Camelina sativa (L.) Crantz as a Promising Cover Crop Species with Allelopathic Potential

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Abstract: The ability of plants to release chemicals that affect the growth and development of other plants offers potential benefits for weed management and sustainable agriculture. This review explores the use of Camelina sativa as a promising cover crop with weed control potential. Camelina sativa, known for its high oil content and adaptability to diverse climatic conditions, exhibits allelopathic potential by releasing chemical compounds that inhibit weed growth. The crop’s vigorous growth and canopy architecture contribute to effective weed suppression, reducing the prevalence and spread of associated pathogens. Furthermore, the chemical compounds released by camelina through the solubilization of compounds from leaves by rain, root exudation, or deriving from microbial-mediated decay of camelina’s tissues interfere with the growth of neighbouring plants, indicating allelopathic interactions. The isolation and identification of benzylamine and glucosinolates as allelochemicals in camelina highlight their role in plant–plant interactions. However, the studies carried out on this species are outdated, and it cannot be excluded that other chemicals deriving from the breakdown of the glucosinolates or belonging to other classes of specialized metabolites can be involved in its allelopathic potential.

Camelina sativa also demonstrates disease suppression capabilities, with glucosinolates exhibiting fungicidal, nematocidal, and bactericidal activities. Additionally, camelina cover crops have been found to reduce root diseases and enhance growth and yields in corn and soybeans. This review sheds light on the allelopathic and agronomic benefits of Camelina sativa, emphasizing its potential as a sustainable and integrated pest management strategy in agriculture.

Keywords: Camelina sativa; allelopathy; cover crop; glucosinolates; weed control

1. Introduction

Allelopathy is the ability of plants to release chemicals that affect the growth and development of other plants. These chemicals can be released into the soil or air and may affect neighbouring plants’ germination, growth, or reproduction [1]. The allelopathic potential of crops has been recognized for many years. There is growing interest in using allelopathic crops for weed management since they can reduce the need for synthetic commercial herbicides, which can have negative environmental and health impacts, promote sustainable agriculture by reducing weed pressure and improving soil health, and be cost-effective for farmers, as they may not need to purchase and apply herbicides [1]. Depending on their activity, allelopathic crops can be classified into direct and indirect. Direct allelopathic crops release allelopathic compounds that directly affect the growth and development of weeds. Indirect allelopathic crops release allelopathic compounds that stimulate the growth and development of beneficial microorganisms, suppressing the growth of weeds [2]. The use of allelopathic crops is a promising strategy for managing herbicide-resistant weeds, even without a detailed understanding of the underlying mechanisms. While understanding the allelopathic effects and optimizing their application is important, mechanistic aspects...
are also crucial to the success of such initiatives. A greater understanding of plant–plant communication, recognition, and the potential non-target effects of allelochemicals, would elevate allelopathic plants from blunt tools for weed control to intelligent components of an integrated weed management program [3]. However, using allelopathic crops for weed management also has some challenges. First, the effectiveness of allelopathic crops may be influenced by environmental factors, such as soil moisture and temperature, which can affect the release and activity of allelopathic compounds [4–7]. Moreover, the development of allelopathic cash crops has also to satisfy the demand for high yields. Agriculture has traditionally prioritized breeding for yield improvement over other traits, so any form of weed suppression must consider its net effect on productivity, given that reduced yield in a weed-free environment can be compensated by the yield benefit provided by effective weed suppression. For example, Kong et al. [8] bred allelopathic rice cultivars that were high-yielding and weed-suppressive, but further research is needed to characterize the trade-offs related to yield and plant defence [9]. Genetic engineering techniques offer a sophisticated but largely underappreciated approach to developing allelopathic crops for weed management [1]. Recent efforts to identify genetic regions involved in sorgoleone biosynthesis in sorghum [10] pave the way for future up-regulation of these genes for a more significant allelopathic effect. There is also evidence that cytochrome P-450 monoxygenases play a role in allelochemical synthesis in various plant species, including sorghum [11] and benzoxazinoid allelochemical biosynthesis in cereals [12], indicating some consistency between species in their genetic tools for allelochemical synthesis. Specific genes involved in allelochemical biosynthesis can also be edited for examination or upregulation of specific compounds in the pathway [1]. Another approach to using allelopathic species for weed suppression is to apply them as cover or intercrops in rotation with a less weed-suppressive cash crop [13]. Recently, besides cereals, huge attention has been paid to brassicaceous species as cover crops with allelopathic activity [14–17]. The Brassicaceae family, or the mustard family, is a diverse group of plants that includes 375 genera and over 3200 species [17]. This family is known for its economic importance, as many members are cultivated as food crops or for their medicinal properties [18–20]. In addition, the Brassicaceae family is known for its allelopathic potential, particularly related to the presence of glucosinolates [17]. Glucosinolates are a class of sulfur-containing compounds found in high concentrations in many members of the Brassicaceae family. When the plant tissues are damaged by herbivores or mechanical stress, glucosinolates are hydrolyzed by the enzyme myrosinase, producing a variety of breakdown products, including isothiocyanates, nitriles, and thiocyanates [21]. The breakdown products of glucosinolates have been shown to have allelopathic effects on neighbouring plants [22,23]. For example, isothiocyanates have been shown to inhibit the germination and growth of various plant species, including lettuce and velvetleaf [24–26]. In addition, isothiocyanates have been shown to inhibit the growth of specific soilborne pathogens [27–29]. The Brassicaceae family includes several species that are known for their allelopathic potential, including *Brassica napus* (oilseed rape), *Brassica juncea* (Indian mustard), and *Sinapis alba* (white mustard). These species have been shown to release allelopathic compounds that inhibit the growth and development of weeds, and they are being investigated as potential allelopathic crops for weed management [30–33]. Research has shown that allelopathic crops, including those in the Brassicaceae family, can effectively reduce weed growth and suppress weed seed production. For example, one study found that using allelopathic cover crops, including *Brassica napus*, reduced weed biomass by 50–90% compared with a fallow control. Another study found that the use of *Brassica juncea* as a cover crop reduced the density and biomass of certain weed species by over 80%.

2. *Camelina sativa*: A Promising Cover Crop

*Camelina sativa* is an oilseed crop that has gained interest as a biofuel and food source due to its high oil content and potential health benefits, and is an annual plant belonging
to the Brassicaceae family. The plant grows up to 60–120 cm in height and has a slender, branching stem covered with narrow, lanceolate leaves (Figure 1).

Figure 1. *Camelina sativa* at flowering stage.

The leaves are alternate, and the plant produces yellow flowers with four petals. Following pollination, the flowers develop into fruits, known as siliques, which contain small, round seeds. *Camelina sativa* is adapted to grow in temperate regions but can also tolerate a wide range of climatic conditions. It is known for its ability to grow in marginal lands with low fertility and limited water availability, making it suitable for cultivation in arid and semi-arid regions. The recommended seeding rate is approximately 10–15 kg per hectare, and the plant requires full sun exposure for optimal growth and development. *Camelina sativa* prefers well-drained soil with a pH ranging from 5.5 to 8.0, and its cultivation offers several environmental benefits. Its deep root system improves soil structure, reduces erosion, and enhances water infiltration. The crop requires fewer pesticides and fertilizers than conventional oilseed crops, reducing potential negative environmental impacts. It is a cool-season crop that can withstand frost and has a relatively short growing season of around 85–105 days [34–36]. One of the primary uses of *Camelina sativa* is for oil production. The plant’s seeds are rich in oil, typically containing 30–45% oil content. Camelina oil is characterized by its high levels of omega-3 fatty acids, particularly alpha-linolenic acid (ALA). It is considered a valuable alternative to fish oil and can be used for human consumption, animal feed, and biodiesel production [37,38]. Beyond its economic and environmental benefits, camelina cultivation has been found to have implications for pathogen management, highlighting its potential as a sustainable and integrated pest management
strategy. In fact, numerous studies have demonstrated the ability of *Camelina sativa* to suppress various plant pathogens, thereby reducing the incidence and severity of diseases. The mechanisms underlying disease suppression include direct antimicrobial properties of plant-specialized metabolites and the activation of systemic acquired resistance (SAR) and induced systemic resistance (ISR) in neighbouring plants. For example, camelina plants produce glucosinolates, which are known to exhibit fungicidal and bactericidal activities against a range of pathogens [39,40]. For example, both jatropha and camelina seed meals possess biofumigant properties and can have varying effects on soil microbial communities, which tend to persist over time. Furthermore, the microbial functional patterns were unaffected. This knowledge will be valuable in appropriately utilising jatropha and camelina SMs for pathogen control while minimizing detrimental effects on non-target microorganisms [41]. *C. sativa* showed good fungicidal activity against *M. phaseolina*, which caused charcoal rot diseases in soybean [42].

One of the most important biotic stresses in soybean production is soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN), a serious pest that affects 90% of the soybean-producing areas in the U.S. [43]. A study conducted by Acharya et al. [43] found that winter camelina and brown mustard are non-hosts for SCN populations and reduced egg numbers [43].

In another study, a three-year field experiment was conducted to investigate the effects of winter cereal rye (*Secale cereale* L.) and winter camelina (*Camelina sativa* [L.] Crantz) cover crops, used either continuously or in rotation on the growth, root disease, and yield of corn (*Zea mays* L.) and soybeans (*Glycine max.* [L.] Merr.). Results showed that corn following a camelina cover crop experienced reduced root disease, a lower *Pythium* fungi population in seedling roots, and exhibited greater growth and yields than corn following a rye cover crop. Furthermore, a winter camelina cover crop grown before corn had less detrimental effects on corn seedling growth, root disease, and final yield compared with a winter rye cover crop preceding corn. This study provides valuable insights into the effects of winter cover crops on root disease and growth in corn and soybeans, suggesting the potential benefits of using camelina as a cover crop before corn cultivation [44]. The integration of cover crops, specifically green manures, alongside cash crops has been widely practised for many decades.

Extensive research consistently confirms the positive effects of green manure cover crops on soil quality, especially in low-carbon or degraded soils. These advantages encompass enriching soil organic carbon content, enhancing soil structure, preventing erosion, and mitigating crop diseases [45]. Furthermore, green manures play a pivotal role in nurturing the soil’s microbial community, improving nutrient availability, and fostering beneficial interactions between crop plants and microorganisms. The presence of green manures creates competition for niches, effectively curbing the proliferation of harmful microbial pathogens and ultimately resulting in increased yields of cash crops [46]. A particular type of green manure, referred to as biofumigants, harnesses the power of certain plants that release toxic compounds into the soil to control crop pests, pathogens, and weeds. Notably, *Brassica* species are known for producing glucosinolates; these compounds break down in the soil, they give rise to isothiocyanates, highly toxic to various organisms, including common crop pests and pathogens [47]. The reduced weed density and biomass observed in crops grown after incorporating brassica cover crops indicate that they can play a role in weed management within agricultural systems.

Green manure and biofumigant crops are widespread in cropping rotations, aimed at maintaining or improving agricultural soil yields by preventing degradation and protecting vital ecosystem services. In recent decades, there have been significant advancements in our understanding of soil microbiomes in agriculture. The use of advanced techniques like metagenomics has enabled researchers to delve deeply into soil microbiomes, providing unprecedented insights [48]. As a result, we now recognize the crucial role of the soil microbiome in delivering essential ecosystem services that are vital for agriculture [49]. This growing awareness has spurred investigations into management practices that aim
to restore, protect, and enhance soil ecosystem health, with organic amendments such as green manure being among the promising approaches. For example, brassica biofumigants release glucosinolates during their growth, which subsequently convert into toxic isothiocyanates in the soil, influencing the structure of soil microbial communities both during the biofumigant’s growth and shortly after its incorporation [47,50]. Glucosinolates can be degraded even without the presence of the hydrolytic enzyme myrosinase, potentially contributing to their bioactive effects. For example, Hanshen and coauthors examined the stability of glucosinolate hydrolysis products derived from Brassicaceae plants and pure glucosinolates in three different soils (a model simulating biofumigation).

Additionally, the research focused on the degradation of pure 2-propenyl glucosinolate and the effect on the soil bacterial community composition. The results obtained showed a significant impact on the bacterial community composition. Interestingly, significant alterations in the soil community due to biofumigant exudates were observed, despite the ferrosol’s high clay and organic matter content. These results suggest that brassica biofumigation might be effective on ferrosols and similar soil types with high clay and organic matter content [50]. In another study conducted by Walker et al. [46] in 2022, the authors investigated the long-term effects of continuous ryegrass green manuring and brassica biofumigation on fertile, clay-rich soil (Red Ferrosol) in an intensive vegetable cropping rotation spanning 10 to 13 years. The results showed that both ryegrass green manuring and brassica biofumigation resulted in alterations to the soil microbial communities, promoting the growth of copiotrophic bacteria and fungi involved in organic matter degradation.

In addition, both treatments significantly increased the relative abundance of arbuscular mycorrhizal fungi compared with the fallow plots. Overall, this study provides valuable insights into brassica biofumigation that positively impacts soil characteristics, microbial communities, and crop yields, making important contributions to sustainable agricultural practices [46]. Furthermore, isothiocyanates have been shown to possess strong inhibitory effects on seed germination leading to stunted seedling growth [51]. Field studies further support the role of brassica residues, including canola, rapeseed, and mustards in weed management [14]. Compared with fallow or other non-brassica cover crops, preceding brassica cover crops have shown effectiveness in reducing weed biomass and weed density in various crops because most weed seeds have considerably smaller masses than the seeds of the crops they infest [52].

Camelina also exhibits a capacity for weed suppression (Figure 2); in fact, Camelina sativa’s vigorous growth and canopy architecture can effectively suppress weed populations, subsequently reducing the prevalence and spread of associated pathogens. However, the careful selection of cover crop species and the timing of interseeding play a crucial role in obtaining advantages while maintaining optimal sugar beet yield. In North Dakota, when camelina was interseeded during the V1–V3 growth stages of corn and the V1–V2 growth stages of soybean (Glycine max (L.) Merr.), there was a reduction of 14% in corn yield and 10% in soybean yield [53].

Furthermore, by releasing chemical compounds that inhibit weed growth, camelina’s allelopathic potential can further contribute to weed control and indirectly influence pathogen dynamics. For example, originating in North America, Ambrosia artemisiifolia L. is an invasive alien species widely recognized as one of Europe’s most harmful plant species. In a study conducted by Scepanovic et al. [54] where the objective was to assess the impact of different concentrations of aqueous extracts from Brassicaceae cover crops (including Sinapis alba, Raphanus sativus, Camelina sativa, Fagopyrum esculentum, and Guizotia abyssinica) on the germination and early growth of Ambrosia artemisiifolia L., allelopathic effects were found to be dependent on the species and concentration of the aqueous extracts. Camelina sativa exhibited the highest potential for inhibiting germination, shoot, and radicle length, and fresh seedling weight. Analysis using liquid chromatography-tandem mass spectrometry identified 15 phenolic compounds in the Brassicaceae, with Camelina sativa having the highest content of vanillin, chlorogenic acid, vanillic acid, caffeic acid, and
syringic acid. These findings suggest that *Camelina sativa* is the most allelopathic among the species used in this study and that the seeds of *Camelina sativa* are particularly rich in allelochemicals [54].

**Figure 2.** Camelina capacity for weed suppression.

3. *Camelina sativa*: A Potential Allelopathic Crop

The allelopathic potential of *Camelina sativa* has been widely investigated in the past. But the analytical and chemical approaches used were mainly based on targeted analysis, which strongly reduced the amount and the complexity of chemicals being characterized. Moreover, there are no available studies focused on the bio-guided fractionation of the phytocomplex of this species aimed at identifying the classes of compounds involved in the allelopathic phenomenon and/or on the phytotoxicity of the plant extracts. Therefore, despite the evidence of camelina’s allelopathy, this field of research is superficially explored, and new coupled to classical approaches should be used to shed light on this phenomenon.

The first study reporting the allelopathic potential of *Camelina sativa* was published by Grummer and Beyer [55], which observed that the presence of camelina in fields cropped with linseed significantly reduced crop yield. They highlighted that this phenomenon was observable when significant rainfall was in conjunction with specific phenological stages of camelina and linseed plants, suggesting that the allelochemical release through the solubilization of compounds from leaves by rain was responsible for the phytotoxic effects observed [55,56]. After almost twenty years, a clearer understanding of this phenomenon was achieved thanks to studies on the effects of camelina’s leaf lixiviates on seedlings’ growth and their interaction with soil bacteria [57]. In particular, such studies demonstrated that under controlled conditions, the lixiviates collected from camelina’s intact leaves were interfering with the growth of germinating *Linum usitatissimum* seedlings.
Moreover, they demonstrated that gram-negative bacteria mediated these growth alterations in the camelina phyllosphere [56,57]. Successively, Lovett and Jackson [58] reported that the effects observed on linseed seedlings were also observable on several species of higher plants, and the main bacteria mediating the effects were Enterobacter cloacae or Pseudomonas fluorescens. Moreover, they highlighted that the bacterial activity rapidly produced the allelochemical involved in plant interference, which probably was an organic compound produced by degrading a more complex metabolite commonly found in plants belonging to the Brassicaceae family. The isolation and identification of this specialized metabolite involved in this plant–plant interaction were achieved only after a year when benzylamine was identified as an allelochemical influencing the association of *C. sativa* with linseed [59,60]. It was demonstrated that camelina leaves contain organic acids that support rapid bacterial growth, breaking complex organic compounds into simpler molecules. One of these compounds, benzylamine, exhibits allelopathic properties, as reported by Lovett and Duffield [59,60]. Successively, it was reported that the release of the precursor, benzyl isothiocyanate, may require damage to the leaves, and the highest concentration of allelopathic activity may occur during the senescent stage of the life cycle when bacterial populations are at their peak. Lovett reported that in Petri dishes, low benzylamine concentrations stimulated germinating linseed seedlings similar to camelina leaf washings, but higher concentrations had an inhibitory effect, as is typical of allelochemicals [61]. Studies on this molecule highlighted its inhibitory effects mainly due to its ability to disrupt cellular membranes and reduce food reserve mobilization [56,62]. More recent studies demonstrated that *C. sativa* only seemed to suppress weeds during the initial growth phase (seedling establishment), as it had a significant impact on annuals such as Sonchus oleraceus, Matricaria recutita, and Fallopia convolvulus but had no effect on the major perennials *Elýtrigia repens* and Cirsium arvens. In row-cropped peas, *C. sativa* played the dual role of a smother crop and weed antagonist without exhibiting phytotoxicity to the crop [63]. More recently, Walsh et al. [64] tested the impact of various camelina plant components, including leaf washings, aqueous extracts, soil-incorporated fresh plant residues, and root exudates, on the seedling growth of different species. Results indicated that *C. sativa* leaf washings increased radish seedling weight, while the germination of wild oat, flax, and radishes was reduced by its aqueous extracts. Wild oat and radish seedlings also displayed a decrease in root weight and an increase in shoot weight in response to aqueous extracts. Incorporating fresh camelina plant residues into growth media increased radish weight, while camelina exudates reduced flax weight. The chemical characterization of the biologically active camelina’s aqueous extract highlighted that the main potential allelochemicals were volatile sulfur-containing compounds, such as methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, which were predominantly present. These results have given a significant turning point to the study of camelina. They have highlighted that, besides benzylamines, the species can produce a plethora of specialized metabolites belonging to the sulfur metabolism with allelopathic activity, such as glucosinolates (GSLs) and their corresponding degradation products [65]. Different organs can differentially produce GSLs depending on the phenological stage of the plant. In particular, it has been demonstrated that the adult plants of *C. sativa* are characterized by an organ-specific production of GSLs. The main organs characterized by their accumulation are the roots and the siliques, whereas the leaves contain a significantly low amount since their content is reduced along with plant development [66]. Three days after germination could be found 60% of the GSLs accumulated in the seeds, and their content dropped to 25% seven days after germination [67]. In addition, it has been estimated that in seed meal, depending on the accession, the GSL content can vary from 19.6 to 40.3 mmol kg$^{-1}$ dry weight [68]. Therefore, it has been suggested that adult plants release GSLs into the environment mainly through root exudation or their decay [69], making the study of the root exudate dynamics and its chemical characterization a crucial step in exploiting the potential use of camelina as a cover crop, since the modulation through genetic improvement of their composition and release can strongly improve its effectiveness in weed control. Moreover,
Quéro et al. [70], studying the glucosinolate profile during seed development, observed that the buildup of glucosinolates primarily takes place within a timeframe of 15 to 25 days following the onset of flowering. The concentration of glucoarabin, glucocamelinin, and gluconesliapaniculatin between these two time points is amplified by factors of 4.0, 3.4, and 2.8, respectively. These three compounds exhibit similar accumulation patterns, maintaining consistent levels between 25 and 35 days after flowering. When examining the glucosinolate profiles of mature seeds, glucocamelinin exhibits a higher level of intensity in terms of area compared with glucoarabinin and gluconesliapaniculatin. The studies aimed at characterizing GSLs in *Camelina sativa* extracts predominantly relied on techniques primarily focused on targeted studies using pure standards. This approach facilitated the identification of three key GSLs (glucoarabin, glucocamelinin, and gluconesliapaniculatin) and a few others (Table 1). However, with the advent of mass spectrometric techniques coupled with gas chromatography or ultra-high-performance liquid chromatography, the exploration of unknown chemicals or biochemical intermediates can expand significantly. These advanced analytical methods offer a unique opportunity to delve deeper into the allelopathic activity of *Camelina sativa* and uncover new aspects regarding its chemical interactions with other species. Moreover, these cutting-edge techniques can shed light on previously undiscovered allelochemicals that may be present in the plant, broadening our understanding of its ecological role and potential applications. By embracing these modern analytical tools, researchers can unveil a wealth of information, opening new doors for further investigations in the field of allelopathy.

### Table 1. Main glucosinolates identified and quantified in different *C. sativa* tissues.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Iupac Name</th>
<th>Tissue</th>
<th>Bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatic</strong></td>
<td>3-(methylthio)propyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>9-(methylthio)nonyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>3-(methylsulfinyl)propyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>4-(methylsulfinyl)butyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>8-(methylsulfinyl)octyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>9-(methylsulfinyl)nonyl glucosinolate *</td>
<td>Whole plant, seeds, root exudates</td>
<td>[66,67,70]</td>
</tr>
<tr>
<td></td>
<td>10-(methylsulfinyl)decyl glucosinolate *</td>
<td>Whole plant, seeds, root exudates</td>
<td>[66,67,70]</td>
</tr>
<tr>
<td></td>
<td>11-(methylsulfinyl)undecyl-glucosinolate *</td>
<td>seed, root exudates</td>
<td>[66,67,70]</td>
</tr>
<tr>
<td><strong>Indole</strong></td>
<td>3-indolylmethyl glucosinolate</td>
<td>Whole plant</td>
<td>[66,67,70]</td>
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<tr>
<td></td>
<td>4-hydroxy-3-indolylmethyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
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<tr>
<td></td>
<td>4-methoxy-3-indolylmethyl glucosinolate</td>
<td>Whole plant</td>
<td>[66]</td>
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<tr>
<td></td>
<td>ds-glucobrassicin</td>
<td>Seedlings</td>
<td>[71]</td>
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<td>ds-4-methoxy-glucobrassicin</td>
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<td></td>
<td>ds-neoglucobrassicin</td>
<td>Seedlings</td>
<td>[71]</td>
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</tbody>
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* The compounds 9-(methylsulfinyl) nonyl glucosinolate, 10-(methylsulfinyl) decyl glucosinolate and 11-(methylsulfinyl)undecyl-glucosinolate are commonly known as glucoarabin, glucocamelinin, and gluconesliapaniculatin, respectively.

### 4. Sulfur Availability and Glucosinolates Production

Sulfur is an essential macronutrient for plant growth and development [72]. Photosynthetic organisms use sulfur to synthesize various sulfur-containing metabolites, including cysteine, methionine, glutathione, vitamins, cofactors, and chloroplastic sulfolipids and a wide variety of specialized compounds, such as GSLs in the Brassicaceae family [73,74]. Recent studies demonstrated that GSLs, generally considered end-products of the metabolism, can serve as a reservoir for sustaining a retrograde flow of sulfur atoms for cysteine production under sulfur deficiency [75]. GSLs are sulfur-rich secondary metabolites with plant-protective and medicinal properties, such as antimicrobial and anticarcinogenic activities [76–78]. Plants suppress GSL biosynthesis under sulfur deficiency, which affects field performance and medicinal quality due to insufficient sulfate supply. A recent study [79] identified the genes *Sulfur Deficiency Induced 1* and 2 (*SDI1* and *SDI2*) as major repressors of GSL biosynthesis in Arabidopsis under sulfur deficiency conditions. The expression of *SDI1*
and SDI2 negatively correlated with GSL biosynthesis at the transcript and metabolite levels. Principal components analysis revealed that SDI1 regulates aliphatic GSL biosynthesis as part of the sulfur deficiency response. SDI1 protein localizes to the nucleus, interacting with MYB28, a key transcription factor promoting aliphatic GSL biosynthesis in yeast and plant cells [79]. Through the formation of an SDI1-MYB28 complex, SDI1 inhibited the transcription of aliphatic GSL biosynthetic genes, leading to the down-regulation of GSL biosynthesis and prioritization of sulfate utilization for the synthesis of primary metabolites under sulfur-limiting conditions. Since plant sulfur nutritional status controls both GSL biosynthesis and degradation in a bidirectional way, the final accumulation of GSLs in plant tissues can be finely modulated through sulfur fertilization, breeding programs, or genetic modifications to improve plant sulfur use efficiency.

5. Biosynthesis of Glucosinolates

Glucosinolates can be categorized into three main classes based on their biosynthesis: aliphatic, derived from methionine; aromatic, derived from phenylalanine; and indolic, derived from tyrosine or tryptophan [80]. The biosynthesis of glucosinolates involves inserting methylene groups into the side chains of aliphatic and aromatic amino acids. The elongated amino acid moiety undergoes reconfiguration through metabolic processes, resulting in the characteristic core structure of glucosinolates, which undergoes further structural modifications [80]. Aliphatic glucosinolates originate from methionine, converted to a 2-oxo acid through amination catalysed by the enzyme BCAT4. This initial synthetic step occurs in the cytosol, while subsequent enzymatic activities in the elongation process occur in the chloroplasts. Within the chloroplasts, the aliphatic chain of the 2-oxo acid is elongated by three enzymes: methylthioalkylmalate synthase (MAMS), isopropylmalate isomerase (IPMI), and isopropylmalate dehydrogenase (IPM-DH). The elongated 2-oxo acid can be transamminated to homomethionine or proceed for further chain elongation (Figure 3a). The overall process generates a range of chain-elongated derivatives of methionine [81–87]. The core formation of glucosinolates involves the participation of homomethionine, which undergoes a series of enzymatic reactions in the cytosol. These reactions are common to all three classes of glucosinolates (aliphatic, aromatic, and indolic) (Figure 3a). Enzymes from the CYP79 gene family, specifically cytochrome P450s, convert the elongated amino acids derived from methionine, tyrosine, tryptophan, and phenylalanine into aldoximes. Different members of the CYP79 family catalyse the conversion depending on the amino acid derivative. Aldoximes are then converted to oxidized forms (aci-nitro compounds and/or nitrile oxides) by cytochrome P450s from the CYP83 gene family. The sulfur donor for conjugation with the activated aldoxime was initially thought to be cysteine, but recent studies indicate that glutathione (GSH) serves as the sulfur donor instead [88,89]. The products resulting from cytochrome P450 activity are conjugated to glutathione by glutathione-S-transferases, forming S-alkyl-thiohydroximates. These are substrates for the enzyme carbon-sulfur lyase SUR1, initiating thiohydroximate biosynthesis [90]. The desulfoglucosinolates produced by UGT74 enzymes are sulfated to form glucosinolates, the second step in glucosinolate biosynthesis [91–98]. Sulfo transferases (SOTs) from the sulfo transferases family catalyse this reaction using 3′-phosphoadenylyl sulfate (PAPS) as the sulfate donor [99–103]. PAPS biosynthesis depends on sulfur nutrition and involves ATP sulfurylase (ATPS) and adenosine 5′-phosphosulfate (APK) kinase [91–98]. Glucosinolate activity is primarily influenced by their side chain structure [104]. Aliphatic glucosinolates can undergo various modifications, such as alkenylations, oxidations, benzoylations, and hydroxylations. Indolic glucosinolates are mainly subjected to methoxylation and hydroxylation [105,106]. These modifications occur in an organ and development-specific pattern [107,108]. Genetic studies have identified three loci involved in the side-chain modification of aliphatic glucosinolates [109]. The Gsl-oxid locus controls the oxidation process, catalysed by the flavin-monoxygenase (FMO) enzyme FMOGS-OX1, converting methylthio- to methylsulfanylalkylglucosinolates [110]. The Gsl-alk and Gsl-oh loci regulate the removal of the methylsulfanyl residue, the introduction of a double bond, and
the hydroxylation of butenylglucosinolate, respectively. Another locus, Gsl-ohp, converts methylsulfanylpropyl- to hydroxypropylglucosinolate in Arabidopsis thaliana [87]. Additionally, three genes (AOP1, AOP2, and AOP3) encoding 2-oxoglutarate-dependent dioxygenases are involved in glucosinolate modification. AOP2 converts 3-methylsulfanylpropyl- and 4-methylsulfanybutylglucosinolate to alkanylglucosinolates, while AOP3 converts 3-methylsulfanylpropyl- to 3-hydroxypropylglucosinolate [111,112].

Figure 3. (a) Schematic representation of the biochemical processes involved in the aliphatic Glucosinolate Chain Elongation machinery; (b) Glucosinolate Core Biosynthesis. Amino acids, including elongated aliphatic methionine-derived molecules, can be converted to aldoximes by CYP79 cytochrome P450 family members to start building up the core glucosinolate scaffold. BCAT4—branched-chain aminotransferase 4; MAMS—methylthioalkylmalate synthase; IPMI—isopropylmalate isomerase; IPM-DH—isopropylmalate dehydrogenase; BCAT3—branched-chain aminotransferases-3; CYP79; GST; SUR1; UGT74; SOT. The pathway was built using the open-source software Pathvisio vs. 3.3.0.

6. Glucosinolate Transport

Transport processes are essential for redistributing specialized metabolites like glucosinolates, which protect vital tissues for species survival. In Arabidopsis, a significant portion of glucosinolates is transferred to maturing seeds [107]. Glucosinolate biosynthesis occurs in both the cytosol and chloroplasts, and their transport involves both short- and long-distance movement facilitated by transport proteins. The bile acid:sodium symporter family protein 5 (BAT5) plays a key role in short-distance transport. BAT5 imports 2-oxo acids into the chloroplast for side chain elongation and exports the resulting products into the cytosol for glucosinolate conversion [83]. BAT5 is activated by aliphatic glucosinolate regulators HAG1/MYB28, HAG2/MYB76, and HAG3/MYB29 [83]. In Arabidopsis, BAT5-defective mutants show reduced aliphatic glucosinolate levels [83,85]. Glucosinolates produced by maternal tissues are long-distance transported and accumulated in seeds [113–115]. In Arabidopsis, the transporters GTR1 and GTR2, belonging to the peptide transporter (PTR/NRT1) superfamily, mediate the movement of aliphatic and indolic glucosinolates between source and sink tissues [116,117]. GTR2 plays a prominent role [117–120]. Additionally, GTR1 and GTR2 are involved in the distribution of long-chain aliphatic glucosinolates between roots and shoots [121,122]. Genetic manipulation of plants to inhibit
the activity of GTR1 and GTR2 offers potential benefits in terms of reducing glucosinolate accumulation in seeds without affecting biosynthesis, thereby maintaining inherent defence capabilities [118,123]. Knock-out mutants of GTR1 and GTR2 in Brassica juncea showed changes in plant phenotype. GTR1 mutants had slightly reduced seed glucosinolate levels and significantly lower levels in source tissues. GTR2 mutants exhibited a significant decrease in seed glucosinolates but increased accumulation in leaves and pods. Moreover, GTR2 mutants demonstrated higher resistance to Spodoptera litura, suggesting the potential for enhancing crop production through manipulation of GTR2 to improve defence mechanisms or reduce anti-nutritional glucosinolate concentrations in seeds [39,118].

7. Glucosinolates Breakdown Product

Glucosinolate activation occurs when plants are wounded, for example, through chewing, which leads to contact between glucosinolates and myrosinases. This interaction triggers the hydrolysis of glucosinolates, resulting in an unstable aglucone that spontaneously rearranges into the corresponding isothiocyanate [124]. Isothiocyanates are highly reactive and toxic to various plant competitors and enemies, including microbes, fungi, insects, nematodes, and other plant species [125–129].

Despite the defensive capabilities of isothiocyanates, many Brassicaceae species have evolved alternative activation pathways through the involvement of specifier proteins. These specifier proteins, characterized by kelch domains, influence the structural outcome of myrosinase-catalysed glucosinolate hydrolysis [130–133]. In the presence of these proteins, the production of isothiocyanates is reduced in favour of other breakdown products, such as simple nitriles, epithionitriles, and organic thiocyanates. This introduces additional structural diversification, besides the biosynthesis process, and may provide further defence mechanisms through direct or indirect effects on plant enemies [134,135].

8. Conclusions

In conclusion, based on the field observations of Camelina sativa’s weed-reducing capacity, more studies are needed to evaluate its allelopathic potential and its effects on sensitive species. The existing studies on the phytochemical characterization of glucosinolates are outdated and rely on dated approaches. Therefore, conducting new metabolomics analyses using mass spectrometry-based techniques is recommended. These analyses would enable the identification of the most abundant metabolites, the glucosinolates produced by Camelina sativa, including metabolic intermediates of GSL and break down products (simple nitriles, epithionitriles, organic thiocyanates sulfides, and thiols), and new classes of potentially phytotoxic compounds involved in the allelopathic phenomenon, which are superficially explored in this species. Therefore, the use of new holistic techniques can expand the knowledge of the bioactive molecules involved in allelopathic interactions.

Furthermore, it is important to note that glucosinolate production in Camelina sativa is strongly influenced by sulfur-based fertilization and regulated by the SDI1 and SDI2 genes. Furthermore, root exudates appear to be the main route by which this species exerts its allelopathic potential. Therefore, it is crucial to deepen the studies on the chemical composition of these compounds, their dynamics during the crop cycle, and their fate once released into the environment (i.e., transformation and/or microbial degradation phenomena).

Consequently, it is advisable to develop fertilization, breeding, and/or genetic improvement strategies using transgenic approaches to enhance the production and release of these specialized metabolites. Adopting such agroecological approaches can significantly improve the sustainable management capacity for weed control.

Author Contributions: Conceptualization, M.G., M.P., F.C., F.F.N., R.P. and F.A.; writing—original draft preparation, M.G., M.P., F.C., F.F.N., R.P. and F.A.; writing—review and editing, M.G., M.P., F.C., F.F.N., R.P. and F.A., Funding acquisition R.P. and F.A. All authors have read and agreed to the published version of the manuscript.
Funding: Agritech National Research Centre and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILLENZA (PNRR)-MISSIONE 4 COM-PONENTE 2, INVESTIMENTO 1.4-D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors’ views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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