Polyacrylamide Hydrogels With Amber for Plants Micropropagation

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Research Article

Keywords: plants micropropagation, polyacrylamide gel, amber, acrylamide toxicity, biotesting, Daphnia magna

Posted Date: October 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2085035/v1

Version of Record: A version of this preprint was published at Plants on March 6th, 2023. See the published version at https://doi.org/10.3390/plants12051196.

Abstract

The *in vitro* cultivation and reproduction of plants is one of the most modern and promising methods of cultivating valuable plants using artificial nutrient media. In this work, a new solid nutrient media for plant micropropagation based on highly dispersed polyacrylamide hydrogel (PAAG) with amber powder was synthesized and investigated. PAAG was synthesized by homophase radical polymerization with grounded amber addition. FTIR (Fourier transform infrared spectroscopy) and rheological studies were used to characterize structural properties of the materials. The synthesized hydrogel showed physicochemical and rheological parameters similar to the standard agar media. The estimation of acute toxicity of PAAG-amber was performed based on the influence of washing waters on the viability of the selected plant seeds (pea and chickpea) and animal (*Daphnia magna*). It proved its biosafety after four washes. The impact on plant rooting was studied using multiplication of *Cannabis sativa* on synthesized PAAG-amber saturated with Murashige-Skoog (MS) medium and compared with agar gel with MS. Developed substrate stimulated the rooting of the plants up to more than 98% in comparison to standard agar medium (95%). Also, PAAG-amber nutrient medium markedly enhanced metric indicators of seedling: root length increased by 28%, stem length – by 26.7%, root weight – by 167%, stem weight – by 67%, root and stem length – by 27%, root and stem weight – by 50%. This means that the developed hydrogel significantly accelerates reproduction and allows obtaining a larger amount of plant material within a shorter period than the standard agar medium.

Key Message

Polyacrylamide hydrogel with amber powder can be used as an agar-agar substitute for micropropagation of plants.

Introduction

Usually, the *in vitro* cultivation and reproduction of plants are carried out in solid nutrient media, and a high-cost agar substrate is the most used in this process (Kaçar et al. 2010; Adhikary et al. 2021; Lubell-Brand et al. 2021; Kaur and Mudgal 2021; Delgado-Aceves et al. 2022; Mood et al. 2022). This material is non-renewable, so for each subsequent passage of plants it must be used *de novo*(Espinosa-Leal et al. 2018). Because of agromarket's needs to propagate plants commercially, the agar substrates are used in large amounts. To avoid such high costs, the search for alternative approaches is demanded. There is a great need to substitute the agar with cheaper disposable materials, such as starch (Henderson and Kinnersley 1988) or gum (Jain and Babbar 2002), or to develop an environmentally friendly solid substrate with optimal physicochemical and functional properties suitable for multiple uses.

These requirements can be met by hydrogels based on spatially cross-linked hydrophilic polymers, which have the inherent ability to sorb and retain a significant amount of aqueous solutions due to adequate swelling properties. This process leads to an increase in hydrogel volume but its geometric shape remains unchanged. The so-called "smart hydrogels" are spatially cross-linked hydrophilic polymers with

a three-dimensional structure capable of responding to various external physical factors, including minor changes in pH, temperature, ionic strength, etc., by changing the swelling degree, volume, sorption and diffusion characteristics (Schmaljohann 2006; Kocak et al. 2016; Samchenko et al. 2018; Goncharuk et al. 2020). They can be synthesized in different consistency and arbitrary shape, particularly in the form of granules of controlled sizes. Such hydrogels are characterized by high hydrophilicity and biological tolerance, as well as improved optical, sorption, and diffusion properties, which can be adjusted by varying the monomer composition and cross-linking density. The listed peculiarities make them suitable for immobilization of a wide range of both organic and inorganic compounds, as well as for use in novel technologies, namely water treatment(Sinha and Chakma 2019), manufacturing of selective membranes (Kosenko et al. 2006; Stadniy et al. 2011), cell cultivation (in particular stem ones) (Kosenko et al. 2006), targeted delivery, and controlled release of anticancer drugs (Schmaljohann 2006). In recent years, the substrates based on such hydrogels found application for plant vegetation under controlled conditions due to their ability to sorb and prolongedly release the necessary bioelements into the environment under the plant root exudates action (Milani et al. 2017; Ramli 2019; Singh et al. 2021; Khan et al. 2022).

Some natural organic compounds with fungicidal and antibacterial properties can be included in hydrogels structure to enhance their functional properties. Primarily succinic acid and products of its transformation can be used as an additional source of microelements, endogenous physiologically active substances, growth or cell metabolism stimulators (Tumiłowicz et al. 2016; Levchyck et al. 2017; Mironov et al. 2018; Shimizu et al. 2020). It is known that amber, in particular, amber chips from the waste of various industries, is an environmentally safe and competitive product, so the inclusion of natural organic compounds in hydrogel substrates can be an effective and cost-effective way to increase the biological activity of the nutrient medium for plants micropropagation. In addition to the increasing bioactivity issue, the problem of ensuring biosafety is relevant for such materials. Since the monomers that compose the basic structural unit of polymers and, particularly hydrogels, are rather toxic materials and part of them always remains unreacted, the problem of synthesized hydrogels purification from residual microquantities of initial compounds is extremely important. The most affordable and effective way to purify hydrogels is to wash them repeatedly with distilled water. The risk control of toxic compounds in washing water is one of the most important steps in the preparation of synthesized material for further use.

Today toxicology uses not only traditional spectrometric methods of analysis, but also a variety of biotesting methods that allow obtaining a comprehensive toxicological assessment of the aqueous environment using living test objects, including plants (cereals and legumes, some algae, etc.) and animals (unicellular, crustaceans, worms, etc.). Bioassays involving aquatic organisms, such as *Daphnia magna*, are common and widely used to study the toxicity of various chemicals (Kim et al. 2015; Olkova and Zimonina 2020). Biological tests on a biomodel *D. magna* are standardized in many countries (2004). Short-term testing allows determining the acute toxic effect of compounds in aqueous solution on the survival rate of branched crustaceans *D. magna*, which is one of the most sensitive biomarkers for determining the toxicity caused by different classes of chemical compounds (2004; Kim et al. 2015; Rand et al. 2020; Olkova and Zimonina 2020).

The aim of the work is to develop the solid nutrient media for plants (namely, *Cannabis sativa*) micropropagation based on highly dispersed polymer hydrogels with biologically active amber compounds, which will meet the requirements of high biocompatibility and suitable moisture content, as well as will demonstrate the environmental safety.

Materials And Methods

Materials

Acrylamide (AA) (C_3H_5NO, "MERCK", Germany); N, N'-methylenebisacrylamide (MBA) ($C_7H_{10}N_2O_2$, "MERCK", Germany); potassium persulfate ($K_2S_2O_8$, "SIGMA", USA); sodium metabisulfite ($Na_2S_2O_5$,"SIGMA", USA) were applied in the study without additional purification. Double-distilled water was used as a solvent in all experiments.

For the synthesis of modified polyacrylamide gel (PAAG), the samples of Ukrainian raw amber from Zhytomyr, Olevsk and Rivne regions (Klesovo and Volodymyrets-Vostochny fields) were used.

Methods

Synthesis of PAAG

The method of homophase radical polymerization in aqueous medium was used for synthesis of hydrogels based on acrylamide (Gertsiuk and Samchenko 2007). Polymerization was carried out at room temperature. The monomer solution was stirred on a laboratory magnetic stirrer (MM-5, 1200 rpm), then the red-ox initiation system (potassium persulfate–sodium metabisulfite) was added. The components' concentrations in the initiating mixture were chosen to provide the polymerization completion within an hour and to prevent excessive composition heating, which could adversely affect the hydrogels' properties. Cross-linking with the spatial network formation occurred due to copolymerization with a bifunctional monomer N,N'-methylene-bis-acrylamide (MBA). The gel was dispersed in a mortar to a granule size of Ø1–2 mm. The components used for PAAG synthesis are summarized in Table 1.

Table 1
Composition of reagents used for synthesis of PAAG samples

Sample	Water, g	AA, g	MBA, g	Amber, %
PAAG	13.6	32	0.04	-
PAAG -A1	11.1	32	0.04	5.8
PAAG -A2	11.6	32	0.04	5.7

The synthesized PAAGs were repeatedly washed from unreacted components of the reaction mixture in distilled water in a ratio of 1 to 50 at a temperature of 45°C for 7 days. The washing process was

monitored spectrophotometrically using a UV spectrophotometer SPECORD M40 (Carl Zeiss) (Gertsiuk and Samchenko 2007). The washing water was further analyzed for the presence of toxic impurities using test objects of plant and animal origin.

Modification of PAAG with amber

The raw amber was grounded using the laboratory mill Kinematica AG (Polymix® PX-MFC 90 D), and then it was divided into fractions sizes from 2–3 to15-20 mm. Milled samples of raw amber were pre-washed in running water and 10% NaCl. After that, the amber samples were cleaned from NaCl residues and dried at room temperature for several days or in a drying cabinet at 40-50^{o}C. The amber samples were differentiated by color into groups: opaque/transparent – the dark amber (samples A1); translucent/transparent – the light amber (samples A2). Amber samples were pre-cooled by liquid nitrogen to prevent the destruction of natural components during mechanical grinding due to overheating.

Synthesis of polyacrylamide gels containing amber and cleaning them from unreacted components were performed according to the method described above. The fine amber A1 and A2 were added to the reaction mixture at a ratio of monomers and amber of 20 to 1 (Table 1).

Biotesting of acrylamide using pea and chickpea seeds

Biotesting of the toxicity of washing waters was performed by the Nelyubov method (1982), which bases on the fact that dyes stain only dead cell plasma (the plasma of a viable cell remains unstained). The viability was determined using pea and chickpea seeds. According to this technique, the seeds were soaked and, after 8 h, were released from the seed coat with a needle without damaging them. Ten peas were placed in the aqueous solutions of acrylamide with a concentration ranging from 0.001–10% and kept at a temperature of 30 ^{o}C for 3 h. Then the peas were transferred to 0.2% indigo carmine aqueous solution and kept for the next 3 h, after which the dye was drained, the peas were washed with distilled water, and their viability was determined according criteria of compliance with the conditions of viability shown in Table 2. Biotesting of washing waters (from the 1st to the 7th washings) was carried out similarly.

Table 2
Conditions for determining the seeds' viability according to Nelyubov (1982):

Viable seeds	Non-viable seeds
all parts are not stained	all parts of the embryo are stained
not stained root	the tip of the root contains unstained spots
not stained area around the root	in the lower part of the cotyledon unstained spots
root and cotyledon are completely/incompletely stained	
cotyledonous stained at the bottom	
cotyledonous is not painted	

Bioassay using Daphnia magna

The acute toxicity of polyacrylamide hydrogel was also determined using the model of the hydrobiont *D. magna* (according to ISO 10706:2000([CSL STYLE ERROR: reference with no printed form.])). This method is based on estimating the influence of aqueous solutions on the *D. magna* mortality rate (%). *D. magna* was kept in ventilated aquariums with carbon-filtered tap water (pH = 7.3 ± 0.3) at a temperature of 18–22 ºC and a dissolved oxygen concentration > 6.0 mg/L ([CSL STYLE ERROR: reference with no printed form.]). The illumination of the cultivation was 400–600 lux at a light period of 16 ± 1 h, and 8 ± 1 h of darkness. The water from the 1st to the 7th washings with an acrylamide concentration of 0.00125 to 0.00001 mmol/L were used for cultivation. Distilled water was used as a control. The experiment used newborns aged 12–24 h, obtained by cultivation. In each 50-ml glass container with 30 ml of the test solution, seven individuals of *D. magna* were placed. The newborn daphnias were fed using *Chlorella vulgaris* or a suspension of baker's yeast 2 h before the experiment and were not fed during the experiments. The mortality of individuals in each beaker was assessed within 24 and 48 h. Specimens that moved freely in the water column or floated to the tank surface no later than 15 sec after light shaking were considered alive ones. The experiments were performed in triplicate. The sensitivity of *D. magna* to the reference model toxicant, potassium dichromate ($K_2Cr_2O_7$), was also determined (for 24 h).This allowed to assess the suitability of *D. magna* culture for biotesting.

Investigation of hydrogel structure and rheological properties

Fourier transform infrared spectroscopy (FTIR) spectra of powdered samples over the 4000–400 cm^{-1} range were recorded using a ThermoNicolet iS10 FTIR spectrometer with a diffuse reflectance mode.

The rheological properties of PAAG were investigated by a rotary viscometer Rheotest 2.1 using a cylindrical system Z in the range of shear rates from 2.43 to 1073 c^{-1} at a temperature of 20°C. For rheological properties investigation, the synthesized chemically cross-linked PAAG were pre-treated in a

ball mill with subsequent sieving to obtain a fraction of gel particles d < 1 mm in the non-swollen state. Then, the dry gel particles were mixed with a given amount of water, which corresponded to the conditions of their use for the microclonal propagation of plants.

Micropropagation

Glesia industrial hemp (*Cannabis sativa*) seeds were selected as test objects. The used variety of monoecious non-narcotic hemp with dense rhomboid inflorescences and seed productivity provides the ability to produce a seed yield of 2.0-2.2 t/ha. The period from emergence to the onset of the phase of technical maturity is 88–93 days, and to the onset of the phase of biological maturity – is 100–120 days. The seeds were obtained from the Institute of Bast Crops of the National Academy of Agrarian Science of Ukraine. The seeds and cuttings of juvenile plants were the primary materials for obtaining sterile (microorganism-free) hemp plants *in vitro*. The cuttings were pre-treated with 70% ethanol for 1 min with the following treatment with 0.5% thiomerosal ($C_9H_9HgNaO_2S$) for 1.5 min. In turn, the seeds were pre-treated with 70% ethanol for 3 min with the following treatment with 0.5% thiomerosal for 5 min. Sterilized seeds and cuttings were transferred to a nutrient medium and germinated at 26°C. The duration of the period before the emergence of seedlings ranged from 14 to 20 days. Grafting of plants was performed in the presence of 3–5 internodes on plants. 1.0–1.5 cm parts of the stem (micropub) with two axillary buds basal part were placed vertically in agar nutrient medium to a depth of 0.3–0.5 cm.

Cultivation was performed on solid substrates: agar-agar (control) and hydrogel substrates: PAAG and PAAG-A2, saturated with Murashige-Skuga (MS) culture medium with the concentration of macronutrients reduced by half (MS/2). This medium contains 0.5 doses of macro- and micronutrients with the addition of 30 g/L sucrose and has a slightly acidic reaction (pH 5.6-6.0). For comparison, the agar-based (7.45 g/L) medium with the same MS and sucrose content was used. The complete cultivation cycle was 60 days. The first stage of introduction to the culture took place at the air temperature of 26–28°C. The obtained specimens were subcultured on MS medium under illumination with fluorescent lamps (2000–2500 lux) with a 16-hour photoperiod at a temperature of 24–26°C and a humidity of 70%.

In order to evaluate the allelopathic activity of aerial parts of hemp *Cannabis sativa L.* extracts, (Grodzinsky et al. 1987) the bioassays were employed. In this method, the one-day seedlings of cucumber *Cucumis sativus L.* cv. Konkurent were used as a test object (Bataineh et al. 2008).

Results And Discussion

FTIR

The FTIR spectra of dried initial PAAG gel, initial amber, and PAAG -gels with amber are given in Fig. 1. The most informative peaks of all studied materials are in the range of 1800–800 cm^{-1}. The spectrum of PAAG shows two bands at 3436 and 2924 cm^{-1}, which correspond with the N–H stretching vibration of

the NH_2 group and C–H stretching vibrations. The bands at 1645 and 1465 cm^{-1} are attributed to the stretching of the C = O group in amide and CH_2 scissoring, respectively (Nakanishi 1962). In the FTIR spectrum of initial amber, the wide shoulder of the 1160 cm^{-1} peak stretching to 1260 cm^{-1} (known as “Baltic shoulder”) corresponds to the high content of succinic acid and other succinate compounds. The intense peak at ~ 1700 cm^{-1} is characteristic of the C = O group of carboxylic acids. The bands at 1445 and 1375 cm^{-1} are attributed to C–H symmetric and asymmetric stretching vibrations. The 887 cm^{-1} band could be assigned to the out-of-plane aromatic C–H bending.(Mänd et al. 2018; Karolina et al. 2022)

In the spectra of polyacrylamide hydrogels with amber, the bands of amide I (1647 cm^{-1}) and amide II (1605 cm^{-1}) of acrylamide have the highest intensity. The bands in the range of 3000–3500 cm^{-1} correspond with symmetric and asymmetric vibrations of amino groups $v_{(NH)}$ of polyacrylamide. The maximum at 2938 cm^{-1} is attributed to stretching vibrations of the methylene group. The intense maximum $v_{(C=O)}$ at 1647 cm^{-1} (Amide I) corresponds with the amide fragment, which overlaps with the bending vibration maximum $\delta_{(NH2)}$ at 1605 cm^{-1} (Amide II) with the formation of a broadened doublet. In the "fingerprint" region, a doublet at 1450–1410 cm^{-1} caused by bending vibrations of the CH group, as well as a broadened band at $v_{(CN)}$ = 1350 cm^{-1} (Amide III) were noted.(Nakanishi 1962; Boldeskul et al. 2009)

Acute toxicity

Synthetic hydrogels, created by polymerization of highly toxic monomers such as acrylamide, contain unreacted residues that should be removed before using them for medical and biological purposes(Gertsiuk and Samchenko 2007). Acute toxicity of the studied compounds of different washes (from the 1th to the 7th) was determined based on the mortality rate (%) of *D. magna.* Data on the survival of individuals in each sample during 24 and 48 h of the exposure are presented in Fig. 2, Table 3.

Table 3
Overall risk assessment of toxic compounds present in washing waters on the viability of biomarkers of plant (legume) and animal (*D. magna*) origin

Washing / AA solution	Conc. AA, mmol/l	Death toll, %					
		PAAG		PAAG-A1		PAAG-A2	
		daphnia	peas / chickpeas	daphnia	peas / chickpeas	daphnia	peas / chickpeas
AA solution	1.4	100*	80/80	100*	80/80	100*	80/90
AA solution	0.14	100*	40/70	100*	40/80	100*	50/70
AA solution	0.014	100*	40/70	100*	40/80	100*	50/70
1	0.00125	100*	10/20	100*	10/20	100*	10/20
2	0.00096	100**	10	28.6**	10	28.6**	10
3	0.00054	42.8**	10	0	10	0	10
4	0.00007	0	0	0	0	0	0
5	0.00003	0	0	0	0	0	0
6	0.00002	0	0	0	0	0	0
7	0.00001	0	0	0	0	0	0
Control (DV)		0	0	0	0	0	0
* - died on the first day of the experiment							
** - died on the second day of the experiment							

The performed experiments indicated that after the first wash of all tested samples, complete death of organisms (100%) was already recorded during 24 and 48 h of the experiment. However, the mortality decreased by 28.6% and 57.2% during 48 h in the second washing water for PAAG-A2 and PAAG-A1, respectively, (Fig. 2). In the third washing water, the mortality of *D. magna* individuals in the PAAG-A1 and PAAG-A2 samples was absent. No cases of mortality were recorded during 24 and 48 h of the experiment. Based on these results, *D. magna* was considered as an organism of high sensitivity to washing water composition, which can be used for diagnosis and risk assessment of the hydrogels and unreacted compounds.

The results of biotesting of the toxicity of washing water by the Nelyubov method are depicted in Fig. 3. It was found that 1–3 washes of hydrogels, in which the concentration of acrylamide was from 0.00054 mol/dm^3 to 0.00125 mol/dm^3 (according to the results of measurements using a UV spectrometer SPECORD M40), were the most toxic for all tested legumes. For peas, 10% of seeds was dead, while for chickpeas, 20%. The results of both biotesting experiments are presented in Table 3. They indicated that

the washing water from the 4th wash is safe for both *D. magna* and legumes. The hydrogels, washed in this way, can be safely used in practice. Tucson et al. also washed PAAG for three days (changing the water every 24 hours), after which the gel was safe for further use as substrates for the study of bacteria (Tuson et al. 2012).

Rheological properties

The structural and mechanical characteristics of hydrogel composites largely determine the application possibilities of these systems. They are related to bioavailability and release of biologically active components from hydrogel materials. Gels are structured systems that demonstrate the structural and mechanical properties of both liquids and solids. Hydrogel, as a dispersed system, acquires the properties of a solid body, that is, shear modulus and elasticity. The most important rheological characteristics of hydrogels include shear stress and viscosity. Effective viscosity is a characteristic of the equilibrium state between the processes of destruction and recovery. Its fluctuation causes a change in the coagulation-crystallization structure of the hydrogel, affecting its performance characteristics. The spatial structure in the hydrogel is determined by measuring the mechanical properties and, in particular, shear deformation under the constant stress. Solids are characterized by a sharp change in the pattern of shear deformation ε depending on the magnitude of the shear stress P. At rather low stresses (less than the yield strength P_k), a free flow with constant and extremely high viscosity η_1 is observed. In this case, the coagulation structure is destroyed, but has time to recover. As the shear stress increases to the yield strength P_k, the viscosity decreases significantly, down to the lowest limit value η_m.

Rheological behaviors observed for agar gel and PAAG are presented in Fig. 4. Non-linear change in effective viscosity values indicates non-Newtonian system properties of studied gels. During the measurement, the destruction of interparticle bonds progresses as the shear rate ($\dot{\gamma}$) increases, which is manifested in the peculiarities of the shape of the flow curves, causing deviations from straight lines. Measuring in the reverse mode indicates recovery of the effective viscosity due to the restoration of the system structure, but the effective viscosity values remain lower than the initial ones. Both dispersed PAAG and agar gel showed thixotropic properties, as evidenced by the hysteresis loops of the dependence of the effective viscosity of gels on the shear rate (Fig. 4a,b).

Table 4 **and** Fig. 4 show the initial and final values of the effective viscosity at the minimum and maximum shear rates for homopolyacrylamide and agar gels. The initial effective viscosity values are close for both gels. During the measurement. The viscosity values at a shear rate of 2.45 s^{-1} at the end of the measurement in the reverse shear rate reduction mode for PAAG and agar are 156.398 and 200.32 Pa•s, which is 37.0 and 40.2% of the initial value, respectively. This means that PAAG had rheological properties and effective viscosity values similar to agar-agar gel.

Table 4
Effective viscosity at minimum ($\gamma = 2.45\ s^{-1}$) and maximum shear rates ($\gamma = 1073\ s^{-1}$)

Sample	η (at $\gamma = 2{,}45\ s^{-1}$), Pa•s		η (at $\gamma = 1073\ s^{-1}$), Pa•s	
	initial	final	initial	final
Agar	498.519	200.32	0.501	0.306
PAAG	422.986	156.398	0.471	0.410

Micropropagation

For micropropagation of plants *in vitro*, all synthesized hydrogels were saturated with a culture medium with a complex of micro- and macronutrients, vitamins, amino acids, growth hormones, etc. This process provided the formation of hydrogel composites with sparingly soluble bioelements, which are localized in the hydrogel pores and can gradually diffuse into the external environment. The experiment showed the acceleration of the rooting time of cuttings on PAAG-A2 gel, which was less than two weeks of incubation. On agar-agar this time equaled three weeks. In addition, an intensification of growth and development of the main shoot was observed on hydrogels: 10 and almost 30% higher on PAAG and PAAG-A2, respectively, compared to the samples grown on agar-agar.

The rooting of the plants on agar medium was 95%, while on hydrogel substrates – higher than 98%. On hydrogel substrates with the addition of amber, there was a 1.7-fold increase in shoot growth intensity, and on substrates without amber – 1.5 times. When the plants remained for 10–15 days in a tall vessel, their height reached 110–130 mm (Fig. 5).

In vitro deposition was carried out with periodic transplantations to the new nutrient medium to avoid drying out and changes in the composition of the environment due to the effect of the products of plant metabolism. After the first cycle, the spent hydrogel material was regenerated – washed in distilled water, sterilized, and used in repeated cultivation cycles. The obtained *in vitro* rooted plants *Cánnabis satíva* are suitable for conversion *in vivo* (Fig. 6).

The increase in the number of the metric indicators of leaf blades were also found. During transferring plants from *in vitro* to *in vivo* it was found that the dose-dependent effect of plant knees on test subjects persists (Fig. 7, Table 5). In general, using a hydrogel instead of agar stimulated the growth of *Cánnabis satíva*. Use of hydrogel-amber substrate increased metric indicators of seedling (in comparison to agar): the root length increased by 28%, stem length – by 26.7%, root weight – by 167%, stem weight – by 67%, root and stem length – by 27%, and root and stem weight – by 50%.

Table 5
Metric indicators of *Cánnabis satíva* seedlings

Experiment techniques	*Cánnabis satíva* seedlings	Root length (cm)	Stem length (cm)	Root weight (g)	Stem weight (g)	Root and stem lengths (cm)	Root and stem weight (g)
In vitro	Agar	3.5	7.5	0.3	0.6	11	0.9
	PAAG	4	8	0.6	0.7	12	1.3
	PAAG + A2	4.5	9.5	0.8	1	14	1.8
In vivo	Soil	15.2	21.8	1.3	1.4	37	2.7

After hydrogel recycling (ten-fold washing with distilled water), all nutrients, succinic acid and products of its transformation were much better absorbed by the plant and stimulated the growth and development of seedlings after transfer *in vivo*. In addition, the allelopathic activity of extract from biological material in 1:10, 1:50, and 1:100 dilutions was studied. It has been shown that allelopathic stress is dose-dependent when used a typical MS medium. Under 1:10 dilution *in vitro*, *Cucumis sativus L*. growth inhibition was observed up to 53%; however growth inhibition was reached 87% (Table 6). Under 1:100 dilution *in vivo* "Suppressive" effect almost didn't fall (95%), which indicates the successful transfer of plants from *in vitro* to *in vivo.* This pattern can be traced on the example of morphometric parameters of *Cánnabis satíva* seedlings (Table 6).

Table 6
Allelopathic effect of aqueous extract of aboveground parts of *Cánnabis satíva* on seedlings of *Cucumis sativus L*.

Dilution of the extract	*In vivo*, (%)	*In vitro*, (%)
1:10	67	53
1:50	75	60
1:100	95	87

Thus, both *in vivo* and *in vitro* a positive effect of amber in the composition of the hydrogel substrate on the main parameters of germination and development of the studied plants was revealed. According to the possible influence mechanism the biologically active components of highly dispersed amber powder affect biological objects at the cellular level, increasing the efficiency of processes in plants, and also take part in forming the microelement balance, i.e., they are bioactive. Highly dispersed amber powder embedded in the copolymer matrix is non-toxic, released gradually, its ionic form quickly includes in biochemical reactions, and therefore, when washing it with MS culture medium, the biologically active amber acid is washed out prolonged and dosed. This fact explains the high bioavailability and biocompatibility of the synthesized nutrient substrate, the possibility of obtaining planting material in a

shorter time, accelerated transition of plants from the juvenile to reproductive phase of development, and increased intensity of growth and development of the main shoot.

Conclusions

A spatially cross-linked polyacrylamide hydrogel with immobilized amber was synthesized. It has been demonstrated that in terms of its physicochemical and rheological properties, the obtained material is similar to agar-agar and can be used as its inexpensive substitute for micropropagation of plants. It is biosafe after four washings. During them, all unreacted toxic monomers and initiator residues were removed from the hydrogel structure, that is, it was purified effectively. The biosafety of new hydrogel was confirmed by the experiments performed using both biological objects, such as pea/chickpea seeds and *D. magna*, and traditional UV spectroscopic method. There was no mortality for both legume seeds and *D. magna* after application of water from 4th washing. The use of new hydrogel, instead of agar, stimulated the growth of *Cánnabis satíva*. For example, the root weight increased by 167%, whilst the stem weight, by 67%.

Thus, the described materials should be considered as novel effective nutrient media, which can accelerate reproduction and allow obtaining a higher amount of plant material within a short period of time in comparison with the standard agar medium.

Declarations

Author contributions LK, OD and OM conceived and designed the study. LK, KS, OG, NP, TP and OD performed the experiments. LK, OG, OS and KSK analyzed the data, contributed inputs, wrote and critically reviewed the manuscript.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Financial interests: The authors have no relevant financial or non-financial interests to disclose.

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Figures

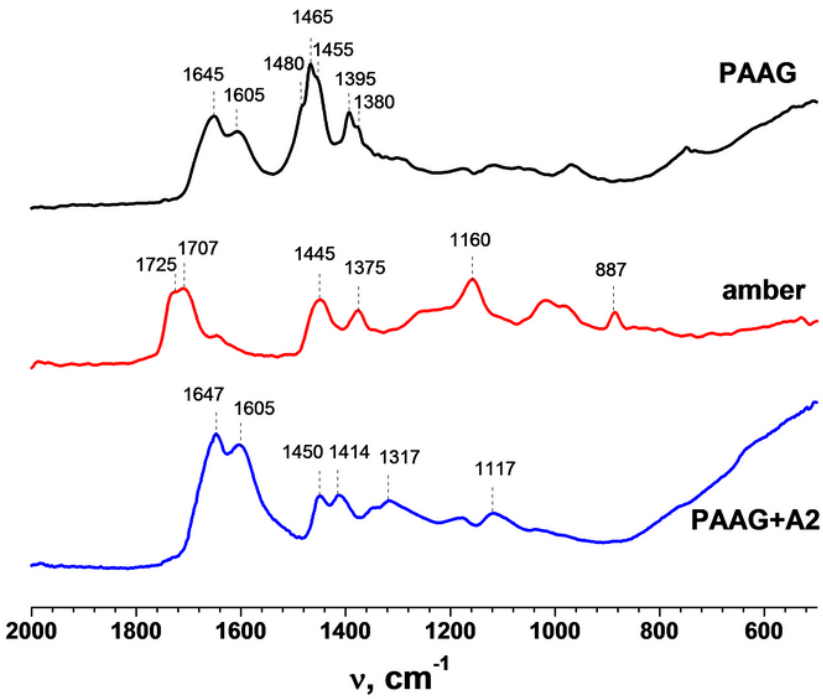

Figure 1

FTIR spectra of amber, polyacrylamide hydrogel and polyacrylamide hydrogel with amber

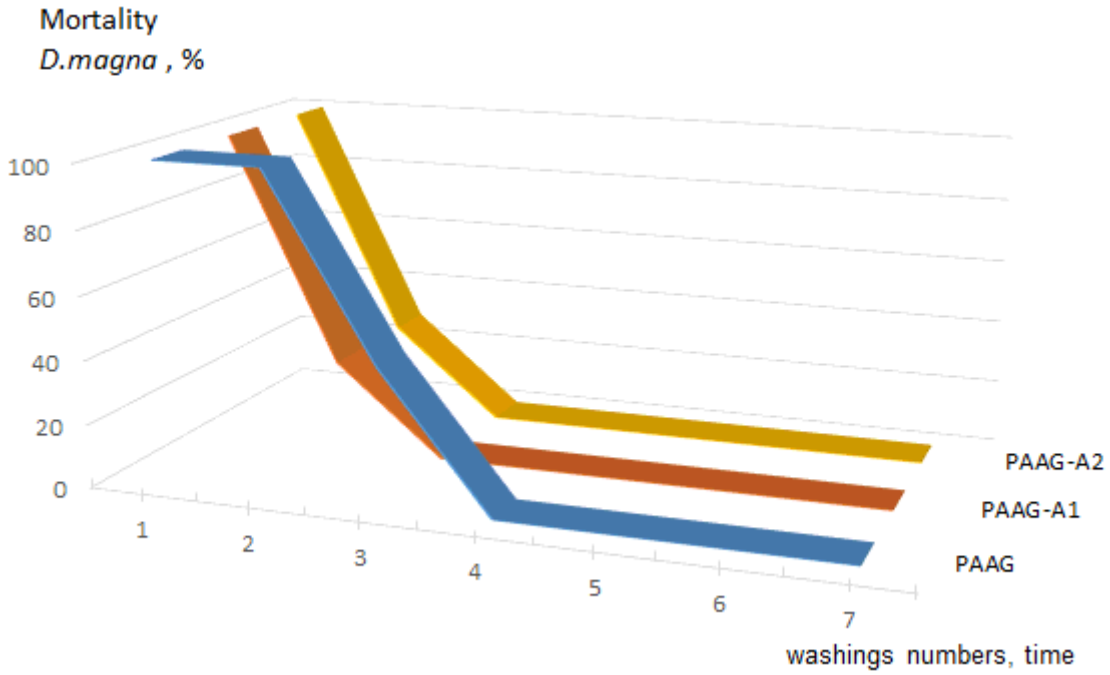

Figure 2

Dynamics of mortality of individuals *D.magna* depending on the number of washings: PAAG – the homopolyacrylamide gel; PAAG-A1 – the homopolyacrylamide gel containing 5.8 % dark amber; PAAG-A2 – the homopolyacrylamide gel containing 5.7 % light amber

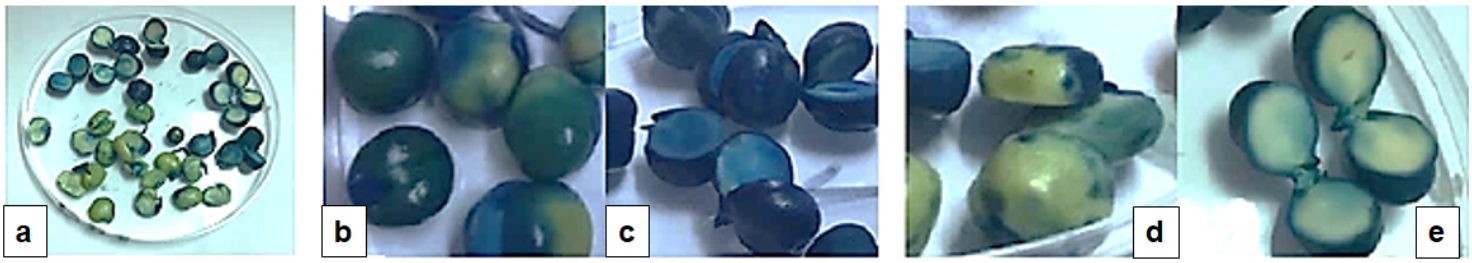

Figure 3

Visualization of Nelyubov method used for determining the viability of peas (b,d) and chickpeas (c,e): a – the control (distilled water), b,c – the first washing, d,e – the second washing. The incubation time was 90 min.

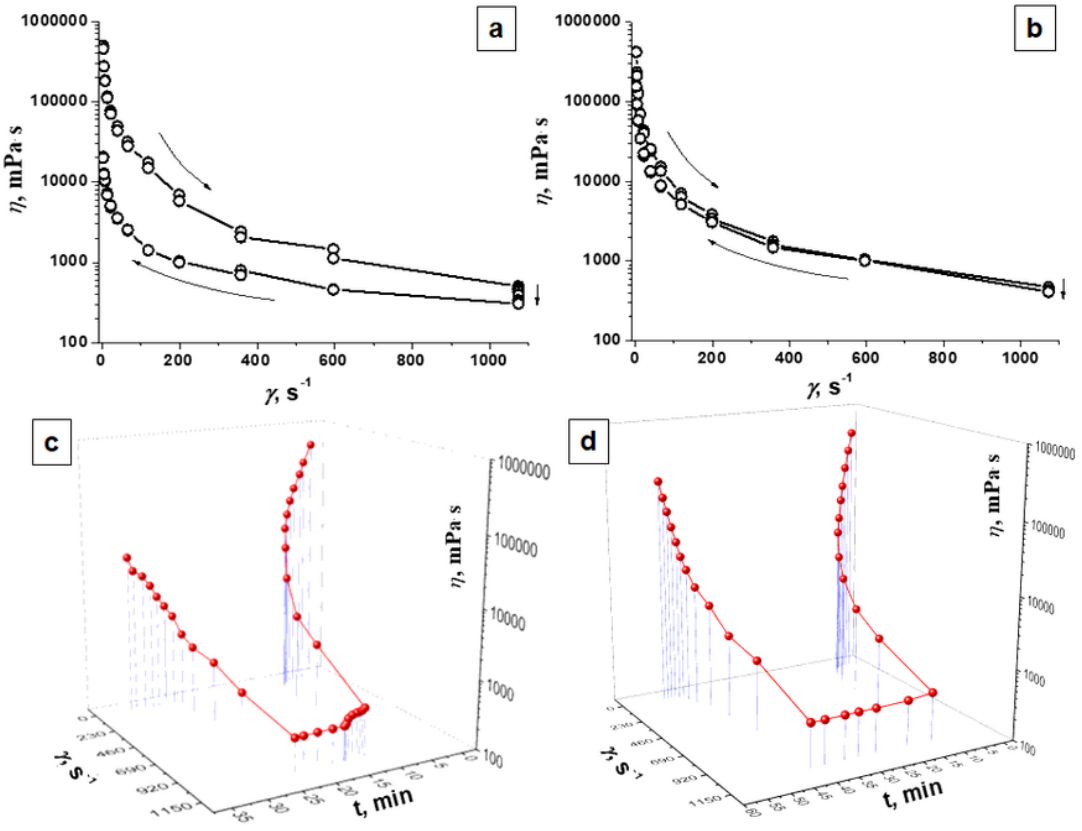

Figure 4

Dependence of effective viscosity (η, mPa • s) of polyacrylamide gel (PAAG) (b,d) and agar gel (a, c); a,b - on the shear rate (γ, s^{-1}); c,d - on the shear rate (γ, s^{-1}) over time (t, min)

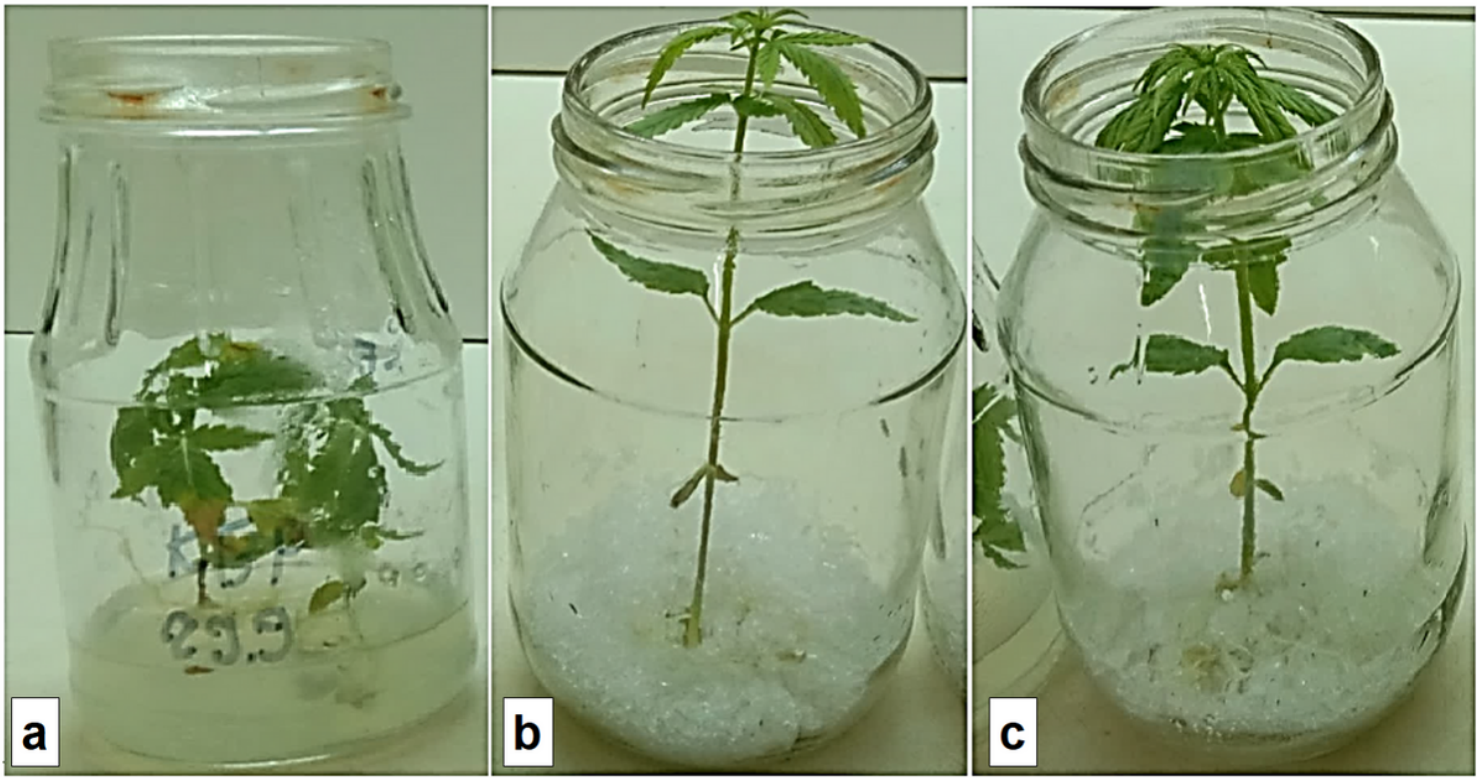

Figure 5

Visualization of the first cultivation cycle of *Cánnabis satíva* (14 days) on agar substrate (a), polyacrylamide (PAAG) (b), and polyacrylamide substrate with the addition of amber (PAAG-A2) (c)

Figure 6

Seedlings of *Cánnabis satíva* after transferfrom *in vivo* to the phytotron – the glasshouse of the M. M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Allelopathy Department

Figure 7

Metric indicators visualization of seedling growth *Cánnabis satíva*, transferred *in vivo:* on agar substrate (1), on polyacrylamide substrate (PAAG) (2),and on polyacrylamide substrate with amber addition (PAAG-A2) (3)