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Synergistic inhibition of the seed germination by crude glycerin and defatted oilseed meals

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ABSTRACT

The main co-products of biodiesel and biolubricant chains are defatted oilseed meals and crude glycerin, generally considered as by-products and mainly used in animal feeding, bioenergy production and other minor uses. However, these co-products could have other interesting features, and high value added outlets could be developed in order to significantly improve the overall economic and environmental impact of the entire biodiesel chain. When defatted seed meals derive from some specific oilseed crops belonging to *Brassicaceae* they are characterized by high levels of glucosidic molecules potentially able to release biologically active compounds by means of an enzymatic hydrolysis reaction. The second biodiesel chain by-product, crude glycerin, contains glycerol and some impurities (including water) up to 20%. This manuscript reports *in vitro* and *in vivo* bioassay results on the application of crude glycerin and defatted seed meals of some *Brassicaceae* species (*Brassica carinata*, *Brassica nigra*, *Barbarea verna*, *Crambe abyssinica*) in seed germination inhibition. These features open new perspectives for obtaining bio-based products for weed control, starting from both main co-products of the biodiesel chain. Indeed, the results confirmed the inhibitory effect of crude glycerin and seed meals used alone and, moreover, showed a synergistic activity when combined. As expected, the inhibitory activity was correlated to glycerol concentrations and to the quali–quantitative release of isothiocyanates from defatted seed meals.

1. Introduction

Due to the rise in global biodiesel production, and more generally of oilseed crops for non-food applications, the amount of defatted oilseed meals (DSMs) and crude glycerin (CG), the main co-products of seed defatting and oil esterification, respectively, have also steadily increased (Katryniok et al., 2009). These products are generally considered as having reduced added value and are mainly used in animal feeding or in the bioenergy chain. Therefore, the identification of high added value outlets should be explored in detail to improve the overall economic and environmental benefits of both co-products for the entire biodiesel chain.

In the meantime, the substitution of conventional chemicals from fossil sources by bio-based materials can provide several benefits for the health of workers and consumers and in general for the environment, depending on the application field and the substitution level. One of the fields of greatest interest for green chemistry is without doubt crop management and protection, due to the following effects: (i) on food quality and safety, (ii) on health conditions for farmers and (iii) on the environmental integrity, in particular considering that pesticides are generally sprayed and dispersed into the environment.

The first by-products of the biodiesel chain are DSMs derived from seed defatting procedures. When derived from *Brassicaceae* species, other than rapeseed (*Brassica napus* L. ssp. oleifera), DSMs are characterized by a high level of glucosinolates (GLs). In their native form, GLs are stable and marginally reactive, while in the presence of water and the endogenous enzyme myrosinase (MYR) (ß thioglucoside glucohydrolase EC 3.2.1.147), they are quickly hydrolyzed with the production of a series of bioactive breakdown products such as isothiocyanates (ITCs) and, to a lesser extent, nitriles, epithionitriles and thiocyanates, depending on the reaction conditions (Fahey et al., 2001; Bones and Rossiter, 2006). Due to these features, *B. carinata* and other *Brassicaceae* species have been widely studied for applications in the so-called biofumigation

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Table 1
Crude glycerin (CG) characterization (mean ± standard deviation).

	Unit	CG
Density at 20 °C	$g\,cm^{-3}$	1.23 ± 0.03
Glycerol	%	80.2 ± 0.6
Water	% w/w	14.7
Ashes	% w/w	4.21 ± 0.19
Cadmium (Cd)	$mg\,kg^{-1}$	<0.01
Chrome (Cr)	$mg\,kg^{-1}$	0.02 ± 0.00
Lead (Pb)	$mg\,kg^{-1}$	0.05 ± 0.00
Copper (Cu)	$mg\,kg^{-1}$	0.58 ± 0.03

technique. After formulation of *B. carinata* DSMs following a patented procedure (Lazzeri et al., 2007, 2010), the Allyl ITC (AITC) released from 2-propenyl GL (common name sinigrin, SIN) was shown to be active for the containment of soil-borne fungi (Lazzeri et al., 2003), nematodes (Lazzeri et al., 2009) and wireworms (Furlan et al., 2010). Some previous experiences showed an inhibitory effect on seed germination and plant growth caused by: (i) DSMs and plant extracts obtained from plants belonging to the Brassicaceae family (Oleszec, 1986; Rice et al., 2007), (ii) solutions of purified GLs and MYR (Angelini et al., 1998), and (iii) pure ITCs (Hara et al., 2010).

The second by-product derived from oil esterification or saponification processes, including in the biodiesel chain, is CG that contains glycerol and up to 20% of different impurities. Glycerol is an organic molecule characterized by a low toxicity when ingested, inhaled or upon contact with the skin; it is also readily biodegradable under aerobic conditions and highly stable in storage (EFSA, 2010). It is completely soluble in water and alcohol, while it is slightly soluble in many common solvents such as dioxane and insoluble in ether, chloroform and hydrocarbons (UNEP, 2002). Glycerol is one of the most versatile and valuable chemical substances known, with several thousands of uses and applications (Pagliaro and Rossi, 2010). It can be used, in fact, in extremely different fields, from feed to chemicals, from biodegradable plastics to lubricants (D'Avino et al., 2015), or after transformation through different chemical synthesis approaches (Wolfson et al., 2009). Some applications require pure glycerol, while for some others the purity of CG is sufficient, and no further refining steps are needed. In the agricultural field, glycerol is used as an adjuvant in formulations, providing anti-evaporation characteristics and greater adhesion to the surface of plant tissues, and more generally for the treatment of crops, including spray solutions (Dissinger et al., 2009). Glycerol esters are applied in a patented formulation of conventional herbicides that play a humectant role and are able to improve the activity of a wide range of chemical active compounds (Krahmer et al., 2006) and are also present as an additive in a lemongrass-based natural product for weed management (Dayan et al., 2009).

The aims of this work were to investigate the potential effect on seed germination inhibition (GI) of liquid formulations containing: (i) CG derived from oil esterification for biodiesel or other fatty-acid productions, as in lipochemistry, (ii) DSMs derived from fatty-acid, biofuel and other bio-product chains, and (iii) the combination of the two products.

2. Materials and methods

2.1. Materials

CG was purchased from Cerealdocks S.p.A., an industrial biodiesel plant (Vicenza, Italy). *Brassica carinata* A. Brown and *Brassica nigra* L. DSMs, produced after seed defatting by an endless screw press in which temperature was kept lower than 75 °C, were purchased from Agrium Italia S.p.A. (Livorno, Italy). DSMs of *Barbarea verna* (Miller) Asch., and *Crambe abyssinica* Hochst came from the CRA-CIN *Brassicaceae* collection and were defatted with hexane on lab level at room temperature (21 ± 1 °C) (Lazzeri et al., 2013); sunflower (*Helianthus annuus* L.) DSM was purchased from Italcol S.p.A. (Castelfiorentino, Firenze, Italy) after an industrial hexane extraction process. The standard AITC was obtained from Aldrich Chemical (Milwaukee, Wi, USA).

Lettuce (*Lactuca sativa* L. cv. Cosmic) was purchased from Tozer Seeds; purslane (*Portulaca oleracea* L.) from B and T seeds (France); garden cress (*Lepidium sativum* L.), cucumber (*Cucuminus sativus* L.), and sorghum (*Sorghum vulgare*, Pers.) from Florsilva Ansaloni (S. Lazzaro di Savena, Bologna, Italy); tomato (*Solanum lycopersicum* L. cv. UC82) from ISI Sementi (Parma, Italy).

2.2. Crude glycerin characterization

CG was paper filtered to remove impurities and analyzed for some minor components by the following procedures:

- Density was measured by gravimetric analysis.
- Water and glycerol content were determined at the Experimental Station for the Industries of Oils and Fats, Milan Italy (SSOG) by using Karl Fischer colorimetric titration and HPLC determination, respectively. An HP1100 (Hewlett Packard, Waldbronn, Germany) system equipped with an HP1047A refractive index detector and an Aminex HPX-87H column (7.8 mm × 300 mm, BioRad Richmond, USA) was used. The mobile phase used was 0.01 N sulfuric acid at a flow rate of $0.8\,mL\,min^{-1}$. The injection volume was 20 μL. An external six-point calibration curve was constructed analyzing standard dilutions of 99.5% glycerin from 10 to $25\,mg\,mL^{-1}$.
- Determination of ash content was performed according to the British and European Standard (BS EN 14775, 2009).
- The presence of heavy metals, such as cadmium (Cd), chrome (Cr), lead (Pb) and copper (Cu), was determined at the Research Institute for Agroindustry (Modena, Italy) by Inductively Coupled Plasma Spectrometry; samples were prepared according to AOAC International (1990) and the analysis was performed according to AOAC International (1988).

2.3. Defatted oilseed meal characterization

The DSMs were analyzed for moisture, nitrogen content, residual oil and GL content by the methods reported in De Nicola et al. (2013). Total phenolic content (TPC) was estimated by a 96-well microplate-adapted Folin–Ciocalteu assay (Weisz et al., 2010). One gram of DSM was diluted in 20 mL of methanol 80% and acetic acid 0.3%. For each well, 120 μL of Folin–Ciocalteu reagent (Sigma) diluted 6-fold were mixed with 2 μL of sample and 80 μL of 7.5% Na_2CO_3. After one hour of incubation, absorbance at 765 nm was measured on Infinite 200-PRO NanoQuant system (Tecan, Männedorf, Switzerland). An external standard curve was performed with serial dilutions of gallic acid and results are therefore presented as gallic acid equivalent.

All characterization data are reported as mean ± standard deviation of four determinations.

All DSMs were formulated by a patented procedure (Lazzeri et al., 2010) aimed at optimizing the enzymatic system that catalyzes glucosinolate hydrolysis, which proved to be almost completely deactivated during oil extraction procedure (Lazzeri et al., 2011). The formulation details must be considered as confidential information and will not be extensively reported.