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Enhancing Greenhouse Tomato-Crop Productivity by Using *Brassica macrocarpa* Guss. Leaves for Controlling Root-Knot Nematodes

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Abstract: Tomato crops are affected in Mediterranean cold-greenhouse agrosystems by soilborne diseases, such as root-knot nematodes (*Meloidogyne* spp.), which represent a serious problem leading to losses in production. Agroecological soil management based on biocontrol agents and natural compounds has had increased grower interest in order to reduce chemical residues in the produce and to adopt environmentally friendly farming methods. In this frame, we evaluate and validate soil biofumigation by the use of glucosinolate (GLS) compounds. Among them, sinigrin showed biocontrol activities against several pests and diseases via nematotoxic action. Among the *Brassicaceae* species rich in sinigrin, we chose *Brassica macrocarpa* Guss. (BM) because its leaves show 90% of all GLSs, and we could better estimate the action of this single GLS. Different dosages of BM leaf flour, containing 200 to 300, 350, 400, 450, and 650 μmol m^{-2} of sinigrin, were inserted into soil already infected by *Meloidogyne* spp. for evaluating their effects on tomatoes grown in cold greenhouses in comparison to absolute control (CTRL) and to the chemical one, Vydate 5G® (CCTRL). The root disease index, caused by nematode attack, was the highest in CTRL, and a reduction of about 50% was observed with the 300 to 650 μmol m^{-2} sinigrin dosage. The CCTRL showed twofold marketable yield increase, and a fourfold increase was found in 650 μmol m^{-2} of sinigrin dosage, in comparison to the CTRL. Biofumigant applications improved tomato plant growth and development, and fruit quality, significantly for dry matter and soluble sugars (°Brix). BM leaf flour inserted into the soil, at a dose of 300 μmol m^{-2} of sinigrin, showed similar effects to the CCTRL on root disease index, root weight, and marketable yield. Data showed the nematotoxic effect of sinigrin for the biocontrol of *Meloydogine* spp. by the use of *B. macrocarpa* leaves, very rich in this GLS compound, which represents a new tool for agroecological soil management and for organic farming.

Keywords: biofumigation; *Brassica macrocarpa*; sinigrin; nematodes; sustainable agriculture

1. Introduction

The tomato (*Solanum lycopersicum* L.) is the most economically important vegetable crop in the world, and its cultivation covers about 5 million hectares, producing about 182 million tons. Italy is the main tomato producer in Europe with about 100,000 Ha, whereas economically important production is concentrated in cold-greenhouse agrosystems (7229 Ha), of which about 50% production is concentered in Sicily [1,2].

In cold-greenhouse agrosystems, nematodes cause serious phytosanitary problems, and losses in tomato production have been associated with pests and diseases, such as *Meloidogyne* spp.

root-knot nematodes [3,4]. During the last few decades, several chemical pesticides have been utilized for controlling soilborne pests and diseases, increasing human and animal health risks as a consequence of their extreme toxicity as air, soil, and groundwater pollutants [5]. Moreover, restriction limits for conventional pesticide products with high environmental impact were outlined by Directive 2009/128/EC, strongly encouraging the use of natural products for plant management and protection in parallel with well-known agrotechniques for limiting soilborne diseases and increasing soil organic matter [6]. Alternative soil management with natural compounds or biocontrol agents for controlling pests and diseases in environmentally friendly farming systems, as well as in conventional ones, enables farmers to avoid and/or reduce the use of chemical pesticides [7]. Among several sustainable agro-echniques proposed for controlling soilborne-disease soil solarization, grafted plants and biofumigation seem interesting, but none could currently be a solution if not combined with different techniques [8]. Matthiessen and Kirkegaard [9] used the term 'biofumigation' to describe the process of growing, macerating, and incorporating *Brassica* species into the soil, leading to the release of isothiocyanate compounds (ITCs) through the hydrolysis of glucosinolate (GLS) compounds contained in plant tissue [10]. This could result in a suppressive effect on a range of soilborne pests and diseases without lingering on products [3]. GLSs are thioglucosidic secondary metabolites that are mainly present in the *Brassicaceae* and *Capparidaceae* families that, after hydrolysis by myrosinase, produce ITC derivatives with a chemical structure similar to soil fumigants with nematotoxic action [11]. The biofumigant effects of *Brassicaceae* biomass on *Meloidogyne incognita* [12,13], *Meloidogyne chitwoodi* [14,15], and *Meloidogyne javanica* [16], have been extensively reviewed [17,18]. In vitro studies showed interactions between pathogens and their sensitivity to different ITCs [19], and that they require different biofumigants for their effective control. Commonly used biofumigant compounds present in several *Brassica* species are represented by several GLSs that release different ITCs [20,21], of which the isothiocyanate allyl seems to be the most active against several pests and diseases [8]. Although some *Brassicaceae* species showed an articulated GLS profile, some of them presented prevalent GLS [22]. Within each *Brassica* species, different cultivars or plant organs may also contain different GLS amounts or profiles [23]. *Brassica* species, such as *Brassica juncea*, *Raphanus sativus*, and *Eruca sativa*, have already been investigated in previous studies, and they have been used as biocide plants for soilborne disease control [15,24,25]. Among *Brassicaceae* species, we have paid attention to *Brassica macrocarpa* (BM) because it has a high concentration of sinigrin in comparison to other species, with about 90% of its GLS profile represented by sinigrin [26]. It is an endemic wild Sicilian *Brassica* relative, present only in the islands of Favignana and Marettimo [27], where its populations are widespread. It has recently disappeared from Levanzo (Figure 1), and conservation and enhancement strategies have been activated [27,28].

BM grows on rocky cleaves, and is endangered by human activity and animal grazing. All three islands of the Egadi archipelago have been monitored for collecting information about the sites and the structure of its populations, such as number of individuals, age, and amount of seeds produced per plant [27]. This perennial wild species has also been used in a breeding program with the aim of increasing aliphatic GLSs in broccoli breeding lines [29], especially glucoraphanin, to provide high amounts of the isothiocyanate sulforaphane, which is the most interesting ITC involved in cancer protection in mammals [26,30,31]. The present work aimed to evaluate the biofumigation effects of BM flour for soil biofumigation by increasing the dose of sinigrin inserted into the soil for controlling root-knot nematodes (*Meloidogyne* spp.) in tomato crops, in comparison to the absolute control (CTRL) and chemical control Vydate 5G® (CCTRL).

Figure 1. Wild *Brassica macrocarpa* Guss. in the island of Egadi.

2. Materials and Methods

2.1. B. Macrocarpa Characterization and Production

B. macrocarpa Guss. seed samples were provided by the Di3A (University of Catania, Italy) ex situ active *Brassica* collection (code accession UNICT 3253). Seeds were sown during the second week of August 2015 in alveolar trays utilizing a peat-based substrate, and placed in a cold greenhouse. The four-leaf-stage plants were transplanted after 60 days from sowing in a cold greenhouse at the experiment farm of Catania University, with a crop density of 2.5 plants m^{-2} (Figure 2). BM basal leaves were collected in February 2016, at the beginning of its reproductive phase, and in March 2016. All harvested leaves were immediately weighed and dried in a ventilated oven at 40 ± 2 °C for about 2–3 days and milled to obtain its flour, fine enough for inserting into the soil. In order to determine sinigrin content, an aliquot of fresh leaves was immediately frozen in liquid nitrogen for GLS analysis.

At the end of April 2016, at the beginning of the seed-dispersal phase, we registered the main biomorphological descriptors (plant height, total number of leaves and ramifications, leaf form, and number of siliques per plant) and seed yield on 50 BM plants. BM flour was divided into five samples for each agronomic replicate for qualitative–quantitative GLS determination by HPLC.

Figure 2. *Brassica macrocarpa* Guss. cropped in cold greenhouse for biofumigant production.

2.2. Glucosinolate Quantitative Analysis

The amount of sinigrin was determined in μmol $g^{-1d.w.}$ on the basis of the EU official ISO 9167-1 method [32] with some modifications [33]. The GLS extraction protocol was carried out using 100 mg of the subsample. It was dissolved in 3 mL 70% ethanol at 70 °C for a few minutes in water-bath heating, vortexed, and subsequently centrifuged for 10 min at 4000 rpm. The supernatant was collected in 15 mL falcon tubes and stored; the pellet was subjected to another cycle in 70% ethanol in order to recover as many GLSs as possible. The obtained supernatants were collected and stored at −20 °C. The extracted GLSs were then converted into desulfo-GLSs by using a microcolumn (BIORAD) as described below: adding 1 mL of resin DEAE e A-25 Sephadex (CAS Number 12609-80-2, Sigma Aldrich, MO, USA) previously conditioned at pH 5.6 in a 25 mM acetate buffer into the column; the column was washed 3 times using 1 mL 25 mM acetate buffer; 1 mL of the sample extract was slowly added in the buffer acetate, prepared, and then washed again; 100 μl of a 0.5% solution of *Helix pomatia* sulfatase type H-1 (Sigma–Aldrich Chemie, Taufkirchen, Germany) was added, and the column was placed at 25 ± 1 °C for 16 h; 3 mL of deionized water was added in the column, and the eluent was finally collected from the column and stored at −20 °C.

Afterwards, analysis was performed with a HPLC Agilent 1200 by Diode Array detector set at a wavelength of 229 nm [32], while the pump LS type was set to a flow rate of 1.0 mL min^{-1}; the used column type was a 250 × 4 mm Lichrospher conditioned at 30 °C.

2.3. Tomato Experiment Field

The experiment trial was carried out at Scoglitti (8 m a.s.l., latitude: 36°53′0″ N, longitude: 14°25′0″ E) on the southern coast of Sicily (Scoglitti, Italy), at the largest concentration of greenhouses in Italy devoted to tomato production. The trial was conducted in a representative farm, in a cold greenhouse characterized by a concrete and wood structure covered with polyethylene film. This type of soil, and the tomato monoculture growing system usually adopted, favors the diffusion of nematode attacks in cold greenhouses in Sicily during the spring and summer seasons. The soil was represented by a

medium loamy sand type consisting of 13.5% clay, 20.8% silt, and 65.7% sand, with 1.6% of organic matter and pH 6.2 ($CaCl_2$).

2.4. *Sinigrin Effect for Controlling Root Knot Nematodes* (Meloidogyne spp.)

The effectiveness of BM flour, and then of sinigrin, was tested for controlling root-knot nematodes (*Meloidogyne* spp.) in a tomato greenhouse by using the cultivar 'Ikram F_1' (Syngenta) susceptible to them but resistant to Fol: 0-1 (*Fusarium oxysporum f*.sp. *Lycopersici* race 0,1), to ToMV: 0-2 (*Tomato Mosaic Virus* strain 0-2), to Va: 0 (*Verticillium* albo-atrum race 1), and to Vd: 0 (*Verticillium dahliae* race 1). The experiment field was established by adopting a completely randomized block experiment design with four replicates. The experiment factor was represented by the sinigrin dose inserted by the BM flour, previously titrated, ranging from 0 (CTRL) to 200, 300, 350, 400, 450, and 650 µmol $g^{-1\ d.w.}\ m^{-2}$ of sinigrin. CCTRL was represented by the Vydate 5 G® (Dupont) fumigant based on Oxamyl 5%, utilized at the dose suggested by the company. Ten days before transplanting and at the end of the growing cycle, soil samples were collected for estimating the root disease index for nematodes in the laboratory according to Cobb's decanting and sieving method [34]. In particular, before transplanting, we fractioned the greenhouse area in 24 sections from which we collected soil samples in a zig-zag pattern as a soil probe until 20 cm deep. Samples were kept in a cool bag until their delivery to the laboratory. At the end of the growing cycle, three soil samples were analyzed for each thesis, corresponding to the studied sinigrin doses.

After that, we inserted BM flour into the first 10 cm of soil at the above doses, whereas we inserted nothing for the CTRL, and the recommended dose of Vydate 5 G® (6.5 g m^{-2}) for the CCTRL. The tomato seedlings were transplanted after 10 days along single rows, adopting a crop density of 3.3 plant m^{-2}; each replicant was represented by 15 plants. The plot size was 4.5 m^2. Thirty days from transplanting, all previously used sinigrin doses were utilized again, burying them about 10 cm deep into the soil in parallel grooves to the rows near the root system. The plants were grown with a single stem, removing secondary shoots, and pruning it above the fifth cluster. The plants were drip-irrigated with an ordinary schedule. A microflow drip-irrigation method was used with dripping wings and distributors giving 2 L h^{-1}, spaced 20 cm along the row. The nutritional requirements were satisfied by a fertigation system coupled with an electronic timer, scheduled on the basis of evapotranspiration values by Penmam–Monteith [35]. The ratio of $N:P_2O_5:K_20$ was 1: 0.4: 1.8 as a whole.

In addition to the ordinary managements for tomato crop (tutoring, suckering, etc.), the root systems and canopy were also monitored in order to observe any signals of attacks attributed to root-knot nematodes.

2.5. *Root Nematode Infestation and Fruit Yield and Quality*

At the end of the growing cycle, 140 days after transplanting, the fresh weight of the root system was recorded for all plants grown in the cold greenhouse. The efficacy of sinigrin treatments was determined by calculating the Disease Index (DI) on the root systems in relation to nematode infection. Referring to nematode attack, the root system was inspected for galls according to a rating scale of 0–5, taking into account gall size and the attacked surface of roots [36].

The DI was calculated as the weighted average of root infestation, rated according to a 0–5 scale: 0 = no galls—healthy plant; 1 = 1–5 galls—very slight damage; 2 = 6–20 galls—moderate damage; 3 = more than 20 galls—medium damage; 4 = root system reduced and root physiology altered by some large galls; 5 = root system completely destroyed [4,36].

The number and weight of the fruits were recorded for each cluster of 10 plants for each plot; the marketable fruits of the harvested ones were detected.

The fruits of the fourth cluster were analyzed for quality parameters: single fresh fruit weight (g); dry-matter content (g 100 $g^{-1\ f.w.}$); color (a*/b*), measured by 'Minolta Chroma Meter CR 400' (Minolta Camera Co. LTD, Osaka, Japan); a* and b* are the Commission International d'Eclairage (CIE) chromatic coordinates that indicate, respectively, the red–green and yellow–blue components. On the

homogenized pulp, soluble solids (SS, °Brix), measured with a digital refractometer (DBR95, Geass, Torino, Italy) and pH were read with a glass electrode pH meter.

2.6. Statistical Analysis

Data were analyzed to Barlett's test for variance homogeneity, and then analysis of variance. Means were statistically separated on the basis of Duncan's test when the F test of analysis of variance for treatment was significant at least at the 0.05 probability level. Significance was accepted at the $p < 0.05$ level. Principal component analysis was performed on the raw data without any prior scaling in order to avoid the generation of a singular correlation matrix, and the highest correlating variables (Pearson's $R > 0.95$) were removed from the model [37].

3. Results

3.1. B. Macrocarpa *Characterization and Production*

BM reached the reproductive phase after 120 days from transplanting. The main morphological and chemical plant descriptors registered after 180 days from transplanting are presented in Table 1, showing a very vigorous plant in comparison with wild ones, which are smaller in natural environmental conditions [38]. The plant after 6 months from transplanting was about 53 cm high, with about 5 secondary branches and about 50 leaves, producing about 11.0 g of seeds and a leaf yield of 473 g (Table 1). The BM leaves collected at 120 (February 2016) and 150 days (March 2016) after transplanting provided a total yield of 1183 g m^{-2} fresh leaves, with a dry matter content of 13.4 g 100^{-1} f.w.. The sinigrin amount was 5.4 ± 0.02 µmol g^{-1} d. m. for the leaves dried in the oven, and 5.9 ± 0.02 µmol g^{-1} d.m. for the freeze-dried leaves. To incorporate the BM flour into the soil to obtain the different dosages, we took into account the sinigrin concentration of the leaves dried in the oven, which represents a faster and easier biofumigation method to transfer to farmers for their own production of biofumigants.

Table 1. Biomorphological characterization of *Brassica macrocarpa* Guss. after 180 days of growth (n = 50 plants).

		IBPGR and UPOCV Descriptors [27]
Plant height (cm)	53.4 ± 7.6	65.3
Number of secondary branches (plant^{-1})	5.2 ± 2.0	7.0
Number of leaves (plant^{-1})	49.6 ± 3.1	50.6
Leaf-blade width (cm)	11.3 ± 0.9	5.7
Leaf-blade length (cm)	30.0 ± 4.6	26.3
Petiole length (cm)	13.6 ± 2.7	24.4
Number of siliques (plant^{-1})	50.0 ± 7.0	
Number of seeds (silique^{-1})	10.0 ± 1.4	
Seed yield (g plant^{-1})	11.0 ± 1.5	
Fresh-leaf yield (g plant^{-1})	473 ± 35	
Dry-matter content (g 100^{-1} f.w.)	13.4 ± 0.6	
Dry-leaf sinigrin content (µmol g $^{-1}$ d.m.)	5.4 ± 0.05	
Fresh-leaf sinigrin content (µmol g $^{-1}$ f.w.)	5.9 ± 0.06	

3.2. Root Nematodes Infestation and Fruit Yield and Quality

The larvae number of second age of *Meloidogyne* spp., expressed per 100 cm^3 of soil, was 1416 ± 50.2 in the soil samples collected before transplanting (T0), whereas at the end of the growing cycle (T1), it was about 1250 ± 35.4 for CTRL and 385 ± 15.2 for CCTRL. The sinigrin dose of 300 µmol g^{-1} d.w. showed the same number of larvae registered for CCTRL and the highest recorded dose of 650 µmol g^{-1} d.w. was about 100 ± 3.5, i.e., twelve times less than for the CTRL, and about four times for CCTRL (Table 2).

Table 2. Number of larvae of second age of *Meloidogyne* spp. (n. per 100 cm^3) at beginning (T0) and end of productive cycle (T1).

Sinigrin Dosage	Larvae of Second Age	
	T0	T1
CTRL	1416 ± 50.2	1250 ± 35.4 [a]
CCTRL		385 ± 15.2 [b]
200		1100 ± 28.0 [a]
300		400 ± 11.3 [b]
350		370 ± 7.8 [b]
400		270 ± 3.8 [c]
450		180 ± 3.1 [d]
650		100 ± 3.5 [e]
Mean		507 ± 14.2
LSD ($p < 0.05$)		14.5
p		***

Values followed by different letters within each column were significantly different ($p < 0.05$) by Duncan's multiple range test ($n = 4$). *** Significant at $p = 0.001$ probability level.

The DI on the root surfaces, due to nematode disease, showed the highest values for CTRL; using BM flour rich in sinigrin led to attack reduction (Figure 1). The highest DI value of about 3.6 was observed for CTRL, which was reduced by about 50% for CCTRL. For all the used sinigrin doses, DI values were lower than those for CTRL (Figure 1). The DI was inversely correlated with root weight (Figure 1). In fact, the lowest DI determined the highest value of root weight, which was 64.4, 75.7, and 102.3 g $plant^{-1}$ for CTRL, CCTRL, and the highest tested dose of sinigrin, respectively (Figure 3; Figure 4). Even if the root system was significantly affected by sinigrin dosages, the registered values for biometric root parameters (number of clusters per plant and stem diameter) 60 days after transplanting to evaluate the effect of sinigrin on plant growth rate during the tomato growing cycle did not show any statistical differences in comparison to the CTRL. In particular, the number of clusters per plant was 4.6 ± 0.3, and stem diameter of 13.7 ± 0.1 mm on the mean of all treatments (Table 3).

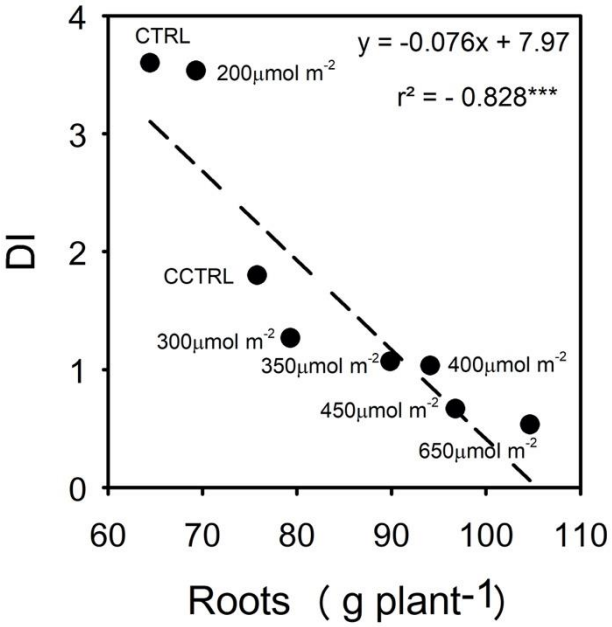

Figure 3. Correlation between root biomass production (g $plant^{-1}$) and nematode Disease Index (DI).

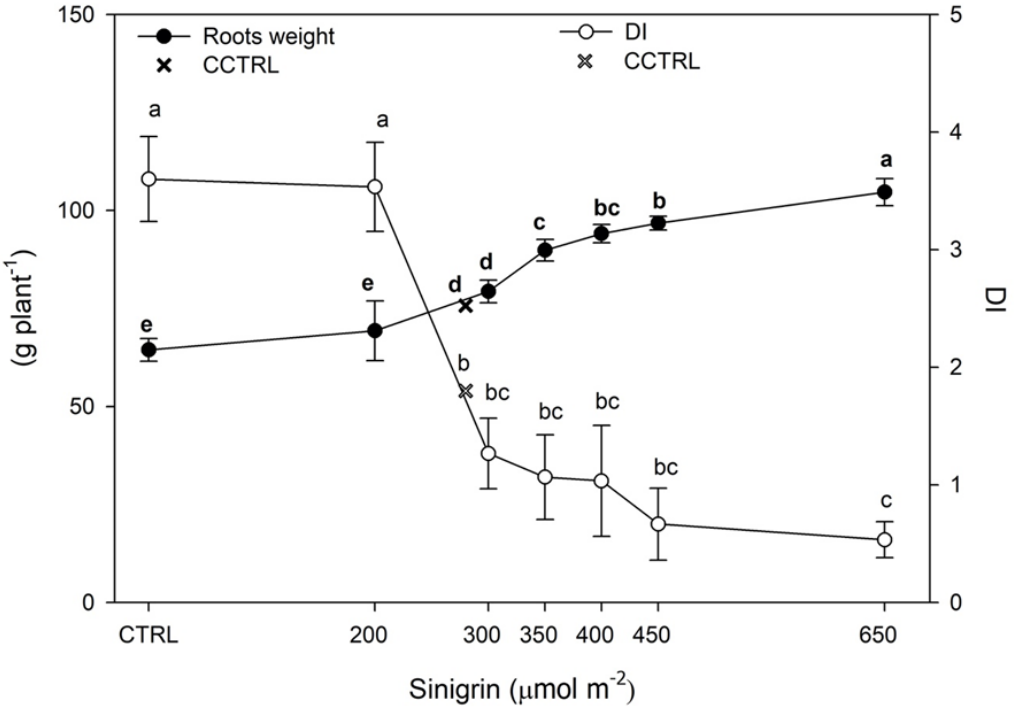

Figure 4. Recorded root weight (g plant^{-1}) and DI in relation to different sinigrin dosages. Different letters indicate statistical differences at $p < 0.05$.

Table 3. Number of fruits and clusters per plant (n.), fruit weight (g), and tomato fruit quality parameters in relation to sinigrin doses at productive-cycle end.

Sinigrin Dosage	Fruits	Clusters §	Fruit Weight	Chromatic Parameters (CIE)		Soluble Sugars	Dry Matter	pH
	(n)	(n)	(g)	(L) *	a/b	°Brix	%	
CTRL	13.9 ± 1.1 ^{e}	4.9 ± 0.5 ^{a}	64.5 ± 5.5 ^{b,c}	39.6 ± 3.9 ^{a}	1.69 ± 0.26 ^{a}	7.1 ± 0.2 ^{a,b}	7.8 ± 0.1 ^{b,c}	5.9 ± 0.5 ^{a}
CCTRL	26.2 ± 0.2 ^{d}	4.5 ± 0.1 ^{a}	67.8 ± 2.3 ^{a,b}	39.9 ± 1.7 ^{a}	1.90 ± 0.18 ^{a}	6.8 ± 0.1 ^{b}	7.7 ± 0.3 ^{c}	6.4 ± 0.3 ^{a}
200	15.3 ± 1.2 ^{e}	4.6 ± 0.2 ^{a}	63.7 ± 1.9 ^{b,c}	36.9 ± 1.9 ^{a}	1.50 ± 0.20 ^{a}	7.3 ± 0.1 ^{a,b}	8.2 ± 0.3 ^{a,b,c}	5.7 ± 0.5 ^{a}
300	30.2 ± 0.3 ^{c,d}	4.5 ± 0.3 ^{a}	74.3 ± 2.6 ^{a}	40.6 ± 3.2 ^{a}	1.58 ± 0.21 ^{a}	7.3 ± 0.5 ^{a,b}	8.3 ± 0.4 ^{a,b,c}	6.5 ± 0.4 ^{a}
350	40.1 ± 0.7 ^{c}	4.4 ± 0.2 ^{a}	59.1 ± 2.8 ^{c}	38.9 ± 2.6 ^{a}	1.51 ± 0.20 ^{a}	7.5 ± 0.4 ^{a}	8.6 ± 0.5 ^{a,b}	6.7 ± 0.3 ^{a}
400	38.5 ± 1.7 ^{c}	4.6 ± 0.1 ^{a}	62.6 ± 5.6 ^{b,c}	37.3 ± 3.0 ^{a}	1.59 ± 0.30 ^{a}	7.4 ± 0.5 ^{a,b}	8.5 ± 0.3 ^{a,b,c}	5.9 ± 0.2 ^{a}
450	44.1 ± 1.7 ^{b}	4.6 ± 0.2 ^{a}	70.1 ± 4.9 ^{a,b}	39.4 ± 4.2 ^{a}	1.75 ± 0.32 ^{a}	7.3 ± 0.2 ^{a,b}	8.3 ± 0.2 ^{a,b,c}	6.1 ± 0.2 ^{a}
650	54.3 ± 1.0 ^{a}	4.6 ± 0.1 ^{a}	66.0 ± 3.6 ^{b,c}	39.0 ± 2.9 ^{a}	1.72 ± 0.34 ^{a}	7.5 ± 0.4 ^{a}	8.8 ± 0.5 ^{a}	6.3 ± 0.3 ^{a}
Mean	32.8	4.6	66.0	38.9	1.65	7.29	8.29	6.19
LSD (p <0.05)	6.96	0.43	6.78	5.27	0.45	0.58	0.80	0.96
p	***	ns	**	ns	ns	*	*	ns

Values followed by different letters within each column were significantly different ($p < 0.05$) by Duncan's multiple range test ($n = 5$); § 60 days after transplanting; (***) (**) (*) Significant at $p = 0.001$; 0.01 and 0.05 probability level, respectively; ns = not significant.

The marketable fruit yield ranged between 890 and 3580 g plant^{-1} for CTRL and CCTRL, respectively (Figure 5). Treatment at 300 µmol m^{-2} of sinigrin increased marketable fruit yield in comparison to CCTRL (Figure 5). The high correlation index (r^2 = 0.898***) indicates the highest dose of sinigrin leading to fourfold yields compared to the CTRL. The lowest dose of sinigrin had the same results for CTRL. The effect of sinigrin dose began to be visible starting from 300 µmol m^{-2}, and it was stable until 450 µmol m^{-2}. The marketable-yield increase was more positively correlated with the number of fruits per plant produced than fruit weight, probably due to the biofumigated healthy root system of the plant that improved the fruit setting (Table 3). Concerning to the number of fruits per plant, which were on average 32.8, value ranged from 13.9 for CTRL to over 50 for the dose of sinigrin of 650 µmol m^{-2}. Fruit weight varied from 59.1 ± 2.8 g to 74.3 ± 2.6 g for the 350 and the 300 µmol m^{-2} doses of sinigrin $^{-2}$, respectively, and it was not different from CTRL, which was 64.5 ± 5.5 g (Table 3).

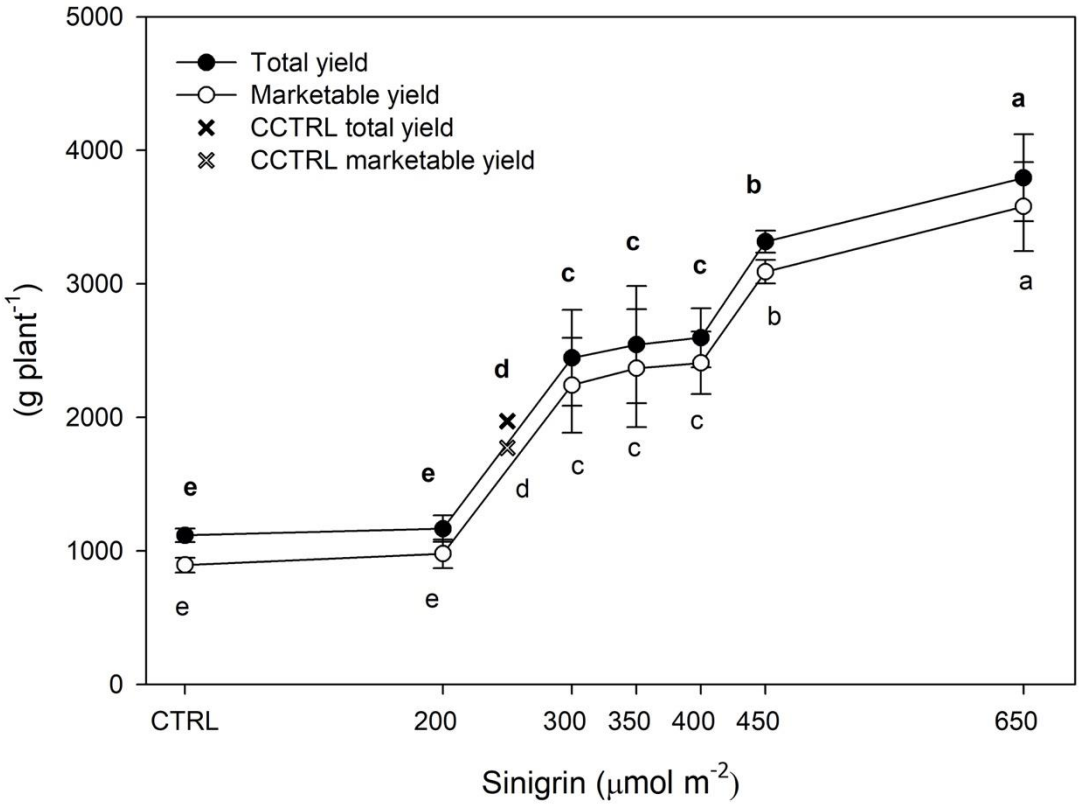

Figure 5. Total and marketable yields of tomato crop (g plant^{-1}) in relation to different sinigrin dosages. Different letters indicate statistical differences at $p < 0.05$.

3.3. Fruit-Quality Parameters

The effect of treatments on fruit quality is reported in Table 2. Color parameter L* and a*/b* ratio were not affected by sinigrin, with mean values of 38.9 and 1.65, respectively. The biofumigant treatments affected fruit quality in terms of dry matter (DM), which positively influenced the soluble sugar content (°Brix). The dry matter was 8.3 g 100 g^{-1} $^{f.w.}$ on the mean of all treatments, while we registered the lowest values for CTRL and CCTRL, 7.8 and 7.7 g 100 g^{-1} $^{f.w.}$, respectively. By increasing the sinigrin dose, fruit DM increased by about 1%, reaching the value of 8.8 g 100 g^{-1} $^{f.w.}$. The soluble sugar value ranged from 6.8 ± 0.1 to 7.5 ± 0.4 °Brix, with an average value of 7.29 ± 0.28 °Brix (Table 3).

4. Discussion

Brassica macrocarpa leaves showed a good amount of sinigrin, suggesting that this species, among other *Brassicacea* species with nematotoxic activity, could be utilized as biofumigant for environmentally friendly farming systems. Inserting different amounts of dried leaves and obviously different doses of sinigrin into the spoil affected DI, root weight, marketable yield, and fruit quality in greenhouse tomato production. The sinigrin dose of 300 µmol m^{-2} showed similar values for roots weight (g plant^{-1}) and DI as those ascertained for CCTRL, reducing DI and increasing root weight for the 650 µmol m^{-2} sinigrin dose.

The marketable fruit yield was correlated with the fourfold DI yield for the highest utilized dose of sinigrin. Several authors have studied the effects of *Brassicaceae* spp. for its possible use as biofumigant [15,39–41], but no data are available related to the amount of sinigrin inserted into the soil by green manure or by seed-oil meal, and to the related effects on tomato-fruit quality of the most important crop grown in greenhouses in Italy.

Nematode attacks in greenhouses are a real threat for farmers in Italy, especially in the southern coastal area of Sicily, where 70% of Italian tomato production is concentered. Several authors evaluated

the effective nematocidal control of biofumigants on crops grown in pots [21,25,42,43]. In order to provide a useful applicative tool for farmers, we focused mainly on marketable fruit yield and tomato health in relation to the sinigrin dose inserted into the soil. Previous studies showed a biotoxic effect of sinigrin applied at transplanting or after one week on tomato growth, so we chose to use this biofumigant two weeks before transplanting. However, the adopted protocol for monitoring the number of larvae in each treatment was not so useful considering nematode motility in relation to soil temperature, water availability, and other variables. In any case, the effect of sinigrin was clear at the end of the growing cycle (end of July), as the number of larvae of second age of *Meloidogyne* spp. per 100 cm^3 into the soil was significantly reduced at the highest utilized dose (lower than 100 larvae using 650 μmol m^{-2}). Our results are in line with Avato et al. [17], who reported that GLS use as biofumigants in potatoes, tomatoes, and grapevines can be an effective tool for the sustainable management of phytoparasitic nematodes. Soil biofumigation with fresh or dry brassicaceous biomasses can be particularly useful in both organic and conventional agrosystems, but it is strictly related to GLS amount and profile. The same results were obtained by [24], using *B. juncea* granulated seed meal for controlling *Melolontha* spp. Grub mortality was significantly dependent on applied GLS concentration with the granulate. In tomato plants, defatted seed meal from *B. carinata* showed good results in terms of efficiency in controlling *Meloidogyne incognita* and agricultural environmental impact. Therefore, GLS application should also be considered as a potential alternative tool for controlling nematodes as it could improve soil biodiversity, offering a potential alternative to chemical fumigants [6]. The percentage of reduction of *M. javanica* larvae into the soil amended with mustard enhanced the quality of tomato fruits, such as total sugars, total amino acids, and total phenols [16]. Daneel et al. [42] tested two CVs of different species of *Brassicaceae* (*Eruca sativa*, *Brassica juncea*, and *Raphanus sativus*) as biofumigants against *M. incognit*a *and M. javanica* in tomato and potato crops. The authors found that none of the *Brassicaceae* biofumigants used for potato crop significantly reduced the population levels of *M. incognita,* whereas some effects were ascertained for tomato crops in relation to used CVs. Our results agree with the positive effect of *Brassicacea* species used as biofumigants even if there are no indications for the amount and the profile of the GLSs of the used plant materials. *Brassicaceae* species are also incorporated as cover crop, but the obtained results could be related to the increase of beneficial nematode assemblages in soils treated with it. It is important to keep in mind that a significant proportion of plant GLSs can persist unhydrolyzed in the soil for several days after *Brassicaceae* biomass incorporation into the soil. Non-ITC liberating GLSs (predominately indolyl GLSs) from rape (*Brassica napus*) and Ethiopian mustard (*Brassica juncea*) were found at lower concentrations than ITC-liberating GLSs, but tended to persist longer in the soil [22]. This means that the time used for the natural decomposition process and the chemical transformation to reactive nematicidal molecules acts as a controlled release translated to a longer residual nematicidal activity under field conditions. Moreover, the risk of development of nematode resistance is relatively very low since the raw material used for soil amendment is a complex cluster of chemically different nematocidal components [8].

5. Conclusions

The use of *Brassica macrocarpa* as a biofumigant against nematodes produced a significant stimulant effect on the roots of tomato plants, which was reflected on tomato fruit yield and its qualitative characteristics. Different authors studied *Brassica* spp. for its potential use as a biofumigant but no data are available for *B. macrocarpa* use with regard to the amount and profile of GLSs incorporated into the soil, and very few reports are related to analysis of fruit-quality characteristics in relation to used biofumigant biomasses.

Our activities indicate the high potential of *B. macrocarpa* for the development of new nematocidal fumigant formulations, sustainable for the environment and human health. In addition, our results suggest that the biocidal activity largely depends on GLS profile and inserted dose into the soil, and show the effects of their use on root weight and nematode disease index. The highest tomato marketable yield and fruit quality were obtained at the highest sinigrin dose we utilized (650 μmol m^{-2}).

Incorporating the dry-leaf flour of *B. macrocarpa* into the soil before the establishment of the susceptible nematode crop is an effective and easy practice to control this pest, which could easily be transferred to farmers for dissemination.

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