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Abstract: Polymeric protein-based biocomposites were used as water dispersions to generate, in situ, biobased mulching coatings by the spray technique, as alternative to low density polyethylene films used for soil mulching. At the end of their lifetime, these biodegradable coatings degrade in soil thank to the microbial community that mineralizes them. Protein hydrolysates (PH), derived from waste products of the leather industry, poly(ethylene glycol) diglycidyl ether (PEG) and epoxidized soybean oil (ESO) were used to make the biodegradable spray coatings.
A field study was carried out using plant test plots in order to investigate the performance of the spray coatings and their possible influence on some aspects of leaf growth, functionality and nutritional quality of the test-plants and on soil properties.
The biodegradable coatings showed agronomic performances comparable with the ones of a commercial low density polyethylene mulching film, maintaining the mulching effect for the requested cultivation period and ensuring a similar rate of plant growth and dry matter accumulation.
At the end of the field test, the soil mulched with the polyethylene film recorded an EC value lower with respect to the soil mulched with the sprayed coatings, which release nutrients in the soil during their decomposition.

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Bari, 06/06/2018

To the Editor of
Science of the Total Environment

Subject: Paper "Preparation and application of spray mulching coatings based on hydrolyzed proteins: effects on soil and plants"
Authors: L. Sartore, E. Schettini, L. de Palma, G. Brunetti, C. Cocozza, G. Vox

Dear Editor,
I would like to submit the research paper entitled "Preparation and application of spray mulching coatings based on hydrolyzed proteins: effects on soil and plants", Authors: L. Sartore, E. Schettini, L. de Palma, G. Brunetti, C. Cocozza, G. Vox, for publication in Science of the Total Environment.

The use of plastic materials in agriculture causes the serious drawback of huge quantities of waste to be disposed. The paper presents the results concerning the creation and application in field of an innovative material for soil mulching. It is easily applicable, releases nutrients to the soil, uses renewable raw materials and can be disposed in the soil. The development of a new generation of materials can overcome critical environmental problems.

Best regards,

Evelia Schettini

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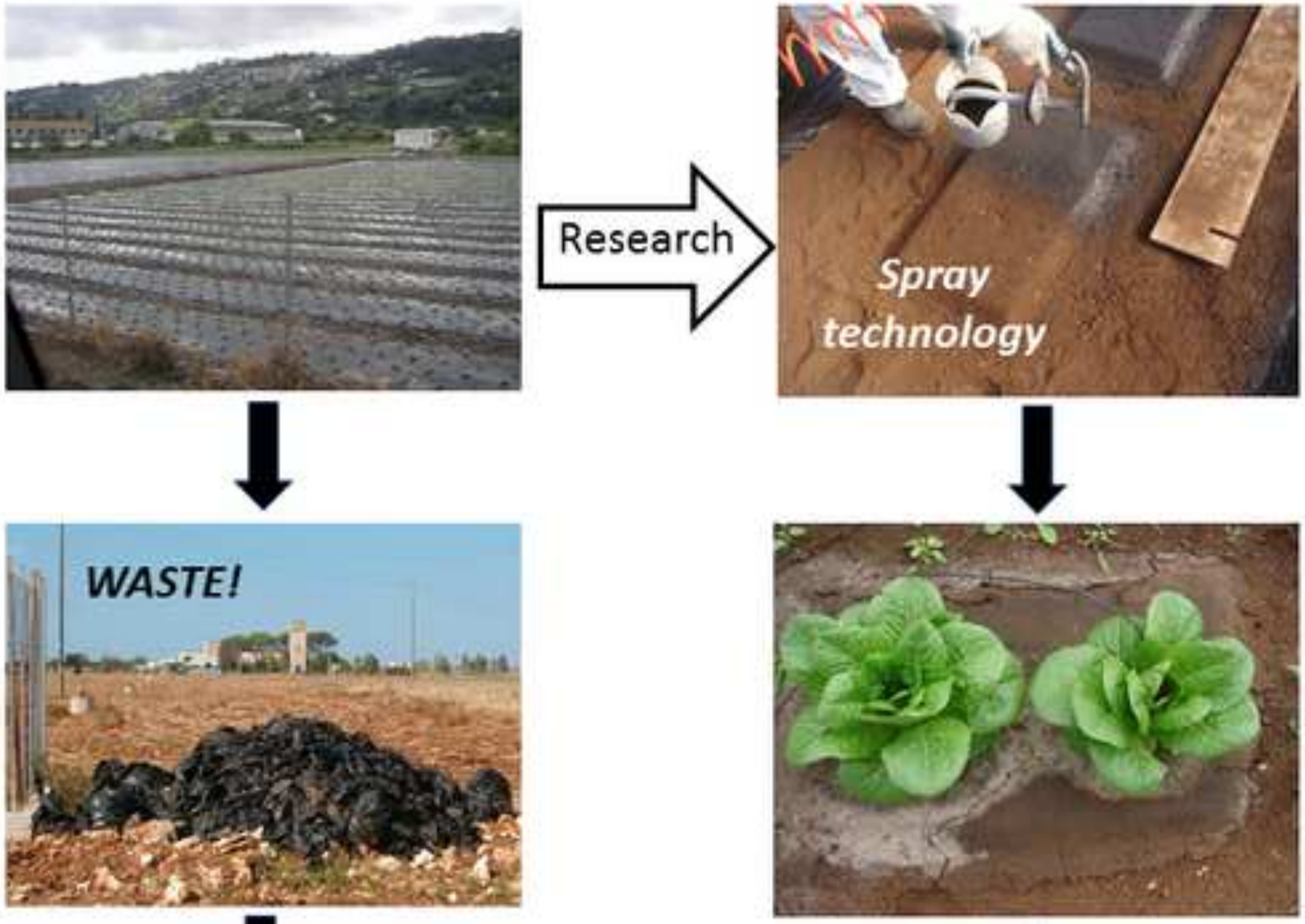
PREPARATION AND APPLICATION OF SPRAY MULCHING COATINGS BASED ON HYDROLYZED PROTEINS: EFFECTS ON SOIL AND PLANTS

*Graphical Abstract



Polyethylene for soil mulching

Polymeric protein bio-based and biodegradable composites

Environmental Problem!

Environmental Sustainability

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

- New biodegradable products manufactured from residues of the leather industry
- Biodegradable mulching coatings made by means of spray technique
- Agronomic performances of biodegradable coatings comparable to those of LDPE films

PREPARATION AND APPLICATION OF SPRAY MULCHING COATINGS BASED ON HYDROLYZED PROTEINS: EFFECTS ON SOIL AND PLANTS

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Abstract

Polymeric protein-based biocomposites were used as water dispersions to generate, in situ, biobased mulching coatings by the spray technique, as alternative to low density polyethylene films used for soil mulching. At the end of their lifetime, these biodegradable coatings degrade in soil thank to the microbial community that mineralizes them.

Protein hydrolysates (PH), derived from waste products of the leather industry, poly(ethylene glycol) diglycidyl ether (PEG) and epoxidized soybean oil (ESO) were used to make the biodegradable spray coatings.

A field study was carried out using plant test plots in order to investigate the performance of the spray coatings and their possible influence on some aspects of leaf growth, functionality and nutritional quality of the test-plants and on soil properties.

The biodegradable coatings showed agronomic performances comparable with the ones of a commercial low density polyethylene mulching film, maintaining the mulching effect for the requested cultivation period and ensuring a similar rate of plant growth and dry matter accumulation.

At the end of the field test, the soil mulched with the polyethylene film recorded an EC value lower with respect to the soil mulched with the sprayed coatings, which release nutrients in the soil during their decomposition.

Keywords: soil properties; physical properties; plant nutritional quality factors; PEG; ESO; biodegradable materials

1 Introduction

The agricultural practice of soil mulching consists in laying a continuous coating over the soil in order to suppress weed, decrease the loss of moisture from the soil and keep plants and edible products clean. Low density polyethylene (LDPE) mulching films are worldwide used due to their mechanical properties appropriate to assure an easy handling and installation, to their functionality and resistance throughout the cropping cycle and to the low cost. LDPE mulching films are generally monolayer films with a thickness ranging from 10 μm to 100 μm and an average lifetime of 2 - 12 months. Mulching films used for weed control must be opaque to prevent the passage of the photosynthetically active radiation (PAR) (Vox and Schettini, 2007). China, Japan and South Korea use about 80% of the worldwide mulching films production in horticulture, with a consumption of about 700,000 tonnes per year.

After their use, the mulching films are dirty of soil, organic matter and agro-chemicals thus they need a correct collection, disposal or recycling process with high costs for the growers. Agricultural plastic waste is often abandoned in illegal dumps, in river basins or burned; as a consequence, plastic residues accumulate in the environment and toxic emissions give off in the atmosphere becoming a considerable threat to human life as well as for terrestrial and aquatic wildlife (Lanorte et al., 2017; Mugnozza et al., 2016).

In order to overcome the problem of huge quantity of plastic wastes, biodegradable and renewable raw materials can be used to make mulches. At the end of their lifetime, biodegradable materials degrade in soil thank to the microbial community that mineralizes them. The lifetime of the biodegradable mulches can be tailored differently through the variation of the thickness in relation with the crop cycle and/or cultivation needs.

Biodegradable mulches can be performed by employing thermo-plasticizing, casting and spraying processes using renewable and biodegradable raw materials such as starch, cellulose, chitosan, alginate and glucomannan (Malinconico et al., 2008; Briassoulis, 2007).

The most recent technique is based on spraying water solutions onto soil forming a biodegradable mulching coating directly in field. Presently, spray mulching coatings based on polysaccharides that are potentially polluting residues of marine and agricultural origin can be obtained, even if they need to be improved mainly in their mechanical properties (Immirzi et al., 2009; Malinconico et al., 2008; Johnston et al, 2016).

Sartore et al. (2013) studied the possibility to obtain spray mulching coatings using protein hydrolysates (PH), derived from waste products of the leather industry, for their intrinsic agronomic values due to their high nitrogen content (Sartore et al., 2016; Schettini et al., 2012). Biodegradable coatings can be obtained by the modification of PH with poly(ethylene glycol) diglycidyl ether (PEG), a water soluble and highly reactive epoxy compound largely used as a cross-linking and insolubilizing agent in water-based systems. They showed very good mechanical and agronomical performances in the field, maintaining their mulching effect for even 12 months (Sartore et al., 2013, Hiroyuki 2014). PEG is a derivative of petroleum, therefore a sustainable solution can be its substitution with a renewable resource such as the epoxidized soybean oil (ESO). Although soybean oil is primarily used as edible oil, there is an increasing demand for its use in industrial applications, e.g. inexpensive and renewable plasticizer, stabilizer, reactive modifier and diluent (Niedermann et al., 2014).

In order to improve the thermo-mechanical properties and decrease water sensitivity while preserving biodegradability, natural fillers from renewable resources, such as wood flour and lignin, were incorporated in mulching coatings obtaining biocomposite materials (Sartore et al., 2016). Lignocellulosic products, mainly cellulose and lignin based materials, have recently attracted much attention due to their renewable nature, wide variety of source materials available throughout the world, low cost and density, high surface functionality and reactivity. Lignin, a byproduct of important processes like paper or biodiesel production, is a natural polymer found in wood and in the secondary cell walls of plants and some algae; it is the second most abundant biopolymer on Earth (Holmgren et al. 2006). Because of its polyphenolic structure, lignin shows antimicrobial (Holmgren et al. 2006; Fortunati et al. 2016) and antioxidant properties (Lora et al. 2002; Rahman et al. 2013) which can be very useful for agriculture applications or packaging with improved life-time.

The aim of this paper is to describe the development and the application of novel biodegradable polymeric materials, based on protein hydrolysate and reagents from renewable sources, for the creation of mulching coatings alternative to LDPE films. Two blends using PH, PEG or ESO and natural fillers were developed. A field study was carried out using plant test plots in order to investigate the performance of the spray coatings and their possible influence on some aspects of leaf growth, functionality and nutritional quality of the test-plants and on soil properties.

2 Materials and methods

2.1. The biodegradable coating materials preparation

The raw materials utilized for the preparation of the mulching coatings were: PEG (molecular weight=526 Da), ESO, ethylene diamine (EDA) and pyromellitic dianhydride (PMDA), supplied by Sigma- Aldrich Co (Milan, Italy) and used as received.

Carbon black (CB) was supplied by Degussa-Hϋls (Dusseldorf, Germany) with an average primary particle size of 23 nm.

Protein hydrolysate (PH) was a slightly yellow, chromium-free, product supplied by Sicit Chemitech S.p.A (Vicenza, Italy) as a by-product of chemical hydrolysis in the leather production process. PH is generated from alkaline hydrolysis of the solid residues generated downstream of leather chrome tanning stage (leather shavings). PH is composed of a mixture of oligopeptides with the typical amino acid composition of collagen and an average molecular weight between 6.000 and 8.000.

The lignin used was Indulin® AT, provided by MeadWestvaco Corporation (Charleston Heights, SC, USA), a commercially available purified powder form of pine Kraft lignin, free of hemicelluloses, characterized by a relatively high degree of purity (97%). This type of softwood Kraft lignin has a specific density of 1.24 g cm^{-3} and an average particle size of 8 μm.

Beech wood flour, characterised by particle size < 180 μm and apparent density equal to 160-210 kg m^{-3}, was supplied by La.So.Le Srl (Udine, Italy) and used as raw material.

Biodegradable polymeric materials were prepared starting from protein-based aqueous solutions, obtained by dissolving the proper amount of PEG or ESO in a 25% (w/v) solution of HP. The desired amount of lignin, natural fillers and water, up to a final 18 wt % concentration, were added under stirring at 50 °C.

Water suspensions were used to made the mulching coatings in field and the specimens for the laboratory tests. The suspensions, kept under stirring for few minutes at 50 °C, were sprayed onto the soil surface after the addition of the proper crosslinking agent. Casted coatings were prepared by slow evaporation of the water suspension of the ingredients in glass trays at room temperature. The dry formulations were hot pressed at 50 °C to make 2 mm thick sheets, from which the specimens for mechanical and radiometric test were obtained.

2.2. The biodegradable coating materials characterization

Tensile tests were performed by an Instron Model 3366 Universal Testing Machine at the University of Brescia. Elastic modulus (E), strength (σ_b) and elongation at break (ε_b) were determined on 2 mm thick and 10 mm wide bars, with a gauge length of 80 mm; crosshead speed was 2 mm min^{-1}. Tensile tests were performed at room temperature after specimen conditioning at 55 °C for 2 h under vacuum. Weight loss was evaluated measuring the weight of dry specimen after immersion in water for fixed intervals as:

$$\text{Weight loss} = \frac{W_1 - W_3}{W_1} 100 \qquad (1)$$

were W_1 and W_3 denote the weight of the starting dry specimen and of the dry specimen after water immersion respectively.

Radiometric tests on the mulches were carried out at the University of Bari by means of spectrophotometers in order to evaluate the spectral transmissivity $\tau(\lambda)$, i.e. the fraction of the incident energy radiant flux that is transmitted at a specific wavelength λ. The total transmissivity, in the wavelength range between 200 nm and 2500 nm, was measured by means of a double beam UV-VIS-NIR spectrophotometer (Lambda 950, Perkin-Elmer Instruments, Norwalk, CT, USA) equipped with an integrating sphere (diameter 60 mm). Radiometric coefficients of the materials were calculated as average values of the spectral transmissivity over different wavelength bands: the solar wavelength range (300-2500 nm), the PAR range (400-700 nm) and the long wave infrared radiation range (LWIR; >3000 nm). The transmissivity coefficient in the solar range was calculated as the weighted average value of the spectral transmissivity using the spectral distribution of the solar radiation at the ground level as weighting function (Vox and Schettini, 2007). Transmissivity in the LWIR range between 2500 nm and 25000 nm was measured by a FT-IR spectrophotometer (1760X, Perkin-Elmer Instruments, Norwalk, CT, USA). The LWIR transmissivity coefficient was calculated as the average value over the wavelength interval between 7500 nm and 12500 nm (Vox and Schettini, 2007).

2.3 The field test

2.3.1 The mulching coatings in the field

The field test was carried out from November 2013 to January 2014 inside a North-South oriented tunnel greenhouse at the experimental farm of the University of Bari, Italy, latitude 41° 05' N. The steel-constructed tunnel (length of 30.00 m, width of 8.00 m, ridge height of 3.20 m) was covered with an ethylene-vinyl acetate (EVA) film (PATILUX, P.A.T.I. Company, San Zenone degli Ezzelini, Treviso, Italy). The film had thickness of 200 μm and the following radiometric coefficients: solar total transmissivity equal to 90.9%, solar direct transmissivity equal to 56.7%, LWIR transmissivity equal to 22.5%.

Greenhouse air temperature was controlled by means of two electric fans positioned in the South end wall and of two sliding shutters in the North end wall; the system was automatically driven in order to maintain internal air temperature under the set point value of 27 °C.

Clay-loam textured soil plots, East-West oriented, each of 0.4 m^2 (0.8 m x 0.5 m), were prepared for transplanting lettuce seedlings (*Lactuca sativa* L., Mortarella selection Romanella variety Duende) as test-plants chosen for their growth readiness.

On 27 November 2013 two different aqueous dispersions, coded PH-ESO and PH-PEG-ESO, were sprayed on the soil to form coatings resistant for the time required by the crop cycle (Figure 1a). The quantity of the sprayed solution determined coating's lifespan. The side slope of the raised bed of each plot was limited in order to avoid possible sliding of the spray mulching coating at the liquid state before the dry process. The solutions, in quantity of 2.25 kg m^{-2}, were distributed on the top of the plots by an airbrush with a nozzle having an internal diameter of 3 mm, using a spray machine at 0.9 MPa of pressure. Control plots coded as LDPE were mulched with a black LDPE film, characterised by a thickness of 40 μm.

The experiment was carried out using a randomised block design with three treatments (PH-ESO, PH-PEG-ESO and LDPE) and ten replications for each treatment.

During the field test, greenhouse air temperature and relative humidity were measured by means of a Hygroclip-S3 sensor (Rotronic, Zurich, Switzerland); solar radiation was measured in the wavelength range 0.3-3.0 μm by a pyranometer model 8104 (Schenk, Wien, Austria). The data, measured with a frequency of 60 s, were averaged every 15 minutes and stored in a data logger (CR10X, Campbell, Logan, USA).

2.3.2 The leaf growth, functionality and factors of nutritional quality

On November 29th 2013, lettuce seedlings were transplanted. The cropping method was the same for all the plots. In particular, each plot received on the whole 8.0 g m^{-2} of N, 2.8 g m^{-2} of P_2O_5 and 16.8 g m^{-2} of K_2O and an automated drip irrigation system ensured water availability to the plants providing 1.5 L $plant^{-1}$ every two days.

In order to test the effects of the mulching treatments on leaf emission and expansion, the number of leaves per plant, the leaf length and width for the 2 biggest leaves per plant were measured 7 times from the transplanting to the harvesting, that occurred on January 27th 2014.

At transplanting, the features of plants assigned to each treatment were recorded. The average leaf number ranged from 3.83 ± 0.17 in PH-PEG-ESO and LDPE to 4.00 ± 0.29 in PH-ESO, leaf length from 10.33 ± 1.17 cm in PH-ESO to 10.67 ± 0.33 cm in LDPE, and leaf width from 3.40 ± 0.30 cm in PH-PEG-ESO to 3.80 ± 0.40 in LDPE.

Possible effects of mulches on soil and leaf temperature were also assessed, since they are critical in controlling plant growth through several biological processes, such as root growth and water uptake, rates of water vapour loss, stomatal aperture, rate of net photosynthesis, etc. (He et al., 2009). From December 10th 2013 to harvest, leaf temperature was measured using an infrared thermometer (3 readings per plant on 1st-to-3rd fully expanded leaves), while soil temperature was measured at ~10 cm deep using a thermocouple thermometer (3 readings per plot). Readings were taken 6 times (at about 10-day intervals), around 10 hr. a.m., under different weather conditions.

Possible indirect effect of mulches on leaf chlorophyll and nitrogen relative content was estimated, on the 2nd and 3rd leaves from the plant outside (Tsiakaras et al., 2014), using a SPAD 502-meter (Konica

Minolta, Inc.): on the largest blade portion, two readings per leaf where taken symmetrically to the midrib.

At harvest, all plant heads were cut and transported to the Laboratorio di Arboricoltura of the University of Foggia. The first two leaves of each plant were collected for chemical analyses and weighted, the remaining portion was also weighed and used to assess the plant dry weight after oven-drying at 65°C until constant weight.

To evaluate the possible influence of mulch types on total polyphenol content (TPP) and total antioxidant activity (AA), as important factors of nutritional value, the first two leaves (used for field measuring of relative chlorophyll content) were trimmed using a tissue ruptor (Quiagen) at 33000 rpm. Leaf extracts were obtained putting in contact 5 g of grinded fresh leaves with 20 ml of methanol solution (80 MeOH: 20 H_2O) for few minutes, and centrifuging for 8' at 4000 rpm (Yurttas et al., 2000). The antioxidant activity was determined using ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) method (Re et al.,1999*)*. $ABTS^{\cdot +}$ solution was diluted with ethanol (1:88) which absorbance, at 30° C and at 734 nm, is about 0.70 (± 0.02) against ethanol as blank. 200 µL of the diluted sample were added to 2 mL of chromogen into a cuvette of p.l. 10 mm and mixed; after 15 min the absorbance at 734 nm was red. Percentage inhibition was calculated as follows:

$$\text{Inib.}(\%) = \left(1 - \frac{\text{Abs}_s}{\text{Abs}_b}\right) \cdot 100 \qquad (2)$$

where: s = sample; b = blank.

The equivalent concentration (µ/L Trolox uM) was obtained replacing, in the Trolox standard curve, the percentage inhibition.

The total polyphenols content was determined using the colorimetric Folin-Ciocalteu procedure. Into a 25 mL flask, 0.1 mL of extract were put followed by 1 mL of Folin-Ciocalteu reagent. After 3-4 min, 10 mL of $NaCO_3$ (7%) were added and the flask filled with water till the mark. The flask was tipped faster several times and then left in dark for 120’ (Slinkard et al., 1977). The absorbance was red at 760 nm against a blank. The absorbance was entered into the standard curve to express values as µ mL^{-1} gallic acid (Oviasogie et al., 2009).

Statistical analyses were carried out with CoStat software (CoHort Software, Monterey, Calif.). Analysis of variance (ANOVA) at 95 percent probability level was applied in order to examine the effect of the coatings on the agronomic responses. When F test was significant at $p = 0.05$, means were compared by Duncan multiple range test at $p \leq 0.05$. Data are presented as average of the replicates ± standard error.

2.3.3 Soil sampling, sample preparation and soil analyses

At the beginning of the experiment, the soil was sampled collecting five cores using a W scheme, air-dried and 2-mm sieved before laboratory analyses.

In order to record any variation of selected properties, sprayed and mulched soils were also sampled at the end of the trial. Therefore, soil sample sets were characterized by conventional analytical methods for their moisture content, pH (1:2.5 w/v), electrical conductivity (EC, 1:2 w/v), total nitrogen (TN) by the Kjeldahl method, organic carbon (OC) by the Walkley and Black method, available P and total carbonates (TC) (Page et al., 1982).

3 Results and discussion

3.1 Laboratory spray materials characterization

Table 1 summarizes the mechanical and radiometric properties of the laboratory samples.

Results from the mechanical tests showed that both the obtained protein-based materials were adequately stiff and strong with low elongation at break (Table 1).

Concerning the behavior of the derivatives in the presence of water, the weight loss of the specimens immersed in distilled water at room temperature was monitored over a period of about thirty days. Unlike the reagents were completely soluble, both the biocomposites possessed limited solubility and degradation rate. PH-PEG-ESO as well as PH-ESO derivatives swelled but for more than 25 days, no significant differences were noticed on the integrity of the corresponding immersed specimens which, in addition, showed a slow release of material gradually increasing with time. This may suggest extensive crosslinking of the two systems and may promote slow release of proteinaceous materials.

Fulfilling the radiometric requirement of a black mulch of being opaque to the PAR radiation, the PH-ESO and PH-PEG-ESO coatings showed a PAR total transmissivity coefficient of 0.00%, as the LDPE black mulching film (Table 1), so that weed growth was inhibited in the period from transplanting to harvesting. The LDPE mulching films must satisfy the EN 13655 standard (EN 13655, 2002), i.e. independently of their thickness, black plastic mulches must have a PAR total transmissivity coefficient < 0.01%. Even if the spray coatings are materials at an experimental stage and their physical properties are not defined by international standards, in the present paper Authors reported very promising results about their PAR transmissivity coefficient, especially in comparison to the literature. In fact, Malinconico et al. (2008) and Immirzi et al. (2009) tested different biodegradable spray coatings that showed a good capacity to reduce weeds growth, even if with different PAR transmittivity coefficient. The spray coatings prepared mixing Locust bean gum and Guar gum, agarose, glycerol and carbon black were substantially opaque to the PAR radiation. In contrast, the coating films made with sodium alginate, seaweeds and bran of wheat showed a PAR total transmissivity coefficient of 6.36%. The spray coatings realized with hydrolyzed proteins by Sartore et al. (2013), with a thickness ranging from 0.6 to 0.8 mm, showed a good capacity to reduce weed growth even with a PAR total transmissivity coefficient ranging from 0.2 to 4.1 %.

3.2 The performance of the spray coatings in the field

The biodegradable spray coatings kept their mulching effect for 2 months from application to harvesting, though some cracks appeared on the surface (Figure 1b). The irregularities appeared on the surface of the coatings around the plants where water was delivered by the drip irrigation system.

Higher values of the humidity of the soil induce faster degradation of the biodegradable coating (Schettini et al., 2007). The LDPE film recorded no variation during the cultivation period.

During the experimental test, the mulches were subjected to a cumulative solar radiation equal to 272 MJ m^{-2}. During the cold period, inside the tunnel the air temperature ranged from a maximum value equal to 29.56 °C (recorded on 16/01/2014) to a minimum value equal to 2.82 °C (recorded on 8/12/2013). Greenhouse relative humidity ranged from 47 % (recorded on 8/12/2013) to 100%, typical value in greenhouse during the night.

The sodium alginate-based spray mulch tested by Immirzi *et al.* (2009) was characterised by a lifetime inside a greenhouse of 6 months, subjecting to a cumulative solar radiation of 2705 MJm^{-2}. This spray mulching coating was composed of sodium salt of alginic acid, polyglycerol, hydroxyethylcellulose, seaweeds and bran of wheat. This coating was characterised by inhomogeneous surface and irregular thickness that varied from 3 to 5 mm. Few cracks on the surface appeared within the first month (Immirzi *et al*, 2009). Schettini et al. (2007) described a transparent spray glucomannan based mulching coating. This coating was applied by spraying a water solution of glucomannans enriched by polyamino-polymers as mulching filler. It was characterised by a thickness of 50 μm. Some little cracks appeared on the surface within the first month but it lasted for 5 months inside a greenhouse (Schettini et al., 2007). Mahmoudpour and Stepleton (1997) tested a coloured synthetic latex mulch base product (‘BN 1849’; BASF. Charlotte, NC, USA), diluted 1:2 (product : water). This mulching coating was used in open air and two weeks after the application it was damaged by a hail storm.

3.3 Influence of the mulches on soil temperature, leaf growth and functionality, factors of nutritional quality

Soil temperature measured around 10 a.m. averaged from 11.32 ± 0.2 °C (recorded on 20/12/2013) to 16.25 ± 0.1 °C (recorded on 27/01/2014). Soil mulched with PH-ESO was significantly cooler (from -1.08 to -1.83 °C) than that mulched with LDPE (Figure 2a), which film was particularly efficient in warming the substrate and retaining the thermal energy. Concerning lettuce, the best dry matter accumulation can be found with 24/24 °C root media/daytime air temperature; in the present trial, the soil temperature was found sub-optimal at all measurements, but PH-PEG-ESO mulch warmed the soil more than PH-ESO coating.

The mulch type, that can influence the surface radiation balance and the plant microclimate (Liakatas et al., 1986), did not affect the leaf temperature during the daylight hours. Leaf temperature averaged from 4.70 ± 0.03 °C (21/01/2014) to 15.15 ± 0.2 °C (10/12/2013) (Figure 2b). Differences among treatments were very small (from 0.11 °C to 0.56 °C) and, depending on the day of measurement, each treatment showed sometimes the highest leaf temperature and sometimes the lowest one.

The type of mulching did not influence the leaf emission and expansion. In fact, seedlings started with similar number of leaves and leaf dimension did not show significant differences during the growing period. At harvest, the leaf number ranged from 27.33 in PH-PEG-ESO to 28.33 in PH-ESO (Figure 3 a), the leaf length from 23.5 cm in PH-PEG-ESO to 24.33 cm in PH-ESO (Figure 3 b), and the leaf width from 15.5 cm in LDPE to 16.33 cm in PH-ESO (Figure 3 c),

Relative chlorophyll content averaged from 32.55 ± 2.3 (12/12/2013) to 40.82 ± 0.4 (30/12/2013). Differences among treatments changed pattern at each measurement (Figure 4); nevertheless, at the last two dates PH-PEG-ESO leaves showed significantly higher readings than other treatments, that is, about 42 SPAD units against 37-39 SPAD units. Tsiakaras et al. (2014) found 42 SPAD units in normal mature leaf tissue of lettuce (romaine type) having similar growing cycle and tested with similar procedure used in our trial. SPAD readings are generally correlated to chlorophyll content and leaf nitrogen status (León et al., 2001). Nitrogen uptake can be stimulated by warmer daylight soil temperature (Verdial et al., 2001); however, in our case, LDPE showed higher soil temperature than PH-PEG-ESO along the growing season, except for the last measurement, but not higher SPAD units, except for the 3rd measurement. Hence, under the trial conditions, other factors should have influenced SPAD readings. This colorimetric technique is suitable to test leaf nutritional status also for other elements, such as phosphorus and calcium (Pitchay et al., 2014), whose level may decline as soil temperature increases (Verdial et al., 2001).

The total polyphenol content (TPP) did not differ between leaves grown in PH-ESO and PH-PEG-ESO plots, but, on average, it was 17% lower than that found in leaves of plants grown with LDPE mulch. The antioxidant activity (AA) showed the same trend and was 11% lower for the two spray treatments than for the LDPE one. Mulch influence on TPP and AA was pointed out in other studies, but most of them were focused on reflective mulches. For not reflective mulches, Morra et al. (2016) found that polyphenols and other antioxidant compounds increased when MaterBi® biodegradable extruded film was used instead of LDPE, but they could not clear which type of environmental factor, or abiotic stress, was correlated to that result. It is well-known that cultural system and environment affect production and accumulation of plant antioxidant compounds (Wang et al., 2002), but, under field conditions, it is hard to separate specific elicitor effects.

3.4 Soil analyses

The soil at the beginning of the trial (T0) was characterized by sub alkaline pH, low EC, very low TC, good endowment of OC, TN and available P_{av} (Table 2).

At the end of the trial, the soil mulched by LDPE showed a significant but slight increase of the pH, while the EC value of LDPE soil was significantly lower with respect to the other treatments. That could be due to the mineralization of the sprayed coatings that released nutrients in the soil during their decomposition. In addition, the soil covered with PH-PEG-ESO showed, at the end of the experiment, a significant but slight increase of the OC, possibly due to the more complex composition of that material with respect to the PH-ESO (Table 1), and that could have played a role in the release of the organic moieties from the PH-PEG-ESO during the trial.

The TN of the PH-PEG-ESO soil resembled the same behavior of OC since that biobased mulching coating showed more N compounds in its composition (Table 1).

The LDPE and PH-PEG-ESO soils showed the highest value of P_{av}. The former soil was characterized by a slight higher pH value that influenced the availability of that element, as reported by Sposito (2008). Instead, the degradation of the biobased mulching coating could have released byproducts useful for the direct complexation of P or for the complexation of calcium inducing, indirectly, a major availability of phosphorous.

4 Conclusions

The laboratory characterization and the field test showed that novel polymeric protein-based biocomposites can be used as water dispersions to generate, in situ, biobased mulching coatings by the spray technique. The biodegradable coatings showed agronomic performances comparable with the ones of a commercial low density polyethylene mulching film, maintaining the mulching effect for the requested cultivation period and ensuring a similar rate of plant growth and dry matter accumulation. The study of the mulch influence on phytochemical content in plant edible organs should be deepened in order to identify eliciting environmental factors and, possibly, improve the new mulching materials looking also to their effect on plant nutritional value.

The sprayed films, in short period, did not modify the pH of the soil, while increased the soil EC value due to their biodegradable nature. Apparently, for its chemical composition and performances, the PH-PEG-ESO has to be preferred because it induced higher availability of N and P, and a temporary slight OC enrichment.

The use of biodegradable hydrolyzed proteins based coatings could increase the sustainability of the agricultural production, by using renewable non-oil raw materials and by enhancing the protection of the rural areas against water and soil pollution.

At the same time raw materials can be obtained both from natural renewable agricultural resources and from potentially polluting residues of the leather industry.

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Figure Captions

Figure 1. Spray biodegradable coating at the experimental field of the University of Bari: (a) at the beginning of the test; 27/11/2013; (b) at harvest, 27/1/2014.

Figure 2: Soil temperature (a) and leaf temperature (b), measured at about 10 a.m., according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error).

Figure 3. Test-plant growth in terms of leaf number (a), leaf length (b), leaf width (c), according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error).

Figure 4. Relative chlorophyll content (as SPAD Units) during test-plant growth, according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error).

Table 1. Composition, mechanical and radiometric properties of PH-PEG-ESO, PH-ESO derivatives and of LDPE mulching film. PH: protein hydrolysates; ESO: epoxidized soybean oil; PEG: poly(ethylene glycol) diglycidyl ether; LDPE: low density polyethylene; PMD: pyromellitic dianhydride; EDA: ethylene diamine; Lign: lignin; WF: wood flour; CB: carbon black; E: Young's modulus; σ_b: tensile strength; ε_b: elongation at break; PAR: photosynthetically active radiation; LWIR: long wave infrared radiation.

		PH-ESO	PH-PEG-ESO	LDPE
Composition	PH [%]	41.2	41.4	
	ESO [%]	10.3	5.2	
	PEG [%]	-	5.2	
	PMD [%]	0.6	-	
	EDA [%]	-	0.3	
	Lign [%]	24.7	24.8	
	WF [%]	21	21	
	CB [%]	2	2.1	
Mechanical properties	E [MPa]	1030±170	2550±190	270±135
	σ_b [MPa]	5.8±0.7	7.6±1.1	20±14
	ε_b [%]	0.7±0.1	0.37±0.1	580±170
Radiometric properties	Solar total transmissivity (%)	0.10	0.09	0.02
	PAR total transmissivity (%)	0.00	0.00	0.00
	LWIR transmissivity (%)	0.01	0.01	11.17

Table 2. Dry matter percentage, total polyphenol content, antioxidant activity in leaves, and soil properties, according to mulching treatments. fw: fresh weight; TPP: index of polyphenol content; AA: index of antioxidant activity; T0: soil at the beginning of the trial; EC: electrical conductivity; TC: total carbonates; OC: organic carbon, TN: total nitrogen; C/N: carbon/nitrogen ratio; P_{av}: available phosphorous. Mean value ± standard error; within row , different letters indicate different mean values at $p \leq 0.05$.

	PH-ESO	PH-PEG-ESO	LDPE	T0
Dry matter (% fw)	5.01 ± 0.58 a	5.00 ± 0.19 a	4.94 ± 0.24 a	
TPP (mg gallic acid/100 g dry matter)	81.01 ± 6.511 b	83.95 ± 7.01 b	98.88 ± 7.45 a	
AA (mg Trolox/100 g dry matter)	1714.88 ± 40.79 b	1683.33 ± 41.21 b	1914.65 ± 87.54 a	
pH	7.85 b	7.85 b	8.15a	7.80 b
EC [dS m-1]	0.83 a	0.64 a	0.20 b	0.74 a
TC [g kg-1]	15 a	18 a	15 a	14 a
OC [g kg-1]	18.7 b	19.7 a	18.6 b	19.0 b
TN [g kg-1]	1.97 ab	2.05a	1.81 b	1.86 ab
C/N	9.5	9.6	10.3	10.2
P_{av} [mg kg-1]	35.4 b	41.3 a	48.2 a	36.7 b

Figure 1a

Figure 1b

Figure 2a

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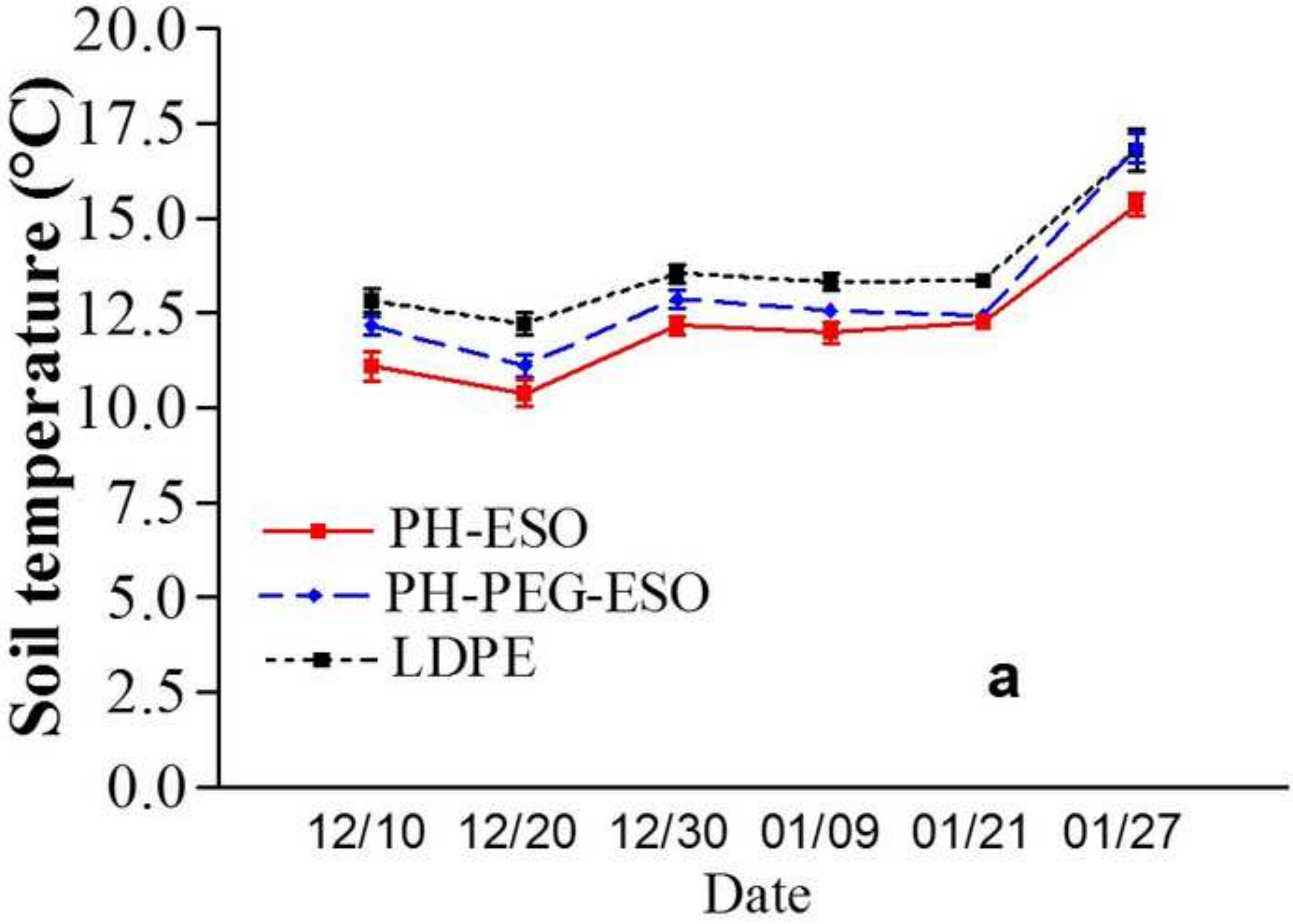


Figure 2b

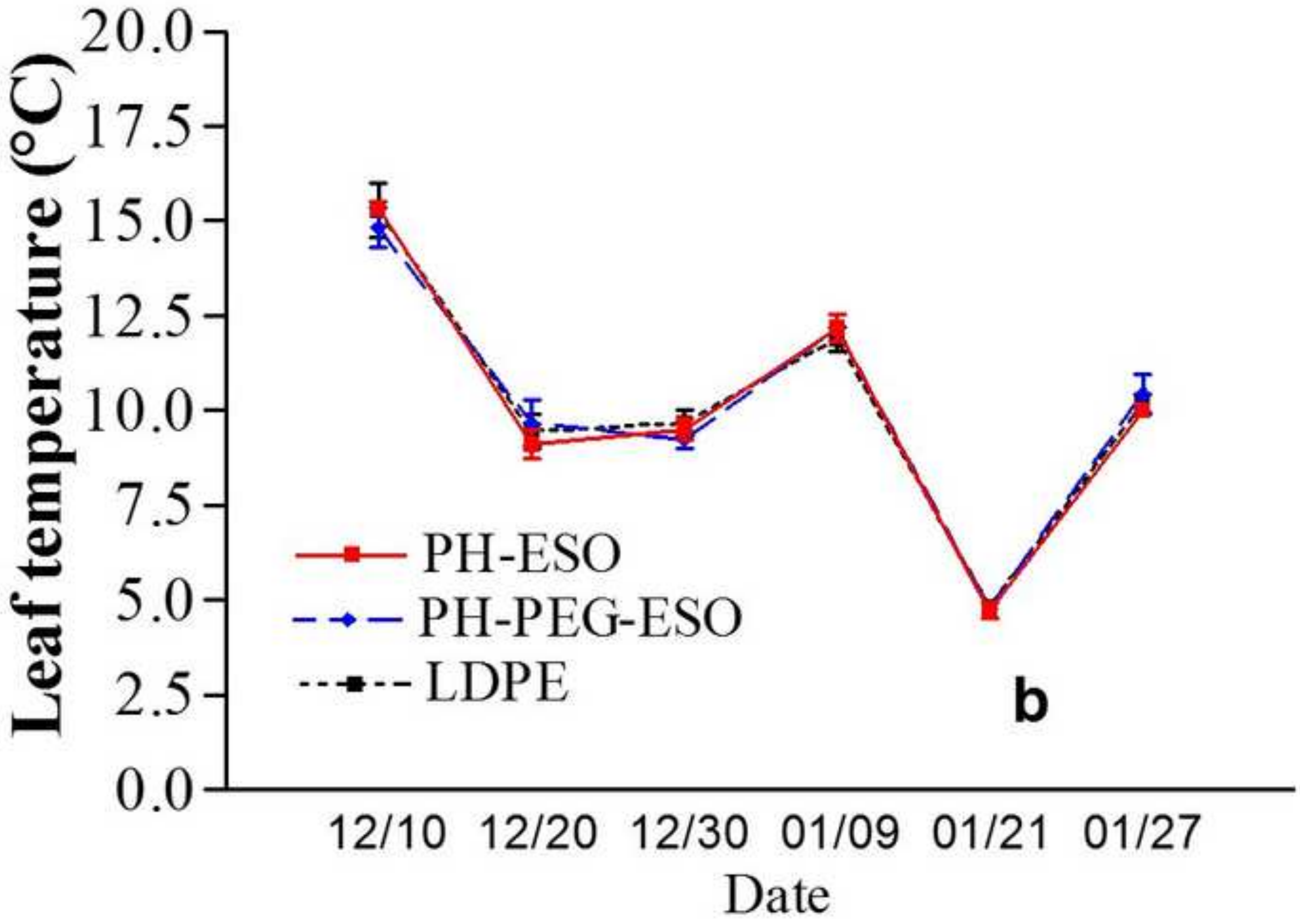
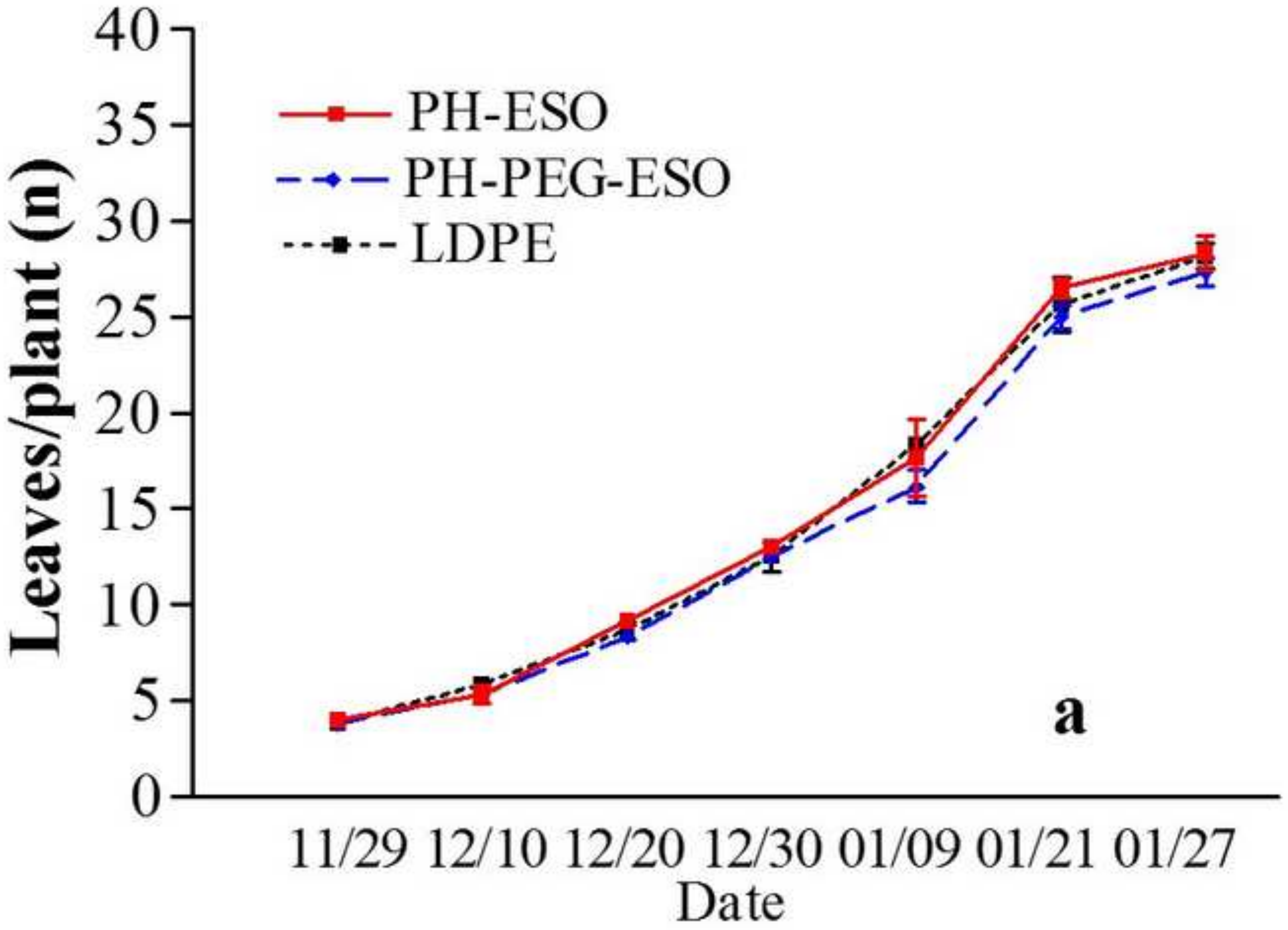

20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Leaf temperature (°C)
PH-ESO
PH-PEG-ESO
LDPE
b
12/10
12/20
12/30
01/09
01/21
01/27
Date

Figure 3a



Leaves/plant (n)
40
35
30
25
20
15
10
5
0
PH-ESO
PH-PEG-ESO
LDPE
a
11/29
12/10
12/20
12/30
01/09
01/21
01/27
Date

Figure 3b

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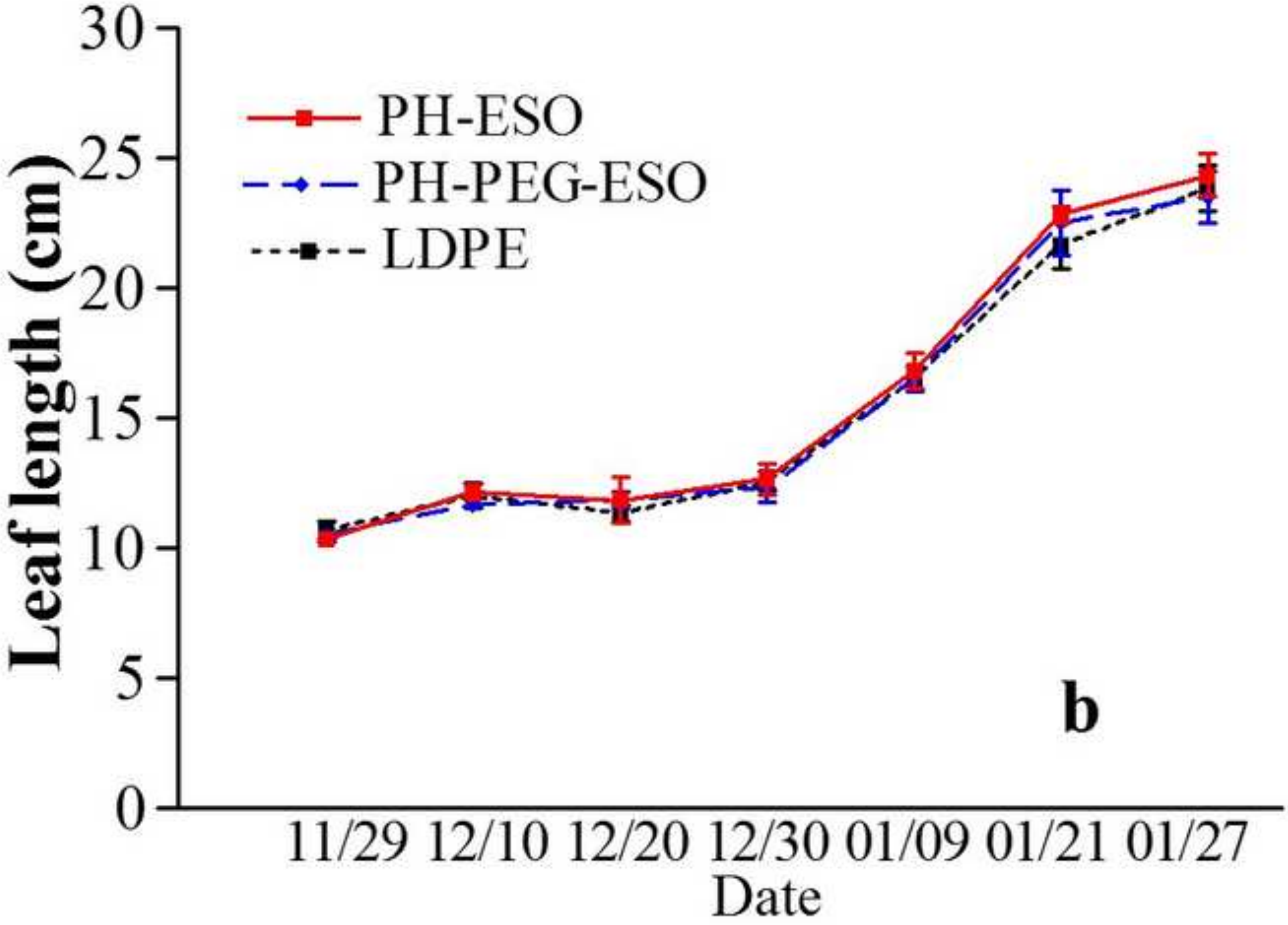

Figure 3c

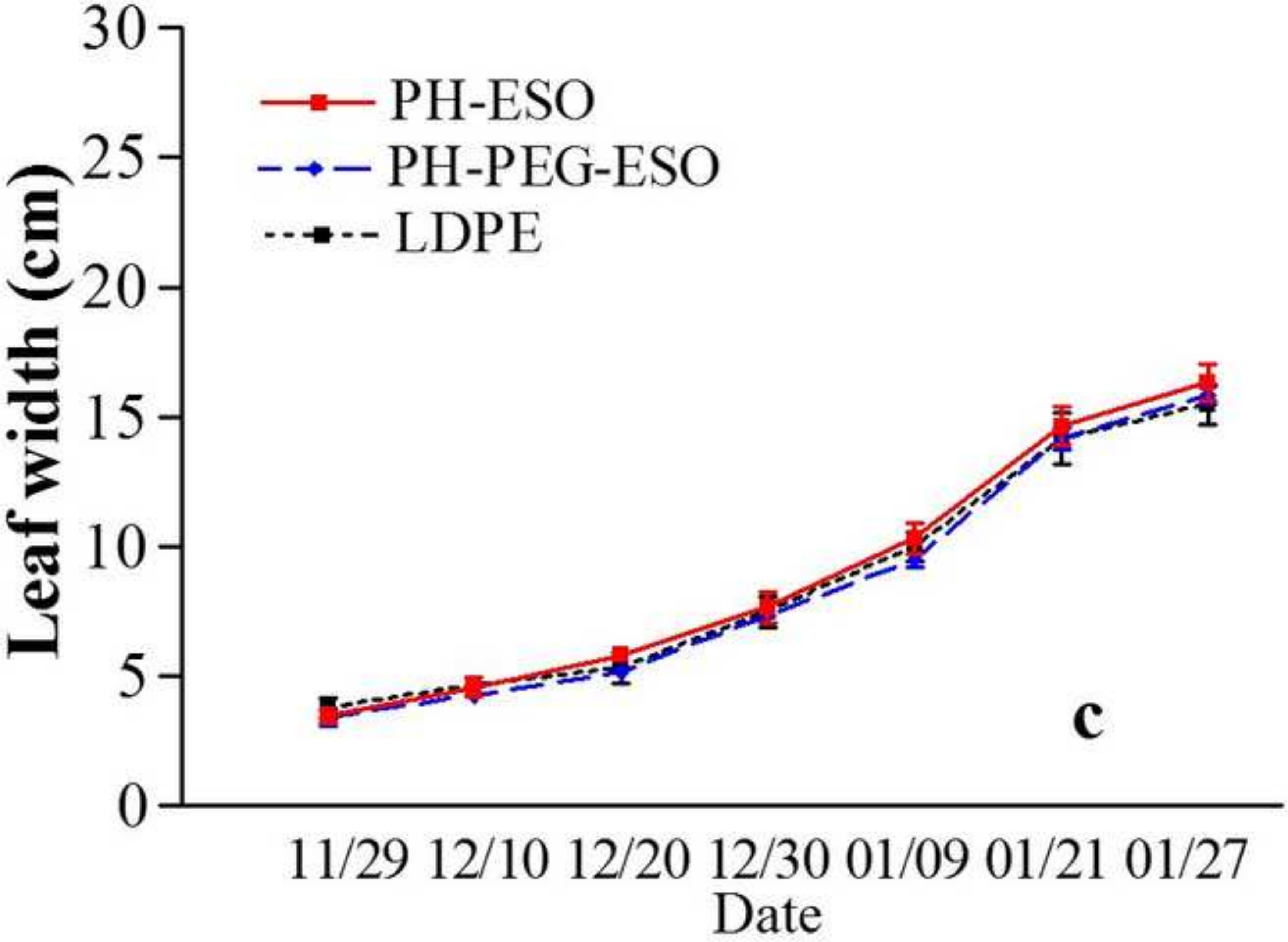
30
25
20
15
10
5
0
Leaf width (cm)
PH-ESO
PH-PEG-ESO
LDPE
c
11/29
12/10
12/20
12/30
01/09
01/21
01/27
Date

Figure 4

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