	410
SD	3.9	1.9	40

Over the trial, 8 samplings were collected, every 4 days, for monitoring total number, yellow and dry leaves, normalized difference vegetation index (NDVI), and weight of the saturated soil samples. At the end of the experiment, additional assessments were performed on seedlings: total fresh and dry weight, total number of leaves, leaf surface, and presence of mould. The leaf surface was measured using an area meter (Li-3100; LICOR, Lincoln, NE, USA) calibrated to 0.01 cm^2. The presence of mould was recorded on a six-point scale, coded 0 to 5 (0 = none; 5 = severe presence of mould). Spectral reflectance of plantlets expressed as NDVI was measured using a handheld GreenSeeker™ optical sensor unit (NTech Industries Incorporation, Ukiah, CA, USA). The sensor unit has self-contained illumination in both the red (656 nm with about 25 nm full width half magnitude FWHM) and near infrared (NIR) (774 with about 25 nm FWHM) bands. The sensor outputs NDVI at a rate of 10 readings per second with travel velocities at a slow walking speed of about 0.5 m s^{-1} [43]. A Greenseeker sensor was used to interpret the NDVI data, expressed in a range between 0 and 1.

2.5. Statistical analysis

The data were subjected to analysis of variance (ANOVA) to evaluate the effect of hydrogels and the specific amount defined in relation to root elongation and seed germination (ORIGIN PRO) (One-way ANOVA). The Fisher Test was performed for mean comparisons (significant for $p < 0.05$). The principal component analysis (PCA) was performed to show clusters in the results of the germination test, using the mean of

each treatment. The Unscrambler® 10.6 (Camo Software, Inc., Oslo, Norway) was used for this analysis.

3. Results

3.1. Germination index

The germination index (GI %) was evaluated in response to the application of different hydrogels (AL, AL Eth, AL Phe, CMC, CMC Eth, CMC Phe) at three amounts (0.1, 0.2 and 0.4 g for 10 g of soil). The obtained results showed a statistically significant difference among the molecules both for root elongation and percentage of germinated seeds. As reported in Fig. 2, after 72 h, the mixtures of molecules based on the presence of CMC and Eth (0.4 g) and CMC and Phe (0.2 g and 0.4 g) exhibited an elevated phytotoxicity values compared to all the other hydrogels containing biofumigant agents tested in this investigation, with the recorded GI % that ranged between 26 % and 42 %. This index is directly influenced by the number of germinated seeds (SG), which ranged from 40 % to 58 %, and by the mean root elongation (mRE), which ranged between 1.18 and 1.51 cm. A different trend was observed for CMC hydrogels, in the absence of isothiocyanate-based micro-emulsions, which recorded a moderate level of phytotoxicity with GI% values ranging between 50 % and 70 %; additionally, an average root elongation between 1.39 and 1.87 cm were detected. By analysing the germinated seeds, a range between 60 % and 95 % is showed. Moreover, AL, AL Eth (at the three different amounts) and AL Phe (0.1 g) exhibited different values from all the other hydrogels showing no phytotoxicity and recording GI% values between 80 % and 90 %, with an average root elongation between 2.06 and 2.26 cm. The SG values ranged from 95 % to 100 %.

Regarding the 144 h monitoring, the hydrogels showed a statistically significant difference only for the average root elongation and not for the percentage of germinated seeds (Fig. 3a and b). The level of phytotoxicity differed significantly from the 72 h monitoring. The CMC Eth (0.2 g and 0.4 g) hydrogels together with CMC Phe (0.4 g) developed a moderate level of phytotoxicity as highlighted by both the number of germinated seeds and root elongation. Regarding SG, the application of these hydrogels led to values between 83 % and 90 % and an mRE between 2.71 and 3.22 cm. GI % values ranged between 65 % and 73 %. The lower amount of CMC Phe (0.1 g and 0.2 g), which were highly phytotoxic in the 72 h monitoring, at 144 h were phytotoxic-free, recording SG values of 88 % and 98 %, and an mRE of 3.39 and 3.70 cm, respectively. The germination index recorded was 92 % and 84 %, respectively. The remaining hydrogels did not exhibit any type of phytotoxicity, exceeding the 80 % threshold. AL (0.2 g and 0.4g), AL Eth (0.2 g and 0.4 g), AL Phe (0.1 g, 0.2 g, and 0.4 g), CMC (0.1 g, 0.2 g, and 0.4 g), CMC Eth (0.1 g), which were phytotoxic-free, recorded GI % values between 83 % and 99 %. The percentage of germinated seeds ranged between 95 % and 100 %, with a mean root elongation between 3.19 and 3.93 cm. A particular case is observed for the AL (0.1 g) and AL Eth (0.1 g) hydrogels, which exhibited a biostimulating effect. In fact, for these hydrogels, the GI values were 103 % and 101 %, respectively, with SG of 100 % and an average root elongation of 3.88 and 3.81 cm, respectively.

In the last monitoring (216 h) reported in Fig. 4, no hydrogel was found to be either toxic or moderately phytotoxic, all reaching phytotoxic-free state, but they statistically differed only about root elongation. Moreover, some treatments showed biostimulant effect; AL hydrogel at two amounts (0.1 g and 0.2 g), achieved GI values of 102 % and 100 %, mRE of 4.41 and 4.46 cm, and SG of 100 % and 98 %, respectively. Other hydrogels exhibiting biostimulant effects in terms of GI % were CMC Eth 0.4 g and CMC Phe (0.1 g and 0.2 g), with values of 112, 119, and 105 %, respectively. The average root elongation was 4.31, 4.95, and 4.67 cm with SG ranging between 88 % and 98 %. The other hydrogels showed GI % values from 83 % to 98 %, percentage of germinated seeds between 83 % and 100 % and average root elongation between 3.74 and 4.48 cm.

The PCA (Fig. 5) explained 100 % of the total variance, as sum of PC1 88 % and PC2 12 %. The 72 h and 216 h incubated seeds seem to highlight good differences for CMC treatments. In detail, all the amounts for CMC Eth and CMC Phe at 72 h were clustered in the quadrant II. Conversely, CMC Eth and CMC Phe at 216 h experimentation, regardless concentration, were clustered in the quadrant I. However, the remaining treatments were grouped together highlighting no specific trend (quadrant I, III and IV).

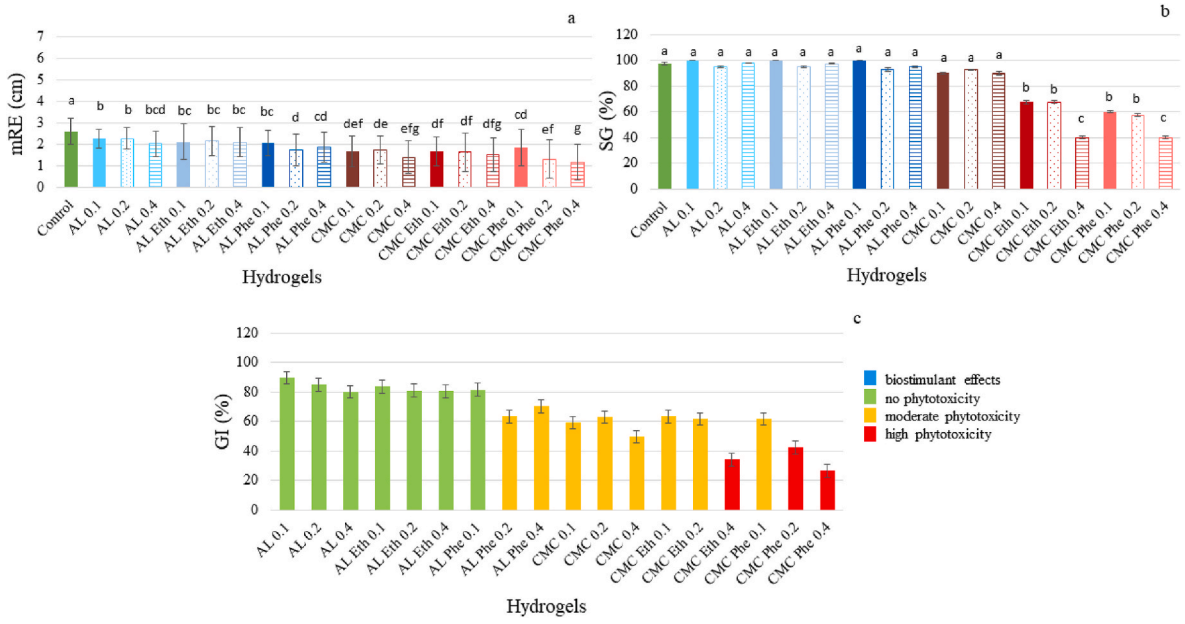

Fig. 2. Values of (a) mean root elongation (mRE, cm), (b) percentage of Germinated Seeds (SG %). and (c) Germination Index (GI %) in relation to six hydrogels application (AL, AL Eth, AL Phe, CMC, CMC Eth, CMC Phe) at three concentration (0.1 g, 0.2 g, 0.4 g) at 72 h. Vertical bars indicate the standard deviation, different letters indicate statistically significant differences *($p < 0.05$)*.

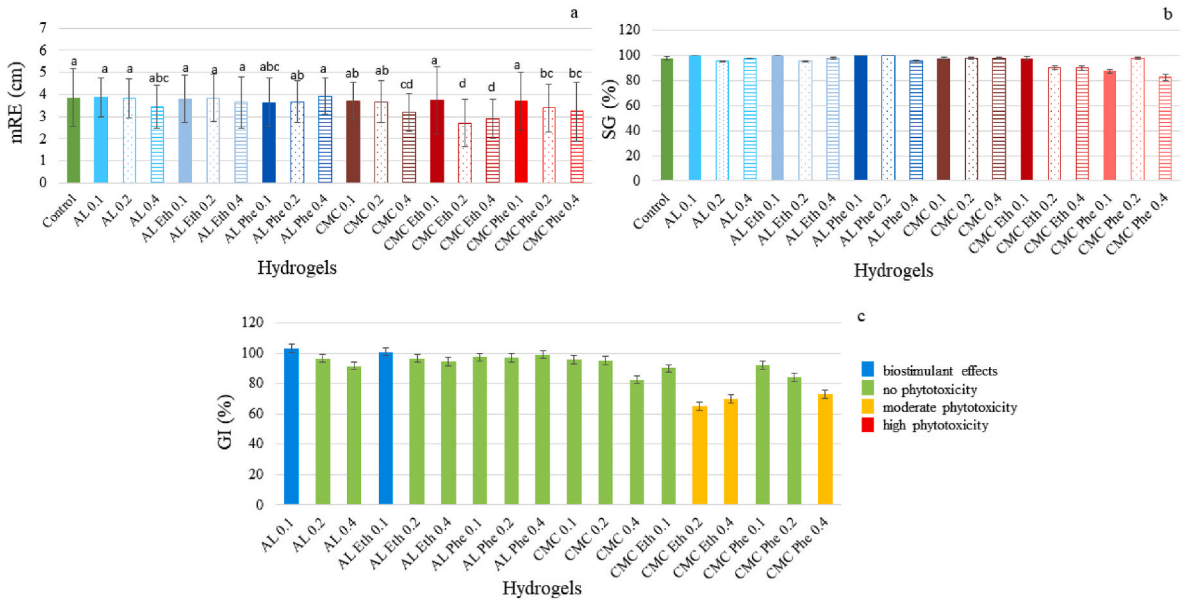

Fig. 3. Values of (a) mean root elongation (mRE, cm), (b) percentage of Germinated Seeds (SG %) and (c) germination index (GI %) in relation to six hydrogels (AL, AL Eth, AL Phe, CMC, CMC Eth, CMC Phe) at three concentration (0.1 g, 0.2 g, 0.4 g) at 144 h. Vertical bars indicate the standard deviation, different letters indicate statistically significant differences *($p < 0.05$)*.

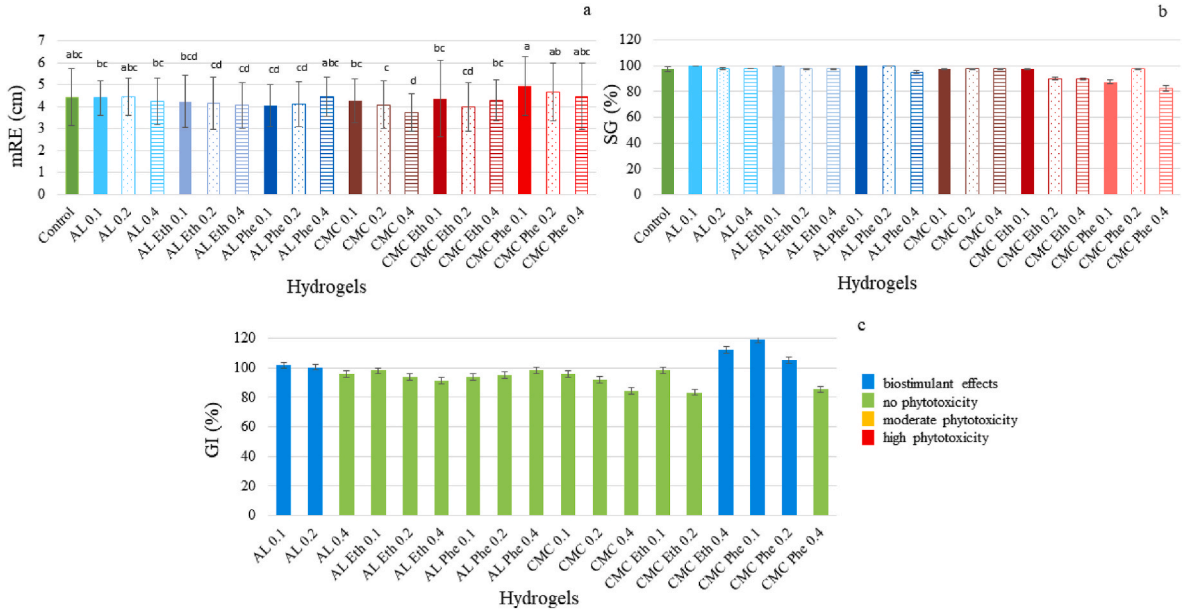

Fig. 4. Values of (a) mean root elongation (mRE, cm), (b) percentage of Germinated Seeds (SG %) and (c) Germination Index (GI %) in relation to six hydrogels (AL, AL Eth, AL Phe, CMC, CMC Eth, CMC Phe) at three concentration (0.1 g, 0.2 g, 0.4 g) at 216 h. Vertical bar indicates the standard deviation, different letters indicate statistically significant differences *($p < 0.05$)*.

3.2. Seedling test

Table 4 reports all the results of the seedling test such as fresh weight (FW), number of leaves, leaf surface, number of dry leaves, presence of mould and NDVI recorded at the end of the experiment (28 days). The highest FW was recorded for AL (8.37 ± 0.64 g), control (7.97 ± 1.44 g) and CMC (7.97 ± 0.69 g). CMC Eth treatment showed fresh weight of 7.58 ± 0.85 g, without any statistical difference, AL Eth and AL Phe recorded a FW value of 7.45 ± 0.76 g and 7.21 ± 0.53 g respectively, statistically significantly lower than the AL hydrogel treatments. CMC Phe showed the lowest fresh weight values of 6.92 ± 0.56 g, significantly different ($p < 0.05$) from AL, control and CMC. Concerning number of leaves, control and CMC obtained the highest average values (10 leaves plant $^{-1}$), followed by CMC Eth, AL and AL Eth with average leaves plant $^{-1}$ values of 9.75, 9.62 and 9.50 respectively. The application of the different hydrogels did not show significantly different values ($p > 0.05$). The lowest values recorded were obtained for CMC Phe and AL Phe with an average value of 8.87 leaves plantlets. The leaf surface had a similar trend as the fresh weight. The AL confirmed a very high trend, with average leaf surface values of about 180 cm^2. High average values were also found for control seedlings with about 174 cm^2, and for seedlings subjected to CMC treatment of about 163 cm^2. This is followed by the average values recorded in the seedlings with AL Eth 148 cm^2, and CMC Eth 141 cm^2. Significant differences were also

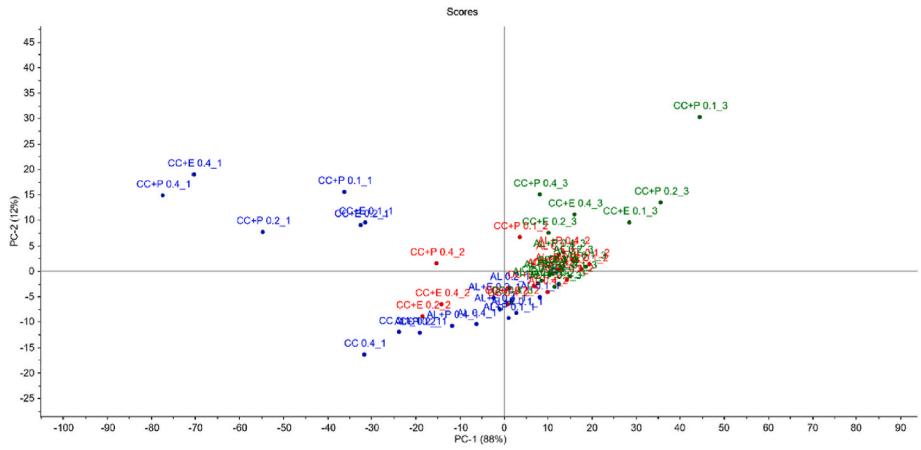

Fig. 5. Principal component analysis (PCA) for the germination test of the investigated hydrogels (AL = Alginate; CC = Carboxymethyl Cellulose; E = ethyl isothiocyanate; P = phenyl isothiocyanate); at different amount (0.1 g; 0.2 g; 0.4 g). Different colours indicate different detection times: 72 h in blue (1), 144 h in red (2) and 216 h in green (3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Data recorded for fresh weight, number of leaves, leaf surface, number of dry leaves, presence of mould and NDVI reporting mean ± standard deviation. Different letters show statistically significant differences among treatments (One Way ANOVA, $p < 0.05$).

Sample	*Fresh weight*(g)	*Number of leaves issued*	*Leaf surface* (cm^2)	*Number of dry leaves*	*Presence of moulds*	*NDVI*
Control	7.97 ± 1.44^{ab}	10.00 ± 0.58^{a}	174 ± 72.4^{ab}	2.00 ± 0.96^{ab}	1.71 ± 0.95^{bc}	0.35 ± 0.01^{bc}
AL	8.37 ± 0.64^{a}	9.62 ± 0.52^{a}	180 ± 18.2^{a}	1.06 ± 0.18^{c}	0.75 ± 1.03^{c}	0.38 ± 0.02^{a}
AL Eth	7.45 ± 0.76^{bc}	9.50 ± 0.75^{a}	148 ± 9.82^{bcd}	1.50 ± 0.27^{bc}	1.50 ± 1.06^{bc}	0.33 ± 0.01^{c}
AL Phe	7.21 ± 0.53^{bc}	8.87 ± 0.83^{b}	129 ± 13.6^{d}	2.00 ± 0.53^{ab}	3.50 ± 1.41^{a}	0.32 ± 0.04^{c}
CMC	7.97 ± 0.69^{ab}	10.00 ± 0.00^{a}	163 ± 10.4^{abc}	1.93 ± 0.56^{ab}	1.25 ± 0.46^{bc}	0.37 ± 0.01^{ab}
CMC Eth	7.58 ± 0.85^{ab}	9.75 ± 0.46^{a}	141 ± 15.8^{cd}	2.06 ± 0.32^{a}	1.50 ± 0.76^{bc}	0.36 ± 0.01^{ab}
CMC Phe	6.92 ± 0.56^{c}	8.87 ± 0.64^{b}	129 ± 13.9^{d}	1.81 ± 0.46^{ab}	1.87 ± 1.12^{b}	0.32 ± 0.03^{c}

found for leaf surface of CMC Phe and AL Phe with the lowest values for leaf surface of 129 cm^2. The average number of dry leaves for most of the products is equal or close to 2.00. The highest value (2.06) was obtained by CMC Eth. This was followed by the control and AL Phe with an average value of 2.00. The application of the formulations CMC and CMC Phe achieved a value of 1.93 and 1.81 respectively. AL Eth and AL hydrogels application showed the lowest values of dry leaves, with an average of 1.50 and 1.06 respectively. Each plant with the different hydrogel treatments was visually examined for the presence of moulds. The AL recorded the lowest average value of 0.75, reflecting almost absence of moulds. Increasing values were obtained from CMC (1.25), CMC Eth and AL Eth (1.50 both), CMC Phe (1.87) and AL Phe (3.50) compared to control (1.71). NDVI showed highest value for AL (0.38). The mean NDVI values for seedlings with CMC and CMC Eth were very close to AL, recording values of 0.37 and 0.36, respectively and no significant difference. The control seedlings showed mean NDVI values of 0.35, while the lowest NDVI values were obtained in the seedlings with AL Eth (0.33), CMC Phe, and AL Phe, with 0.32 both.

4. Discussion

Hydrogels have significant applications in agriculture due to their ability to retain large amounts of water as they are able to release active molecules, growth regulators, or beneficial microorganisms. This promotes seed germination, root growth, and overall plant growth [44,45]. On the other hand, isothiocyanates may have a potential role in ecological biofumigation [46]. Although these tools may be pivotal for agricultural purposes, little research has been carried out on the ecotoxicological effects of hydrogel with biocidal components on cultivated crops. The use of hydrogels in agriculture offers an environmentally sustainable solution to various challenges. According to Tariq et al. [45], agricultural productivity, sustainability, and resilience can be improved through various methods such as water retention, nutrient release control, seed coating, crop protection, and yield increase. However, seed germination and plantlet development are critical stages in the early growing and establishing of any plant species [47]. They are delicate steps as plants can undergo to mortality due to drought or soil-borne pathogens [48]. Therefore, we carried out a germination test and seedling experiment using *Lactuca sativa* L. seeds and plantlets, respectively. We tested hydrogels of AL and CMC loaded with aqueous microemulsions based on Eth or Phe ITCs to evaluate the effect of bio-compounds on agronomic traits. During the initial 72 h monitoring period, significant differences were observed among the hydrogels, particularly in root elongation values and the percentage of germinated seeds. Hydrogels containing combinations of CMC with Eth or Phe at the highest amount (0.2 and 0.4 g) exhibited significant phytotoxicity. This suggests inhibitory effects on both seed germination and root elongation, with rates as low as 45–58 % for root elongation and 45–50 % for seed germination. It would seem that isothiocyanates loaded in CMC hydrogels can be released faster and the volatilization might be a way for ITCs to reach seeds in the soil as also reported by Petersen et al. [10] on different ITCs on weed seeds. Hydrogels that exhibited moderate phytotoxicity showed a 25–35 % reduction in root elongation compared to the control. It has been showed a small decrease (2–7 %) in seed germination for AL Phe at 0.2 and 0.4 g and CMC at 0.2 and 0.1 g, while a higher diminution (31–38 %) for CMC Eth at 0.2 and 0.1 g and CMC Phe at 0.1 g hydrogels. This moderate phytotoxicity could indicate that the application of such hydrogels is not very impactful on seedling development. Our findings align with Petersen et al. [10] on the inhibition of germination by high amounts of ITCs, likely due to an interaction with seed enzymes related to development [49]. These temporal dynamics highlight the importance of extended monitoring to accurately capture the nuanced effects of hydrogels on plant development. During the 144 h monitoring period, a change in phytotoxicity dynamics was

observed for all compounds, with particular evidence in the case of CMC Eth and CMC Phe hydrogels. The latter showed a significant increase in root elongation compared to the previous 72 h monitoring of 50 %, associated with a higher percentage of germinated seeds (30–50 %). The combination of AL and AL Eth at the lowest concentration showed a biostimulant effect, with a 3 % higher percentage of germinated seeds than the control. The last monitoring was performed at 216 h. All hydrogels showed a phytotoxicity-free profile, indicating a gradual dissipation of the inhibitory effects over time. However, there was a persistent variation in root elongation observed between different amount of the various hydrogels. The results confirm that low amount of ITCs delay germination and induce secondary seed dormancy, with ungerminated seeds still retaining viability [10]. Some hydrogels, including AL (0.1 and 0.2 g), CMC Eth (0.4 g) and CMC Phe (0.1 and 0.2 g), have consistently demonstrated biostimulatory effects. Of particular interest are the hydrogels of CMC Eth (0.4 g) and CMC Phe (0.1 g), which exhibited a biostimulating effect by increasing root elongation by 21–30 % compared to the control, despite a lower percentage of germinated seeds than the control. The observed biostimulant effects of select hydrogels highlight their potential as growth-promoting agents in agricultural practices. These results are in accordance with Tariq et al. [45], who reported that hydrogel beads provide a more controlled and targeted approach to water retention, improve soil fertility, and stimulate the development of better root systems, resulting in more vigorous plant growth. Furthermore, biostimulant effects of hydrogels was observed on weed seed emergence by Norsworthy and Meehan [50], who experienced a biostimulant effect of sublethal amount of isothiocyanate.

Laboratory toxicity experiments on ITCs do not always reflect field outcomes [51], for these reasons, a seedling test on *Lactuca sativa* L. plants was carried out with the same hydrogels (2 g of hydrogel per plant, equivalent to the 0.4 g used for the germination test). The analysis of fresh weight revealed significant variations between the different experimental treatments. The control and CMC treatments had similar average fresh weight values of 7.97 g. The AL treatment showed an increase of over 5 %, while the AL Eth, AL Phe, and CMC Eth treatments had a 5 %–10 % reduction in fresh weight compared to the control. AL treatment showed the same results obtained on two cellulose derivatives, sodium carboxymethylcellulose and hydroxyethylcellulose by Montesano et al. [52] on cucumber plants. The CMC Phe treatment had the lowest fresh weight value, with a reduction of 14 % compared to the control. The number of leaves per plant was consistent across most treatments. CMC achieved the highest value (100 %) compared to the control, while other hydrogels containing antimicrobial agents showed a lower presence of fresh leaves, ranging between 5 % and 10 %. Our findings support those of Idrissi et al. [53] on tomato, who reported a significant rise in leaf count from the third week after transplantation, resulting in a noticeable difference in leaf presence at the end of the growth cycle. The leaf surface measurements reflected the trends observed in fresh weight. AL showed the highest leaf surface values, 3 % higher than the control, followed by CMC. Differences in leaf surface were observed for CMC Eth, AL Eth, CMC Phe, and AL Phe, indicating variations in leaf area development with a decrease ranging between 17 % and 34 %. The number of dry leaves per plant remained relatively stable between treatments, with most products averaging about 2 dry leaves per plant. However, CMC Eth exhibited the highest average value of 3 % compared to the control. The other hydrogels resulted in a lower dry leaf percentage of 50–70 % related to the average of 2 dry leaves. The evaluation of mould presence on the plants showed varying degrees of contamination, with the control seedlings exhibiting moderate mould presence. The application of AL hydrogel exhibited an average value that was approximately 50 % lower than the control, while AL Phe showed twice the value of mould compared to the control, despite ITCs are generally inhibitors of fungi colonization [15]. This suggests that hydrogel treatments may affect susceptibility to fungal infections, displaying variable effect related to compounds with which they are associated [15,54]. The NDVI can increase or decrease due to the measurement time. According to Ashraf et al. [55], NDVI index close to 1 indicates stronger vegetative growth. However, in this case, the doses applied resulted in lower values (0.32–0.38) than the lettuce standard (0.82–0.85) of NDVI. Galieni et al. [56] found that untreated lettuce plants exhibit reduced NDVI values.

5. Conclusions

The present paper reports the effect of six hydrogels (four loaded with biocidal compounds and two empty) at three different amounts on seeds and plantlets of lettuce (*Lactuca sativa* L.), for evaluating their agronomic and ecotoxicological impact. Germination index result showed that hydrogels containing carboxymethylcellulose loaded with ethyl- or phenyl-isothiocyanate exhibited moderate or high phytotoxicity after an incubation time of 72 h, while at the same time, alginate hydrogels loaded with phenyl showed a moderate phytotoxicity. However, after 216 h, all the samples treated with hydrogels in this study revealed a biostimulant effect and absence of phytotoxicity.

Concerning seedling test, loaded hydrogels showed an inhibitory effect on the agronomic parameters (fresh biomass, number of leaves, leaf surface), on the contrary, the empty hydrogels highlighted a biostimulant effect on the same features. The Alginate hydrogel was the only able to reduce the incidence of mould.

It can be claimed that the effects of hydrogels on lettuce could consist in a temporary inhibition effect on seeds able to induce a reduction of crop growth traits. Biopolymeric hydrogels have significant potential for use in agriculture, however, the timing and dosage of hydrogels on seeds and crops need to be refined to maximise their effectiveness and minimise potential negative impacts on crops and the environment. Further research is essential to fully understand the long-term impact of such technologies on crop yield, soil health and the agricultural ecosystem as a whole.

CRediT authorship contribution statement

Marika De Angelis: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Pasquale Napoletano:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martina Cofelice:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis. **Erika Di Iorio:** Validation, Methodology, Data curation. **Claudio Colombo:** Writing – review & editing, Validation. **Michele Baglioni:** Writing – review & editing, Validation, Methodology. **Claudio Rossi:** Writing – review & editing, Validation, Methodology, Funding acquisition. **Elena Sorrentino:** Writing – review & editing, Validation. **Arturo Alvino:** Writing – review & editing, Validation, Supervision, Conceptualization. **Francesco Lopez:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition. **Stefano Marino:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marika De Angelis, Pasquale Napoletano, Martina Cofelice, Erika Di Iorio, Claudio Colombo, Michele Baglioni, Claudio Rossi, Elena Sorrentino, Arturo Alvino, Francesco Lopez, Stefano Marino reports financial support was provided by Italian Ministry of Agricultural, Food and Forestry Policies. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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