Research Article

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Gelatin–chitosan–PVA hydrogels and their application in agriculture

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Abstract

BACKGROUND: Hydrogels are materials with great potential in agricultural applications. Biodegradable hydrogels are used as preparations, for example to increase the substratum water capacity, and improve soil structure or agrochemical-controlled release. This work aimed to develop biodegradable hydrogels based on chitosan, gelatin and polyvinyl alcohol (PVA) for releasing inulin isolated from Dahlia tubers to induce protection in chili plants against *Phytophthora capsici*. The hydrogels were characterized by water absorption capacity, Fourier transform infrared (FTIR), scanning electron microscopy (SEM) and stereomicroscopy microscopy, and degradation capacity using the soil burial test with sterile and inoculated soil.

RESULTS: This work demonstrated the ability of a fabrication process in the preparation of gelatin-chitosan-PVA hydrogels for potential agricultural applications. The hydrogels showed a dense, tridimensional, interconnected and reticulated structure that was more evident in the hydrogel loaded with inulin. The hydrogels showed a water absorption capacity of $\leq$12 times its mass. FTIR and light microscopy demonstrated that the hydrogels were biodegradable. The percentage of degradation of hydrogels in inoculated soil was higher than in sterile soil using the soil burial test. Hydrogel loaded with inulin was found to be capable of inducing resistance in chili plants against *Phytophthora capsici*.

CONCLUSION: The hydrogels prepared for the described methodology have great potential for use in the agricultural sector as a reservoir for agrochemicals and inductors in plant resistance treatments. Furthermore, the hydrogels were proven to be biodegradable, offering a promising tool in crop protection.

Keywords: biodegradable; biotreatment; degradation; green chemistry; plant

INTRODUCTION

Chilli (*Capsicum* spp.) is one of the most cultivated vegetables worldwide. Mexico is the second biggest producer of chili with an estimated total production of 2 732 635 tons (2017). The chili crop is susceptible to the pathogen *Phytophthora capsici*,[1] which causes the disease known as chili wilt, which spreads through irrigation water and rain through oospores and zoospores.[2] This pathogen causes losses in the world production of peppers that exceed US$10 million annually.[3] The characteristic symptoms of this disease are delay in the growth or wilting of the plant, and characteristic brown or black coloration to the roots, stem and fruits.[4]

Disease control is based largely on the use of fungicides, bactericides and insecticides, many of which are toxic chemical compounds to plant invaders, causative agents or vectors of plant diseases. Fungicides, for example metalaxyl, mancozeb, propamocarb, cymoxanil, etc., have been used extensively for controlling fungal pathogens of plants; however, its excessive and uncontrolled use had led to increase resistance of pathogens.[5] Several natural phenomena of induced resistance could help to protect chili plants from disease. One of the environmental strategies for the wilt control in chili plants is the use of inducers or elicitors that activate chemical defense in plants, such as peptides, polysaccharides, lipids, among others. Elicitors induced a defense response, which includes changes at the physiological, molecular

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or epigenetic level, because of the perception of stimuli related to a process of pathogenesis, where the plant generates a response of resistance that keeps it protected against subsequent attack from pathogens.[6]

Fructans are a class of elicitors that show a beneficial effect on the control of diseases, for example in cucumber plants, because they induce resistance mechanisms and control postharvest diseases; they also present antimicrobial activities against pathogens.[7–9] The inulin from *Dahlia* (I) is a fructan that can act as a molecular pattern associated with damage and activate the innate immune response of the plant through receptors.[10] The green chemistry revolution of reducing environmental impact is causing change in many industries. In the agriculture sector, there is a strong need to provide green approaches for protecting crops and increasing their productivity based on renewable resources.[11,12] Fructans are a very attractive renewable option for crop protection.

The use of hydrogels in agriculture has become very popular and, under the green chemistry approach, an essential demand in their preparation is the replacement of petroleum-based materials with materials derived from natural sources.[13] The most common hydrogel applications in agriculture are in preparations to increase the substratum water capacity, to improve the soil structure,[14] as carriers of agrochemicals including fertilizers,[15] pesticides,[16,17] and seed coatings,[18] with the intention to promote crop protection. Hydrogels are tridimentional (3D), hydrophilic, cross-linked polymeric networks able to absorb large amounts of water. The acrylate-hydrogels are the most common products on the market; however, they are not biodegradable, and some concerns exist regarding their toxicity for use in agriculture.[14,16]

The use of biodegradable hydrogels has become very popular for the controlled release of agrochemicals. The active agent can either be dissolved, dispersed or encapsulated into the hydrogel.[19] Natural polymers are mainly preferred over synthetic polymers for preparing biodegradable hydrogels due to their safety, low toxicity, low cost and eco-friendly properties. Nevertheless, they also are mechanically weaker and more susceptible to degradation than synthetic polymers.[18,20]

Chitosan (CS) is one of the most abundant natural polymers in nature. It is a polysaccharide composed of glucosamine and *N*-acetyl glucosamine by β-(1–4)-linkage, which is derived from the deacetylation of chitin, and is obtained from the cell walls of fungi and crustaceans. CS is nontoxic, biocompatible and biodegradable, presents antimicrobial activity, and is used mainly in agriculture for crop protection due to its effects on plant response as an elicitor.[21]

Gelatin (Gel) is defined as a mixture of peptides and proteins that are generally derived from partial hydrolysis of collagen obtained from connective tissues of animals, which can include skin and bones (Gelatin Manufacturers Institute of America, 2012). Gel is soluble in water and in most polar solvents, and has shown its use as a biostimulant in plant growth.[22]

Polyvinyl alcohol (PVA) is one of the very few vinyl polymers that is soluble in water and is susceptible to ultimate biodegradation in the presence of suitably acclimated microorganisms. The preparation of PVA-based materials and its film-forming capacity make it attractive for a wide range of industrial applications.[23]

Recently, the use of ternary blends based on CS, Gel and PVA polymers has shown promise for improving the physiochemical and mechanical properties of biodegradable hydrogels.[24–27] However, the most common applications of the ternary CS–Gel–PVA blends are in biomedical applications.[28,29] To our knowledge, no research exists addressing the use of such hydrogels for agriculture applications. Thus, the main objective of the present study is to provide a simple and inexpensive method for developing biodegradable hydrogels based on both natural (CS and Gel) and synthetic (PVA) polymers for releasing inulin isolated from Dahlia tubers. The secondary objective is to investigate the use of these hydrogels in the protection of chili plants against the *Phytophthora capsici* pathogen. Additionally, the hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR), their water uptake properties were investigated under different conditions, and, finally, their biodegradation was assessed using the soil burial test.

MATERIALS AND METHODS

Materials

Reagents

Gelatin from bovine skin (Gel; type B, Bloom ~75), CS low molecular weight (92.2% degree of deacetylation), PVA (89 kDa, 99.8% hydrolysis), inulin from *Dahlia* tubers (I, M_r~5000), phosphate buffer saline (PBS), chloride acid, 2,3,5-Triphenyl-tetrazolium chloride solution (TTC), and Trypan blue cell cultured and tested were all bought from Sigma-Aldrich (St. Louis, MO, USA).

Biological material

Soil-borne fungi (*Fusarium oxysporum*, *Trichoderma spp*, *Penicillium spp* and *Aspergillus spp*) and soil-borne bacteria (*Bacillus subtilis* and *B. thuringensis*) CIATEJ, A.C. strain collection were used. The *Phytophthora capsici* (CH11) strain was isolated by Sylvia Fernández Pavia of the Universidad Michoacana de San Nicolás de Hidalgo, México.

Vegetal material

Serrano chili cv. Camino Real was obtained from the Harris Moran Seed Company.

Hydrogels

The hydrogels were produced according to the methodology reported by Rodriguez-Rodriguez et al. (2019).[28] Gel, CS and PVA solutions were prepared individually by at concentration of 2.5 wt%. Gel was dissolved in deionized water using mechanical agitation at 37 °C for 2 h, CS was dissolved in acetic acid solution (0.4 mol L^{-1}) at 25 °C for 24 h, and PVA was dissolved in distilled water at 80 °C under stirring for 3 h. Hydrogels were fabricated by mixing the polymer solutions at a weight ratio of 1:1:1. The polymer blend was stirred at 20 °C for 3 h and its final pH was adjusted at pH 4.0, below the pKa value of CS and the isoelectric point of Gel, to obtain a homogeneous polymer blend solution. Approximately 2 mL of the blend solution was placed into silicone mold, and frozen at −80 °C for 24 h. The samples were freeze-dried using a Telstar LyoQuest Freeze Dryer (Terrassa, Spain). The dried samples were placed into a NaOH solution 0.1 mol L^{-1} for 30 min. After that, the samples were treated with xylene for 15 min. The samples were washed with ethanol gradients from 100 to 0% to remove the solvents.

The hydrogel cubes (H) were dried using a freeze-drier (Telsar LyoQuest Freeze Dryer, Terrasa, Spain) at −80 °C for 24 h. A homogeneous inulin solution was prepared at a concentration of 20 mg mL^{-1}; 200 µL of this solution was injected inside the dry hydrogels. Next, the loaded hydrogels (HI) were lyophilized to produce dried samples.

Physicochemical characterization

Water uptake and pH-sensitivity study

The swelling capacity of the hydrogels was evaluated in distilled water in distilled water (pH 5.5) and PBS with different pH values (5.0, 6.0 and 7.0). The dry hydrogels (0.5 cm × 0.5 cm squares) were immersed in the PBS solutions at 37 °C for 24 h. The hydrogel was removed from the solution and the excess solvent on the surface was removed by blotting quickly with absorbent paper and weighed. The swelling ratio was calculated as follows:[29]

$$S\,(\%) = \frac{(W_s - W_d)}{W_d}\ (100\%) \quad (1)$$

where S is the swelling percentage; Ws is the swollen sample mass and W_d is the dried sample mass.

Equilibrium water content in soil

Dried samples were correctly weighed and buried in 10 g dry soil (Sunshine®-mix 3) placed in plastic cups. Then 15 mL deionized water was added into each cup which were kept under identical conditions at room temperature and samples were not irrigated again. The hydrogels were removed after different times (0, 1, 2, 3, 6, 12, 18, 24 h) over a period of 24 h from the soil. Excess solvent on the surface was removed using a filter paper and the hydrogels were weighed. The following equation was used to calculate the equilibrium water content (EWC):

$$EWC\,(\%) = \frac{(W_s - W_d)}{W_d}\ (100\%) \quad (2)$$

where W_s is the mass of hydrogel after different durations of hydration and W_d is the mass of the dried samples.

Microscopic morphology analysis

The hydrogels before and after the biodegradation study (burial test with and without sterile soil) were freeze-dried for morphology analysis. Samples were cut using a cold knife. Samples were then examined by stereoscopy microscopy (Leica EZ4 HD Digital Stereo Microscope, Republic of Singapore).[30] In order to observe surface morphology of the dry hydrogles with (HI) and without inulin (H), the samples were examined by Scanning electron microscopy (SEM, Jeol JSM-6010LA, Tokyo, Japan).

Fourier transform infrared spectroscopy

Analysis by FTIR was performed in lyophilized hydrogels using the attenuated total reflectance (ATR) technique. Spectra were collected in the range from 4000 to 400 cm^{-1} using a Perkin Elmer Model Spectrum GX. For the acquisition of the ATR spectrum, each sample was placed onto the ATR crystal. The crystal was cleaned with acetone after the acquisition of each spectrum. Spectra were obtained in triplicate with 24 scans and 4 cm^{-1} resolution, and normalized with an ordinate limit of up to 1.0 of absorbance using the tool available in the spectrometer software (SPECTRUM, v5.01, Perkin-Elmer, 2003).

Biodegradation study by soil burial test

The degradation of the hydrogels was studied by means of the burial test proposed by Saruchi (2016)[31] with modifications on a laboratory scale. The soil burial test lasted for 28 days using two soil treatments: sterile and no sterile (inoculated with microorganism). The soil used was Sunshine®-mix 3 substrate. The soil was sterilized twice using an autoclave (Market Forge Industries Inc. Model STM-EL, New York, USA) at 120 °C for 40 min. The inoculated soil contained microorganisms with soil-borne fungi (*Fusarium oxysporum*, *Trichoderma*, *Penicillium*, and *Aspergillus*) and soil-borne bacteria (*Bacillus subtilis* and *Bacillus thuringensis*). The final strain concentrations in the inoculated soil were 1×10^6 spores mL^{-1} for fungi and 1×10^6 colony forming units (CFU) mL^{-1} for bacteria. The soil was placed into plastic containers with tiny holes perforated at the bottom and sides to increase air and water circulation. The moisture of the soil was maintained with water and stored outside the room throughout the test. Hydrogels (H and HI) were buried in sterile soil and with microorganisms (five hydrogel samples per block) at a depth of 0.5 cm and thus subjected to the action of microorganisms which are normally present in the soil. Samples were withdrawn from the soil after 7, 14, 21, and 28 days of incubation. After the test, the samples were removed, washed with distilled water and freeze-dried, then kept in a desiccator until analysis. Samples were analyzed by FTIR spectroscopy, visual examination by stereoscopy, and mass loss analysis. The experiment was carried out in duplicate.

Mass loss analysis

The degradation of the samples was evaluated using the mass loss ratio, calculated from the average value of the mass change of five measurements. The mass loss ratio of the samples is provided by the following equation:[32]

$$W_L = \frac{(W_0 - W_f)}{W_0}\ (100\%) \quad (3)$$

where W_L is the mass loss percentage, W_0 is the initial mass of the samples before the soil burial test and W_f is the final mass of the samples after the soil burial test.

Evaluation of crop protection

Serrano chili cv. Camino Real seeds were disinfected by submerging for 3 min in 0.2 mol L^{-1} of sodium hypochlorite (NaClO), then rinsed with distilled water and placed for germination in trays with the sterile substrate Sunshine®-mix 3. The seeds were germinated in an acclimation room at 26 ° C with a photoperiod of 16:8 h, light:dark. Similar conditions were maintained for 30 days before the plants were transplanted into plastic bags with the sterile substrate. At the time of the transplant, the defense induction was performed where the plants were distributed in treatments of five blocks with ten repetitions each: control plants (ø) treated with 10 mL distilled water; plants inoculated only with *P. capsici* (P); plants treated with 10 mL inulin from *Dahlia* tubers (200 µmol L^{-1}) and inoculated with *P. capsici* (I + P); plants with three hydrogels each and inoculated with *P. capsici* (H + P); and a treatment with three hydrogels loaded with 20 mg ml^{-1} inulin and inoculated with *P. capsici* (HI + P). All treatments were applied to the base of the root. After 10 days of the induction, the plants were inoculated with 10 mL zoospore suspension of *P. capsici* (1×10^4 zoospores mL^{-1}). The oomycete was grown in the clarified V8 medium for seven days under dark conditions at 26 °C and subsequently cut into 1 cm^2 fragments and placed in a flood with sterile distilled water for 10 days for the generation of sporangia at 24 °C in the dark. Subsequently, they were left at 4 °C for 2 h for the release of the zoospores. These were quantified in a Neubauer chamber and resuspended in sterile distilled water at a concentration of 1×10^4 zoospores mL^{-1}. A photographic record was taken

at the end of the experiment to determine the incidence of the disease based on characteristic symptoms of the disease, and the height and fresh mass of the plants were recorded.

TTC assay

The root viability was considered by a modified TTC method from Wang et al. (2013).[8] The reduction of TTC to 2,3,5-triphenylformazan (TTF) was used for the visual determination of root viability.[8] Root samples were taken at the end of the experiment and washed with distilled water. Their surfaces were dried carefully with absorbent paper. The roots were placed in a small beaker and incubated with 10 mL 1:1 (v/v) mixture of 1% TTC solution and 0.l mol L^{-1} phosphate buffer (pH 7.0) at 37 °C for 1 h in the dark. The reaction was stopped by the addition of 1 mL of 1 mol L^{-1} H_2SO_4. The TTF was evaluated by the change in the color in the roots; red indicated the viability of the roots.

Trypan blue staining

Plant roots were collected at the end of the experiment and were gently washed in distilled water to remove soil particles before staining following the protocol of Phillips et al. (1970)[33] with minor modification. Briefly, the roots of each seedling were placed in a 15-mL Falcon tubes and bleached with 1.7 mol L^{-1} KOH at 50 °C for 30 min. Next, they were rinsed with sterile distilled water twice and 0.5 mol L^{-1} H_2O_2 at 50 °C for 15 min and finally stained with 0.05% (w/v) trypan blue in lactoglycerol (lactic acid: glycerol: H_2O = 1:1:1 v: v:v) at 50 °C for 30 min. These were distained with sterile 50% (v/v) water: glycerol several times and stored at 4 °C. The samples were observed under a light microscope (Olympus model BH-2, Tokyo, Japan).[33]

Effect of hydrogels on mycelial growth of P. capsici

Mycelial plugs (5 mm diameter) excised from the margin of 7-day-old colony of the isolate oomycete (randomly selected) were placed in the center of petri dishes (9 cm diameter) containing minimal medium with I (200 µmol L^{-1}), HI and H. Petri dishes with glucose (10 g L^{-1}) were used as control. Inulin and glucose were filtered through a 0.20-µm filter and the hydrogels were sterilized in an autoclave. After three days at 25 ° C in the dark, the mycelial formation of *P. capsici* treated with glucose, inulin, hydrogel without inulin and hydrogel with inulin was observed by stereoscope microscopy (Leica E24 HD, Digital Stereo Microscope). The experiment was conducted three times with three replicates.[34,35]

Statistical analysis

STATGRAPHICS CENTURION XVI v16.1.11 software (StatPoint Technologies, Inc., Warrenton, VA, USA) was used to carry out a two-way ANOVA test, followed by a Tukey post-hoc test using a 0.05 significance level (*P*-value).

RESULTS AND DISCUSSION

Physicochemical characterization

Preparation of hydrogel

In this study, we aimed to fabricate hydrogels from a Gel–CS–PVA blend to act as an agrochemical reservoir of Dahlia inulin for use in a green strategy for chili plant protection against the *Phytophthora capsici* pathogen. The protocol followed for the preparation of hydrogels is outlined in Fig. 1.

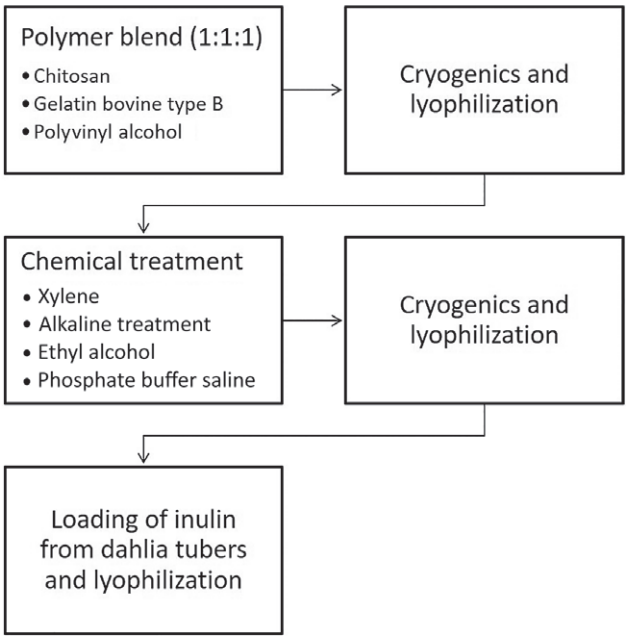

Figure 1. Diagram of the process of obtaining the hydrogel.

Table 1. Parameters of plant growth at the end of the experiment (fresh weight and height)

Treatment	Fresh weight (gFW)	Height (cm^{-1})
ø	0.58 ± 1.47^{c}	16.5 ± 0.05^{b}
P	0.11 ± 0.85^{a}	9.5 ± 0.0^{a}
I + P	0.20 ± 0.90^{b}	10.0 ± 0.06^{a}
H + P	0.17 ± 1.43^{a}	11.0 ± 0.05^{a}
HI + P	0.27 ± 1.15^{b}	11.0 ± 0.03^{a}

ø, plants without treatment; P, plants inoculated with *P. capsici*; I + P, plants with 200 µmol L^{-1} inulin and inoculated with *P. capsici*; H + P, plants with hydrogels without any molecule and inoculated with *P. capsici*; and HI + P, plants with hydrogels with inulin and inoculated with *P. capsici*.

The CS, Gel and PVA polymers were used as part of the formulation due to their biocompatibility and biodegradability.[28] Moreover, CS and PVA are able to form physical cross-linked and highly porous hydrogels (36). CS is typically soluble in acidic aqueous media due to the protonation of amino group (-NH_2). In order to induce the formation of physical cross-linking, the samples were freeze-dried to form junction points in the form of crystallites and interpolymer complexation.[36] In addition to this, the samples were immersed in NaOH to favour a physical gelation throughout the deprotonation of the amino groups of CS (pKa = 6.3–7.0)[37] Xylene was used to induce porosity in the physical cross-linking hydrogels.[38]

Water uptake and pH sensitivity evaluation

Soil pH is an important parameter that affects the performance of a hydrogel, influencing water uptake and swelling behavior. The typical pH range in a chili crop is between 5.5 and 7.0.[39] Water uptake of the hydrogels was evaluated by the measurement of the liquid amount absorbed by the material until saturation (swelling equilibrium). Figure 2 shows the water uptake for hydrogels in PBS solutions at different pH ranging from 5.0 to7.0 and distilled

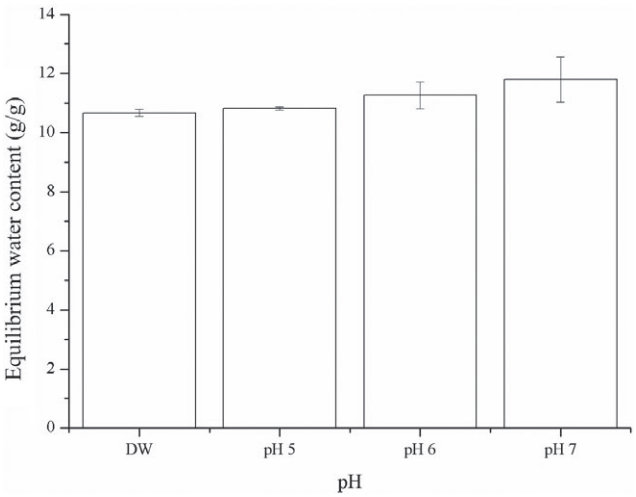

Figure 2. Swelling ratios of the hydrogel at different pH values.

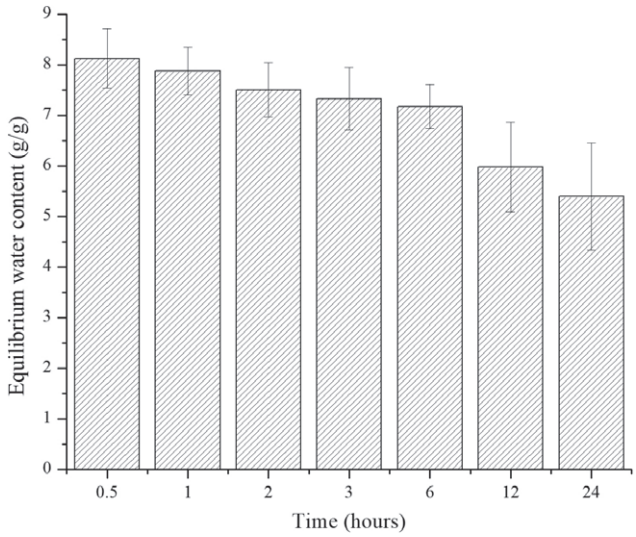

Figure 3. Equilibrium water content in soil.

water as the control (pH 5.5). The swelling equilibrium was reached after 24 h. The hydrogels showed an equilibrium water content from 10.8 to 12 times their own mass in all pH ranges. The statistical analysis showed that there was no significant difference ($P > 0.05$). Similar results were reported by Rodriguez et al.,[28] who mentioned that the ionization degree of the amine and carboxylic groups of the hydrogel, and the formation of new bonds during the preparation process were responsible for the swelling of the hydrogel at different pH conditions. The phenomenon of water sorption by hydrogel depends mechanistically on the diffusion of water molecules into the gel matrix and the subsequent relaxation of macromolecular chains of the hydrogel.[40]

Equilibrium water content in soil

The equilibrium water content of the hydrogels (Fig. 3) is related to its ability to absorb irrigated water and rainwater through the polymer network for agricultural applications.[41] It was found that the water content of hydrogels decrease in the soil after 24 h probably as a consequence of soil dehydration. Sunshine ® mix-3 allows good water retention because it contains fine-grade sphagnum peat moss, vermiculite and dolomitic limestone[42] yet it was irrigated only once during the experiment. Furthermore, the hydrogel swelling capacity depends on the polymer ratio due to the presence of hydrophilic groups attached to the polymer backbone (e.g. amino, carboxylic and hydroxyl groups).[28]

FTIR

The FTIR spectrum of H, I and HI exhibited several characteristic bands (Fig. 4). The dominant component of the hydrogel was identified mainly by the amide I (at 1630–1600 cm^1), amide II (at 1540–1350 cm^{-1}), amide III (at 1250–1300 cm^{-1}) bands present in CS and Gel. The characteristics bands of saccharide structure in CS were observed at 1150, 1060, 1030 and 890 cm^{-1}.

The spectrums of the hydrogel showed the following bands: (a) typically, a shift of the broad 3600–3000 cm^{-1} band towards lower wave numbers has been described as indicative of water-mediated hydrogen bonding formations; (b) amine and amide groups of Gel and CS at 1680 and 1480 cm^{-1}; (c) hydrogen bonding interactions between the amino and carboxyl groups at 3450 and 1600 cm^{-1}; and (d) the backbone of the polymeric network (CH and CH_2) at 1370 and 835 cm^{-1}.[27,29,43–45] All of those changes indicate chemical interactions between the components. In addition, the inulin

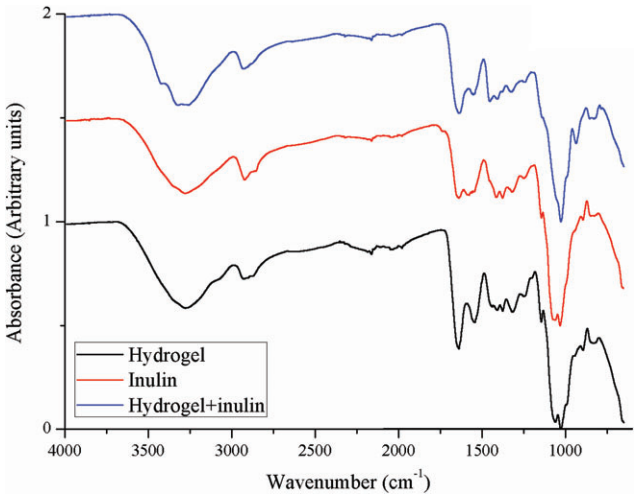

Figure 4. FTIR spectra of the hydrogel, hydrogel (H), inulin from Dahlia tubers (I) and loaded hydrogesl (HI).

characteristic bands at ~1130–1030 cm^{-1} were correlated to the stretching vibrations of the C–O, C–O–C groups and ring vibrational modes in the composition of cyclic structures. The deformations of the CH, CH_2 and OH groups of the fructose ring were observed at ~1330–1400 cm^{-1}.[46] In HI, the presence of inulin was detected by specific bands at 1040–1140 cm^{-1} (related to the fructose ring of inulin) suggesting weak interactions with the polymer network.

Surface morphology of hydrogels

The hydrogels have a polyhedral irregular shape. Figure 5(a) shows that the surface morphologies of the hydrogels revealed the formation of a reticulate heterogeneous network. When the hydrogel was loaded with inulin and freeze-dried again, inulin aggregates between the macro channels of the structure [Fig. 5(b)].

Biodegradation test of the hydrogel

The degradation of the hydrogels (H and HI), that were buried in the sterile and inoculated soil were monitored after 7, 14,

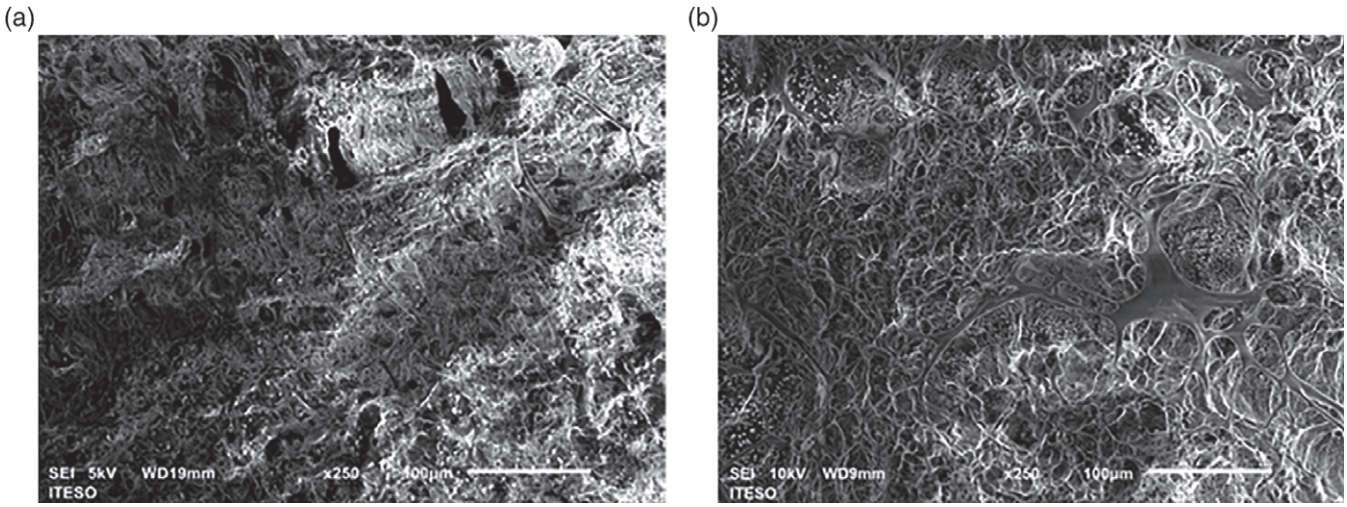

Figure 5. SEM micrographs of (a) hydrogel (H) and (b) loaded hydrogel (HI).

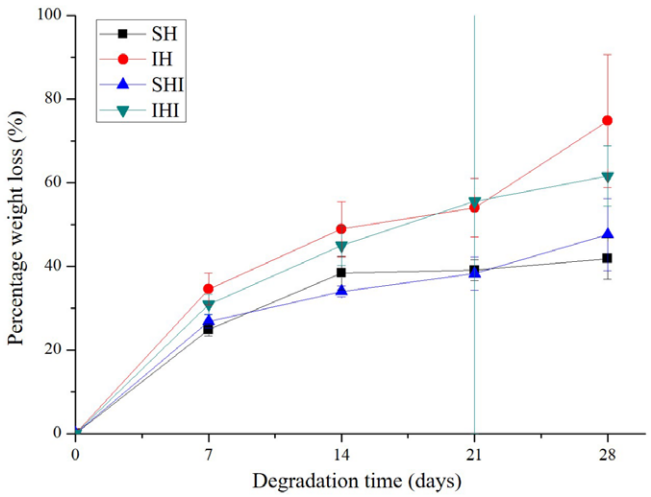

Figure 6. Biodegradation studies: SH (hydrogel) and SHI (loaded hydrogel) in sterile soil. IH (hydrogel) and IHI (loaded hydrogel) in inoculated soil.

21 and 28 days (Fig. 6). A simple and quick method to measure the biodegradation of hydrogels consist in determining mass loss during a soil burial test. From Fig. 6, it is observed that, with increasing of incubation time (days), a reduction in mass was observed in all cases after the biodegradation burial test in sterile and inoculated soil treatment. Statistical differences were found between the soil treatments ($P > 0.05$). It is well-known that degradation of polymers depends on wide range of factors such as temperature, oxygen content, pH, humidity, macro- and micronutrients, and the complexity of soil ecosystems hosting bacterium, fungi, protists, insects and other life-forms.[47]

The burial test showed that the highest biodegradation occurred in the inoculated soil, which can be explained based on its higher bacterial population, in addition to many factors including humidity and the greater availability of nutrients in this environment increasing the growth and metabolism of microorganisms.[48]

Although inulin presents a carbon source for microorganisms, its presence does not promote the growth of microorganisms, and therefore does not affect the mass loss. In sterile soil treatment, H and HI showed 40–47% mass loss. A slight variation in weight loss was observed on all days of biodegradation (1–28 days). No statistical differences were found between H and HI hydrogels with and without inulin ($P > 0.05$). The penetration of water into the body of the hydrogels favors hydrolytic attack on the surface of the hydrogel, favoring its erosion, which results in the hydrolytic degradation of the samples.[49]

By contrast, in the inoculated soil treatment, the H showed a 74% mass loss and the HI showed 61% of mass loss. No statistical differences were found between the hydrogels ($P > 0.05$). The initial biodegradation in the soil involves the colonization of the polymer surface by microorganisms present in the inoculated soil. The bacterial population in the burial environment accelerates the degradation process due to microorganism invasion that promotes the breaking of intermolecular bonds inside the hydrogels, causing the collapse of their structure.[50]

Figure 7 shows the FTIR spectra of the hydrogels before and after biodegradation test in sterile and inoculated soil after 28 days of the biodegradation. Bands in the region between 3750–3000 cm^{-1} assigned to OH stretching vibration, and bands between 1680–1480 cm^{-1} (amides I and II) correlated with CS and Gel functional groups observed in all of the samples. The band intensity in the OH region tended to decrease with increased burial time, which indicated that dehydration and erosion occurred upon buried. The spectrum of the samples in the presence of the bacterial population showed decreased intensity in the bands at 1680–1480 cm^{-1} suggesting the utilization of nitrogen by the soil microorganisms as a nutrient.[51]

Figure 8 displays the stereoscopic images of the degradation of the hydrogels before and after the soil burial test in both soil treatments. Figure 8(a) shows the hydrogel at the beginning of the experiment. Figure 8(b) and (c) illustrate hydrogels after 28 days of burial, in sterile soil, whereas Fig. 8(d) and (e) illustrate the equivalent time points in inoculated soil. Upon visual examination, the samples were whitish with a smooth surface, but with time, the color changed from white to dark brown and the surface turned rough and heterogeneous. In the inoculated soil, these changes were associated with microbial growth. The degradation rate of the hydrogel in the inoculated soil burial test was faster than it was in the sterile inoculated soil burial test. These results agree with the results of the biodegradation test and FTIR.

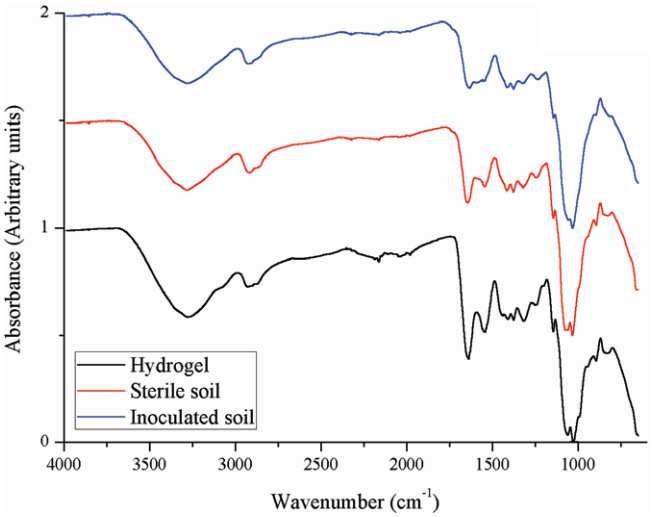

Figure 7. FTIR spectrum of biodegradation using the soil burial method of the hydrogel (H) and loaded hydrogel (HI) using the soil burial test with sterile and inoculated soil.

Crop protection evaluation

Figure 9 shows the plants after ten days of application of the treatments. Figure 9(a) shows healthy plants without the addition of *P. capsici* (ø), where there was no evidence of wilting disease. Figure 9(b) shows the dead plants due to infection with *P. capsici* (P). Figure 9(c) shows the surviving plants with inulin infected with *P. capsici* (I + P), where only 40% of the plants survived but those that did showed symptoms such as chlorosis and reduction in height. Figure 9(d) shows the 20% of plants that survived with the hydrogels without inulin (H + P). These results were like treatment P, which indicates that there was no protection in the plants. Figure 9(e) shows 80% survival of the plants treated with hydrogels loaded with inulin (HI+P); Disease symptoms were not visible, and the height of the plants decreased when compared to the control (ø). We can infer that inulin from *Dahlia* had a positive effect in reducing disease severity but did not inhibit the characteristic symptoms of wilt.

Several authors who have used defense inductors have observed a decrease in the development and growth of plants, so it was concluded that the activation of defense mechanisms through the use of inulin as an inducer could lead to additional energy expenditure, which is why plants and products of lower weight and size were observed.[52] In this work, we also evaluated the growth parameters height and weight (Table 1). It was observed that the control plants had statistically ($P > 0.05$) better growth than those that were infected, and energetic stress was the inferred cause. A statistically significant difference ($P > 0.05$) in weight was observed between the two groups: the control plants presented the highest biomass, and the plants inoculated with the pathogen and those with the hydrogel recorded lower weights in comparison to the other treatments. However, the use of fructans such as inulin may be able to improve and delay diseases in plants as they that can act as a molecular pattern associated with damage and active defense mechanisms that prevent attack by pathogens.[10] For example, fructans isolated from *Arctium lappa*, activated pathogen-response proteins, like β-1,3 glucanases and peroxidases in cucumber plants.[9] Therefore, the use of inulin as an elicitor for disease control is an alternative to keep plants protected due to the delay in infection by pathogens.

Figure 9(f–j) shows the root damage visualized by the TTC technique, where a reddish coloration indicates viable roots, but the absence of color shows the death of the plant. Chili plants treated with the hydrogel loaded with inulin (H + I) and healthy plants showed root viability, whereas the plants treated with inulin (I) samples showed slight root viability. Plants with hydrogel and the pathogen showed a compatible plant–pathogen interaction.[8] In addition to having proven the infection through the TTC test, it was observed that the plants in which the disease developed completely presented a necrotic stem and in the case of those that were still alive but infected, the number of roots decreased due to the destruction of the vascular bundles caused by the pathogen.[4,53]

In order to confirm infection by the oomycete, obstructions of the vascular bundles of the oomycete mycelium were observed by light microscopy.[39] Figure 9(k–o) demonstrates the presence of mycelium in the infected plants. Therefore, it was assumed that H + I was able to retain the inulin molecules and prevent plant infection by the pathogen.

The effect of inulin as an elicitor was associated in this work to the increase in the activity of enzymes related to the pathogenesis with activity of β-1,3 glucanases and peroxidases against the infection caused by *P. capsici*.[54] In addition, to verify the antifungal effect of the H + I against *P. capsici* a simple experiment was carried out. The *P. capsici* inoculum treated with glucose, I, H – I and H + I after three days was observed by stereoscope microscopy [Fig. 10(a–d)]. In all cases, mycelial formations around the inoculum were observed. However, the presence of glucose caused excessive branching in mycelial formations, whereas this was not the case with the other treatments [Fig. 10b–d)]. These results thus show that neither inulin nor hydrogel have antifungal properties, which underlines the potential induction effect of inulin against *P. capsici*.

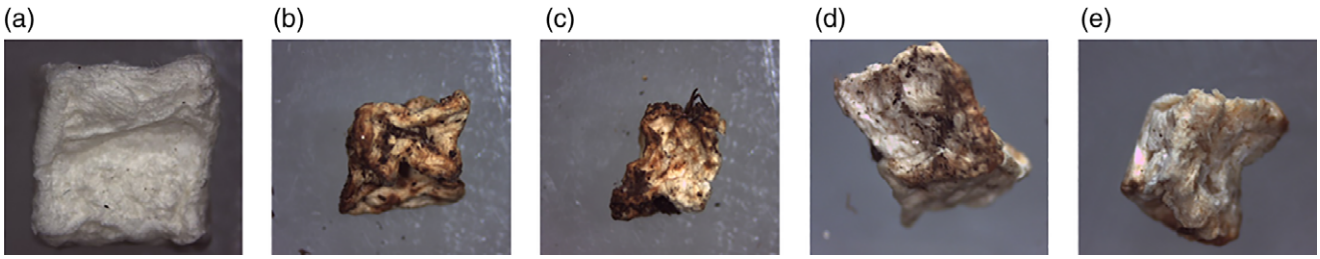

Figure 8. Stereoscopic microscope photographs of biodegraded hydrogels using the soil burial method. (a). Hydrogel (H) at initial day of the experiment. Buried after 28 days in sterile soil: (b) H and (c) loaded hydrogel (HI). Low-magnification objective lens (12.5X). Buried after 28 days in inoculated soil: H (d) and HI (e). High-magnification objective lens (16X).

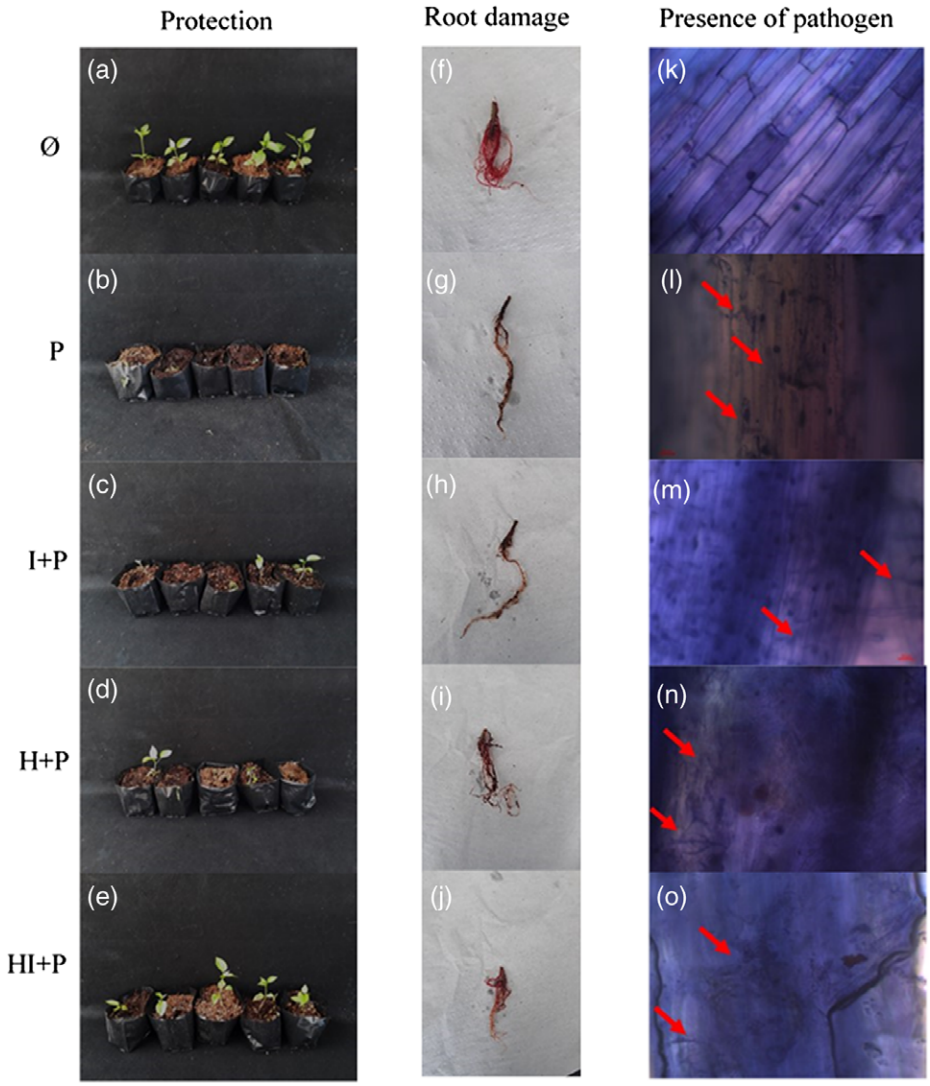

Figure 9. Effect of hydrogels on Crop protection: Ø: plants without treatment; P: plants inoculated with P. capsici; I+P: plants with 200 µmol L^{-1} inulin, H+P: plants with hydrogels without any molecule and inoculated with *P. capsici*; and HI+P: plants with hydrogels with inulin and inoculated with *P. capsici*. Symptoms disease observation (protection, a–e); TTC assay (root damage, f–j) and tripan blue staining (presence of pathogen, k–o, arrows indicate oomycte).

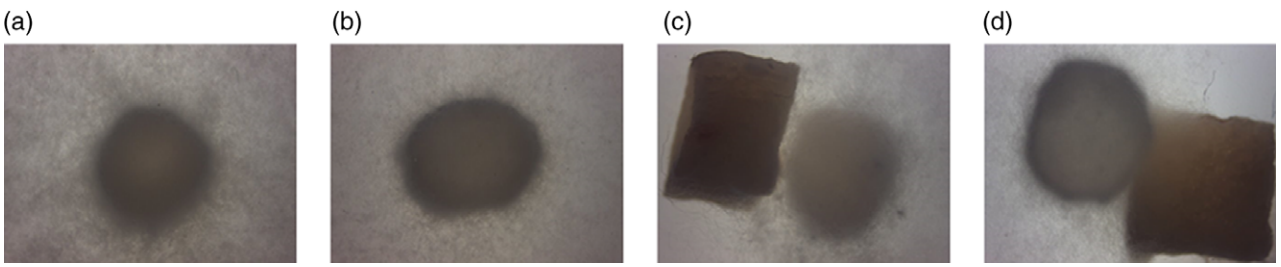

Figure 10. Antifungal properties of inulin (I), hydrogel (H) and loaded hydrogel (HI): (a) *P. capsici* in medium with glucose, (b) *P. capsici* in medium with inulin, (c) *P. capsici* in medium with H and (d) *P. capsici* in medium with HI.

CONCLUSIONS

This work demonstrated the ability of the fabrication process for the preparation of a hydrogel based on CS, Gel and PVA for potential agricultural applications. The hydrogel showed a dense, tridimensional, interconnected and reticulated structure that was more evident in the hydrogel loaded with inulin. The hydrogels showed a water absorption capacity of ≤12 times its mass. Biodegradation analyses demonstrated that hydrogels were biodegradable. The percentage of hydrogel degradation in the inoculated soil was higher than in the sterile soil in the soil burial test. The hydrogels loaded with inulin were found to be capable of inducing resistance in chili plants against *P. capsici*. The hydrogels showed the protective capacity of inulin *Dahlia* molecules that allowed it to be bioavailable for plants, and a

protection percentage of 80% of the disease caused by the pathogen was observed with respect to the plants that received different treatments. This suggests that the implementation of the hydrogel as a carrier of inulin has a potential use in agriculture, since it allows plants to be protected against the attack of pathogens in the production of vegetables such as serrano peppers. Furthermore, hydrogels have potential in agricultural applications as agrochemical carriers and inductors in plant resistance treatments.

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