

CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF BROWN MUSTARD (*BRASSICA JUNCEA*) BIOMASS

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ABSTRACT

The last decade has shown the decrease of chemical inputs use in agriculture. Indeed, REACH recommendations, advised the lowering of numerous chemical molecules which were used in agriculture. The chemical registration conditions have been hardened. This fact allows searching “green” molecules extracted from plants which are environmentally friendly. The botanical family of Brassicaceae is known as potential source of bioactive molecules with antifungal, bacterial as well as nematocidal effects. All the Brassica species are rich in glucosinolates (GLs), in their seeds as well as in the green parts. The degradation of GLs is catalysed by Myrosinases allowing to the production of several volatile compounds among them thiocyanates, isothiocyanates and nitriles, which present effective biocidal activities

The aims of this study were to examine the chemical composition of the several plant parts at different phenological stages of brown mustard (*Brassica juncea*), and to test the efficacy of their glucosinolates (GLs) as an antifungal potential.

The HPLC analysis determined that two main compounds (Sinigrin and Gluconapin), which represented 99.5% of total GLs, were present in the different plant parts. As expected, the grain (110 µmol.g-1DW) is the richer organ in GLs and 10 times higher than the green plant parts, at ripening stage.

In order to study the effect of glucosinolates and their degradation compounds as antifungal molecules, biofumigation assays were performed against *Fusarium solani*, *Botrytis cinerea* and *Rhizoctonia solani*. Four GLs concentration (1, 3, 5 and 10 µmol.g-1DW) were tested against three fungi culture of 10 days old. The brown mustard powder, which was activated by adding water, revealed remarkable antifungal effect against the three studied fungi. Indeed, the growth and spore germination inhibition was complete at 10 µmol.g-1DW for the all fungi and for *Rhizoctonia* at 5 µmol.g-1DW. The GLs and their degradation products had also a strong detrimental effect on spore germination of all the tested plant pathogens along with concentration as well as time-dependent kinetic inhibition of *B. cinerea*. Thus, the results obtained in this study demonstrate that *B. juncea* possess a wide range spectrum of fungicidal activity and could become an alternative to synthetic fungicides for controlling certain important plant fungal diseases.

Introduction

Last decade has shown the decrease of chemical inputs use in agriculture. Indeed, REACH recommendations, advised the lowering of numerous chemical molecules which were used in agriculture. The chemical registration conditions have been hardened. This fact allows searching “green” molecules

extracted from plants which are environmentally friendly. The botanical family of Brassicaceae is known as potential source of bioactive molecules with antifungal, bacterial as well as nematocidal effects. All the Brassica species are rich in glucosinolates (GLs), in their seeds as well as in the green parts.

The degradation of GLs is catalysed by myrosinases (glucothiosidases) allowing to the production of several volatile compounds among them thiocyanates, isothiocyanates and nitriles depending in pH condition. The breakdown glucosinolates compounds present effective biocidal activities as reported by several studies (Lazzeri et al., 1993; Manici et al., 1997; Lazzeri et al., 2004; Yu et al., 2007).

The aims of this study were to examine the chemical composition of the several plant parts at different phenological stages of brown mustard (*Brassica juncea*), and to test the efficacy of their glucosinolates as an antifungal potential and to test a faster method to assess GLs.

MATERIALS AND METHODS

1- Plant material

The plant samples of *Brassica juncea* arise from plants from field growing culture during spring and summer 2008 in Auch (Gascony, SW of France). The samplings were made on whole plants, carved in several green

fractions, dried separately at 30°C, and stored in sealed freezer pockets, then crushed before use. Two samplings were made, the first, from the bloom, the other one, during the full bloom.

2- Extraction and measurements of glucosinolates

Two HPLC process methods were compared: the method of extraction following the ISO 9167-1 1992 versus a method described by West et al. (2002) and slightly modified.

In brief, power was added to warm water (>100°C) in order to denature the myrosinase. The mixture was filtered for elimination of big fragments. The obtained mixture was filtered across a 1µm filter. The samples obtained are used for HPLC injection.

3- Antifungal activities

Fungicidal effect was tested based on the bio fumigation method derived from that described by Yu et

al. (2007). Fungi were cultivated in PDA medium and therefore incubated at 25°C for 10 days. After this period, mustard powder was deposited in the lid of the Petri dish. Distilled water was added and the dish was immediately closed and sealed. Incubation was carried out during 3 days at 25°C and fungi development was checked in comparison with control. Five treatments were tested 0, 1, 3, 5 and 10 µM. The control was tested by used by inhibition of myrosinase and by adding water as the other treatments.

RESULTS AND DISCUSSION

The HPLC analysis determined that two main compounds (Sinigrin and Gluconapin), which represented 99.5% of total GLs, were present in the different plant parts (Fig 1). As expected, the grain with 129.8µmol.g⁻¹DW) was the richer organ in GLs. The green plant parts, at ripening stage, were 4 times less rich in GLs than grain. The less rich plant parts are the roots (4.4µmol.g⁻¹DW).

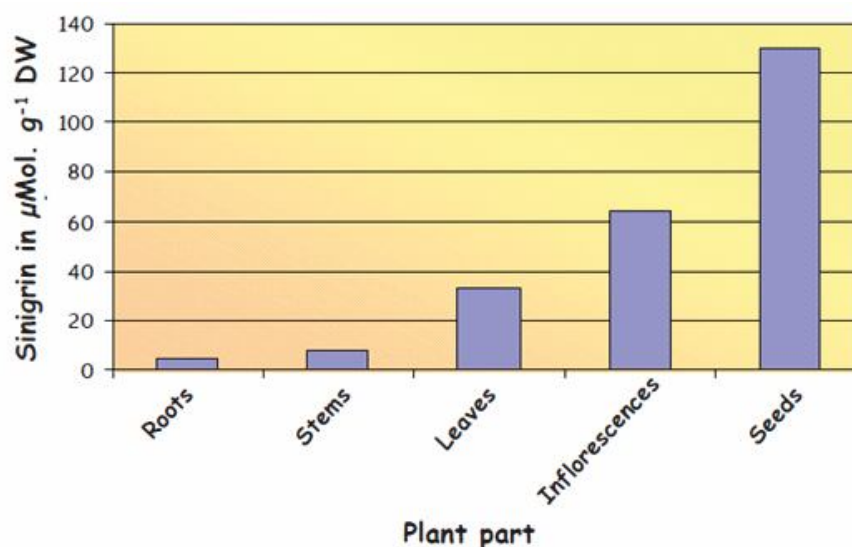


Figure 1. Glucosinolates content measured in different parts of *Brassica juncea* cultivated in Auch (SW France) in 2008.

Sinigrin (prop-2-enyl-glucosinolate) represented the most dominant GLs with the mean averaged proportion being 92.1% of total glucosinolates. Glucopnabin (but-3-enyl-glucosinolate) was the other most present GLs representing more than 7% of GLs content (Table 1). Other glucosinolates were present in very small amounts in *Brassica juncea*.

Seed GLs, sinigrin and glucopnabin content values are quite similar to those reported by Merah et al. (2004) in large collection of brown mustard genotypes and those of Lionneton et al. (2004) in segregating population for genetic study.

All the green part of *Brassica juncea* synthesizes and

stores GLS. Our results showed that a migration of these molecules occurs during the growth since stems then leaves and finally in the inflorescences. Every part seems to inherit from the stock constituted previously and increases its stock by its own synthesis. So, after complete maturation of the seed, it does stay GLs neither in siliqua nor in dry straw and leaves.

Organ	Sinigrin	glucopnabin	others
Roots	4,07	0,31	0,02
Stems	8,88	0,67	0,05
Leaves	34,41	2,60	0,19
Inflorescences	60,31	4,56	0,33
Seeds	120,07	9,09	0,65

Table 1. Glucosinolates content (in $\mu\text{Mol} / \text{g}$ of dry weight) and composition of different brown mustard organs

Our method of adapted extraction turned out as reliable to the ISO method. It is however easier of use because faster and less expensive.

Biofumigation assays were performed against *Fusarium solani*,

Botrytis cinerea and *Rhizoctonia solani*. (Fig.2). Four GLs concentrations (1, 3, 5 and $10\mu\text{mol.g-1DW}$) were tested against three fungi culture of 10 days old. The powder, which was activated by adding water, revealed a marked

antifungal effect against the three studied fungi. Indeed, both growth and spore germination were completely inhibited at $10\mu\text{mol.g-1DW}$ for the all fungi and for *Rhizoctonia* at $5\mu\text{mol.g-1DW}$.

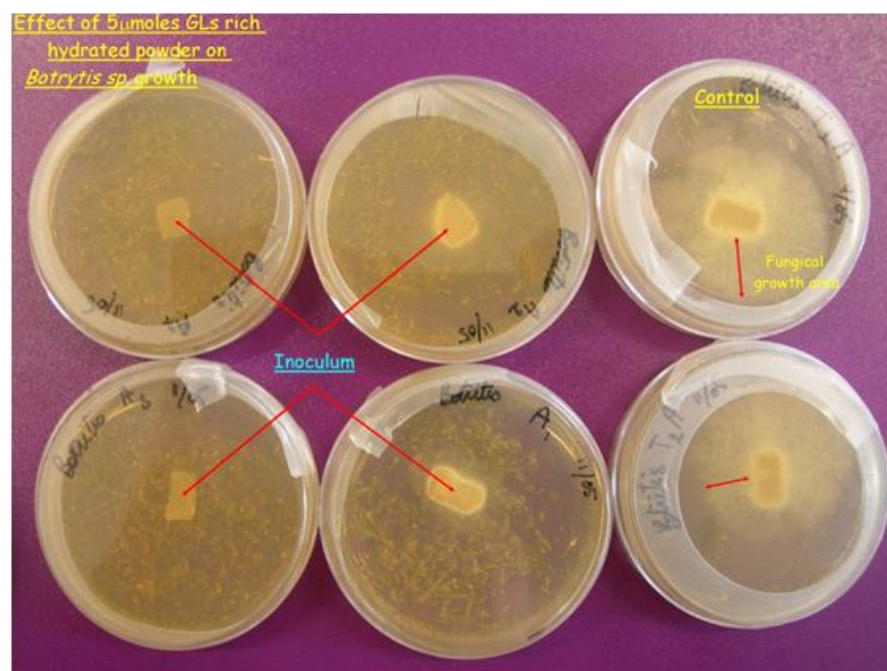


Figure 2. Bioassays of fungicidal effect of brown mustard powder performed on *Botrytis cinerea* at right the control (without treatment) in middle $5\mu\text{mol}$ and at left $10\mu\text{mol}$ treatments.

Treatment (μM)	Fungi					
	<i>Botrytis cinerea</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>			
	SG	Growth	SG	Growth	SG	Growth
0	+++	+++	+++	+++	+++	+++
1	+++	++	+++	+++	++	++
3	++	++	++	++	+	+
5	+	+	+	+	--	--
10	--	--	--	--	--	--

However, these GLs, whatever their place of storage and/or synthesis seems to have the same efficiency, as is on pathogenic fungi or on Nematodes. Our results suggest that the volatile phase of the ITC is active *per se*, without be solubilised. Mari, et al. (1996). Lazzeri, et al. (1993) reported GLs activities on *Heterodera schachtii*. Even there, our results highlighted that the volatile phase is active as well at low concentration.

CONCLUSION

Gls and their degradation products had also a strong detrimental effect on spore germination and growth of all the tested plant pathogens along with concentration as well as time-dependent kinetic inhibition of *B. cinerea*. Thus, our results demonstrate that *B. juncea* possess a wide range spectrum of fungicidal activity and could become an alternative to synthetic fungicides for controlling certain important plant fungal diseases.

The green biomass use of *B. juncea* can be envisaged to fight against the tested pathogenic fungi.

What opens rather promising perspectives, because the quantity of useful biomass appears then as important and not limited to the only seeds. Therefore, it is necessary to develop new cultivars with high GLs level in green part.

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Table 2. Fungicidal effects of different GLs levels on growth and spore germination of three pathogen fungi.

+ correspond to growth and -- correspond to absence of any growth or germination

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