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Bio-Management of Root-Knot Nematodes on Cucumber Using Biocidal Effects of Some *Brassicaceae* Crops

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Abstract: Biofumigant and crop sequencing are effective and safe control system activators that maintain soil fertility and reduce pest populations. The study goals were to find new pesticide-free therapies for root-knot nematode management on cucumbers to maintain high yields and protect the environment and human health. In 2018 and 2019, the research employed a fully randomized block design under field conditions with five treatments and control: two bio- fumigants, cultivation of cucumber after broccoli plantlets incorporation (BPI) and radish plantlets incorporation (RPI), two crop sequence treatments (cultivation after broccoli (BCS) and radish (RCS), and nematicide treatment). Cucumber cultivation after BPI treatment exhibited the best horticultural traits, which reflected positively on early and total productivity. The increased yield was gained by suppressing all nematode parameters, the number of nematode larvae, galls, and egg masses, as well as egg hatching reduction. The most effective biocides, total phenols, myrosinase activity, total glucosinolates (GSLs), and isothiocyanates (ITCs) in brassica crops were estimated for their pesticide properties. The highest amount was released with BPI treatment, compared to adult plants and radish in its two stages. The bio-managed treatments revealed superior effectiveness compared to nematicide application and control to suppress the nematode population while enhancing cucumber growth and production.

Keywords: biofumigant; crop sequence; cucumber; root-knot nematode; myrosinase; glucosinolates; sothiocyanate

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1. Introduction

Cucumber is a major vegetable crop in Egypt. It belongs to the *Cucurbitaceae* family. It is a cash crop that the farmer depends on for immediate profit. Soil-borne diseases, especially root-knot nematodes (*Meloidogyne* spp.), cause economic losses in the cucumber crop. Uncontrolled root-knot nematode numbers will ultimately degrade crop yield and quality. Root-knot causes plant fragility and degradation, leading to quantitative and qualitative crop losses [1,2]. Chemical control, such as pesticide treatment, is the simplest and quickest technique for nematode management. While this approach is quite successful, it is costly, as well it creates an imbalance in the environment. Environmentally acceptable methods of nematode control in food crops have become required and important. Thus, efforts to increase the food supply must be oriented toward producing a percentage of safe and healthy food [3]. In a sustainable agricultural system, integrated nematode management techniques

should comprise a variety of nematode control approaches, such as crop rotation and resistant cultivars. Unfortunately, these tactics are restricted because of *Meloidogyne* spp. host range, which makes developing a practical and successful cropping sequence challenging. It is vital to develop alternatives as a sustainable and integrated approach to nematode control in cash crops that result in nematode pathogen suppression and growth and yield enhancement via the use of biofumigants and crop sequence techniques in combination [4]. According to Youssef [5], biofumigation occurs when volatile pesticide-like chemicals are generated during the degradation of plant components. The biofumigation effects are a result of residues containing glucosinolate molecules. The toxicity is related to an enzyme that promoted the analysis of glucosinolates [6]. Glucosinolates are a group of chemicals that are decomposed into isothiocyanates that inhibit nematodes by interfering with their reproductive cycle [7]. The mechanism of action is based on glucosinolate degradation, comparable to the chemical fumigant nematicide metam sodium, which degrades into methyl isothiocyanate in the soil [5,8]. The crop sequence refers to the practice of growing many crops in succession on the same field, as opposed to monoculture, which includes the cultivation of the same crop repeatedly in the same area [9,10]. Crop sequencing information is used to regulate nematode populations and maintain them below a crucial threshold [5]. Brassicas have been shown to have nematode suppressive properties that help the succeeding crop in the sequence. Kirkegaard and Mathiessen [4] showed that sequential cucumber culture not only helps prevent nematode populations from reaching nematode economic thresholds but also aids in the management of other plant diseases and insect pests. Similar results proved that biocidal green manure crops can be employed as an agronomic strategy for root-knot nematode management in horticultural sequences [9]. In comparison to the chemical control oxamyl, biofumigant spray reduced nematode infection in tomatoes. Isothiocyanate is a decomposing product of GSLs that is similar to the metham sodium breakdown product, methyl ITC, in the soil [11,12]. The study aimed to manage the root knot nematode, *Meloidogyne* spp., by using two of the best and safest management strategies: biofumigation and crop sequencing. In addition, studying their effects on cucumber crop development and production is a viable method of sustainable agricultural farming.

2. Materials and Methods

This study was conducted during two successive seasons of 2018/2019 and 2019/2020 under field conditions on soil highly infested with *Meloidogyne* spp. at a private farm in Wardan village, Giza Governorate, Egypt. The experiment layouts were designed as randomized complete blocks, with three replications. The initial nematode populations were 12,350 and 11,700 s-stage juveniles per 250 g of soil in the first and second seasons, respectively. This study was conducted to study the efficiency of biofumigation treatment by broccoli and radish plantlet incorporation and crop sequence approach compared to nematicide application on cucumber, which was transplanted in a highly infested field with root-knot nematode.

2.1. Pre-Cultivation Treatments

On 15 December 2018, and 2019, seeds of broccoli (Centauro F_1) and radish cv (Balady) were directly dispersed in soil to determine the biofumigations effect. The plantlets were tilled into the surface layer of soil after only one month from germination. The beds were covered with polyethylene plastic shelters to encourage high decomposition and prevent volatile evaporation [4]. Regarding crop sequence effects, on 1 November 2018, and 2019, broccoli and radish seeds were directly sown. Broccoli seeds were sown in lines (7 m in length and 0.8 m in width). The space between seeds was 75 cm. Radish seeds were sown in six rows on beds (7 m in length and 1 m in width), spacing 5–7 cm between seeds. All agricultural practices were done as the Ministry of Agriculture's recommendation in Egypt for broccoli and radish production. The yields for broccoli and radish were harvested at a suitable time for commercial production. In addition, Nematicide Rugby treatment

nematicide (rugby, organophosphates group 10% Ebufos) with contact action was added to the soil with soil preparation as a granule at the rate of 5 g/m^2 and control plants were transplanted. All treatments were presented in three replicates.

2.2. Cucumber Cultivation

Seeds of cucumber hybrid F1 (Madaen, Skata Seed Company) were sown in an open field on 20 January 2018 and 17 January 2019. Plantlets were transplanted after 25 days into two rows in the bed (one 7 m in length and the other 1.0 m in width). The space between the plants was 50 cm. The experimental plot contained 15 plants/5 m^2. Common agricultural practices (irrigation, fertilization, and pest control) were carried out according to the Ministry of Agriculture's recommendation for cucumber production in Egypt.

2.3. Horticultural Characteristics Assessment

The traits were recorded on five randomly chosen plants from each replication: vgetative growth as the main stem length (cm), plant fresh weight (g), and leaf area (cm^2). The flowering character is expressed as the number of days until the first female flower anthesis. In addition, fruit characteristics such as fruit set percentage, fruit length (cm), fruit diameter (cm), and average fruit weight (g) were measured for 50 fruits from each plot, and the averages only were recorded. Early yield was determined as the weight (kg) and the number of fruits/plants harvested during the first two weeks of harvesting. Total yield was determined as the total weight (kg) and the number of harvested fruits /plants.

2.4. Nematode Parameters Assessment

The initial population density in the infested field was estimated in soil by modified Sieving and Bearman's plate technique [13].

Reproduction factor

$$\mathbf{RF} = \frac{\mathbf{FP\ (final\ population)}}{\mathbf{IP(initial\ population)}}$$

Cucumber plants were uprooted at the end of the season; their roots were rinsed with tap water and stained with acid fuchsine [14]. On stained roots, the number of galls, egg masses, and eggs per root system were counted. On the cucumber, root galling, egg masses, and fecundity (egg deposition) were counted to be utilized as measures of the broccoli and radish's effectiveness as biofumigants and in crop sequences. The reduction in root galling, egg mass, and eggs was evaluated using bioassay techniques in comparison to the control [15].

2.5. Chemical Compounds Assessment

Three plant samples were randomly taken from each replicate within the plots, with plantlets for biofumigants and adult roots for crop sequence treatments.

Total phenols content was determined using the Folin–Ciocalteu method. Plant extracts were prepared according to a standard protocol. Total phenols were determined by the method of Du et al. [16]. In an Eppendorf tube, 7.9 mL distilled water, 100 µL plant extract, and 500 µL Folin–Ciocalteu reagent (1:1 with water) were added and mixed. After exactly 1 min, 1500 µL of sodium carbonate (20 g/100 mL) was added, and the mixture was mixed and allowed to stand at room temperature and darkness for 2 h. The absorbance was read by a spectrophotometer at 765 nm. For the calibration curve, Gallic acid was used. Results were expressed as mg GAE/100 g FW.

To measure glucosinolates, the method described by Jia et al. [17] was used. The samples were prepared. The samples were overnight treated with 100 µL of 0.1% (1.4 units) aryl sulphatase to convert the glucosinolates into their desulpho analogs. The desulphoglucosinolates were eluted with 2 × 0.5 mL water. The HPLC system was used to perform the HPLC analysis consisting of a Waters 2695 separations module and a Waters 2996 photodiode array detector (Waters Corp., Milford, MA, USA). A Hypersil C18 column (100 × 4.6 mm × 5µm) was used with A, water at a flow rate of 1.0 mL/min and B, a mobile

phase of acetonitrile. The time program was 5% B: 0–1 min, 5–25% B: 1–4 min, 25–60% B: 4–5 min, 60–5% B: 5–6 min, 5% B: 6–8 min. An autosampler injected a sample (40-µL) into the column and the absorbance was detected at 226 nm. For the internal standard for the HPLC analysis, Sinigrin (Sigma St. Louis, MO, USA) was used. The comparison of retention time and quantified by peak area were used to determine desulphoglucosinolates. Brown et al. [18] reported relative response factors to correct absorbance differences between the standard and the other glucosinolates for calculating molar concentrations of individual glucosinolates. The glucosinolate concentration was expressed as µmol/g fresh weight (FW) of plantlets and roots for both broccoli and radish.

Isothiocyanates were assessed by incubating the samples at room temperature for 30 min. Then, the material was centrifuged at 4415× *g* for 4 min. The supernatant was collected in a separate tube and the pellet was re-extracted with 3 mL of buffer solution after shaking for 5 min and centrifuging at 4415× *g* for 4 min. The new supernatant was added to the collected supernatant and, 200 µL was mixed with 12 µL of 1-butanethiol reagent (99%) and incubated in a heating block at 50 °C for 2 h, shaking the tube from time to time. Then, 4 mL of methanol and 1.5 mL of the solution were relocated to the HPLC vial. Isothiocyanate conjugates were analyzed using an HPLC equipped with an X Bridge RP18 column (3.0 × 100 mm, 5 µm) with an injection volume of 10 µL and a flow rate of 0.4 mL/min. The tray temperature was 15 °C and the column oven temperature was 30 °C. Eluent A was 0.1% formic acid in Millipore water and eluent B was 0.1% formic acid in acetonitrile, the injection volume was 10 µL at 15 °C. The time program was 50% B: 0–6 min, 95% B: 6–7.1 min, and 50% B: 7.1–10 min. The estimation was conducted at the Center of Applied Research and Advanced studies in Cairo. Uni. Fac. of Pharmacy according to the protocol of Baenas et al. [19].

The samples were homogenized with 0.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) in an ice bath to determine the activity of myrosinase. The supernatants were used as crude enzymes after centrifugation at 15,000× *g* for 20 min at 4 °C. The protein amount in the crude enzymes was determined using the Bradford assay [20] using bovine serum albumin as a standard. The test was carried out with 1 mM sinigrin and 20 µL of supernatants in a total volume of 100 µL. After 15 min of incubation at 37 °C, the reaction was stopped by boiling at 100 °C for 5 min. The reaction mixture was diluted with methanol, and the concentration of the remaining sinigrin was measured using a spectrophotometer at 227 nm. One myrosinase unit was equivalent to 1 mM sinigrin transformed per minute. The specific activity was measured as units/mg protein.

2.6. Statistical Analysis

Analysis of the data was statistically done using analyses of variance (ANOVA) with the Stat Soft Statistical Package (MSTATC) software program (Michigan State University, East Lansing, MI, USA). The least significant difference (LSD) ($p \leq 0.05$) was used to determine the probabilities of significance among treatments and means according to Steel and Torrie [21].

3. Results

3.1. Effects of Biofumigants and Crop Sequence Approach on Horticultural Characteristics

3.1.1. Vegetative Characteristics

Data in Figures 1 and 2 indicated that cucumbers grown following the incorporation of broccoli plantlets had the longest main stem length, followed by treatment with nematicide. All treatments, with the exception of cultivation after radish, had better vegetative qualities than the control. When comparing cultivation after broccoli plantlet incorporation to control and other treatments, data on plant fresh weight indicated the most significant increase. Except for cultivation after radish, all treatments differed significantly from the control. There was a substantial difference between cultivation after BPI, nematicide treatment, and control when it came to leaf area measures. Both seasons' data were equivalent, with the best vegetative metrics coming from cultivation after BPI.

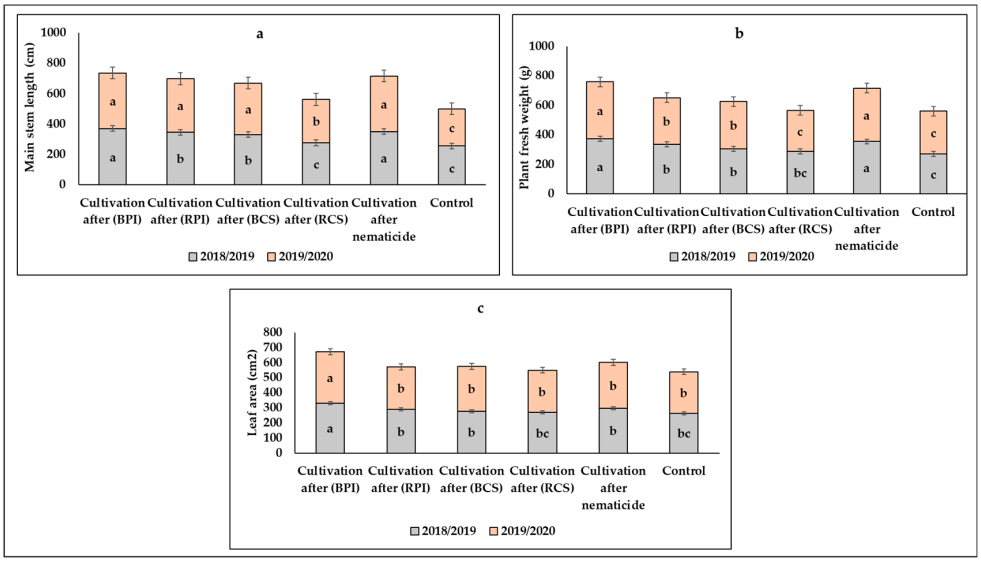

Figure 1. The effects of biofumigation, crop sequence, nematicide treatments compared with control on cucumber vegetative growth, in two seasons 2018/2019 and 2019/2020. ((**a**) Main stem length (cm), (**b**) plant fresh weight (g), (**c**) leaf area (cm^2)). Means (±SE) followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

Figure 2. *Cont.*

Figure 2. The effects of the incorporation of broccoli plantlets treatment on cucumber vegetative growth (**A**) and cucumber vegetative growth in control treatment (**B**).

3.1.2. Flowering and Fruiting Characteristics

The findings in Figure 3 revealed that all the treatments had a significant effect on the number of days until the first female flower opened in the first season. Furthermore, the significant differences in treatment were seen across all of this character's appearances in the second season. All treatments were statistically different from the control, with the exception of cultivation after radish, which was not. There were no statistically significant changes between cultivations after BPI and nematicide application treatments. Furthermore, with biofumigant treatments, the fruit set percentage increased significantly, especially after broccoli plantlets incorporation, which had a fruit set percentage of 78–75.5% for the first and second seasons, respectively. The chemical nematicide achieved a success rate of 76.4% and 72% in the first and second seasons, respectively. The three most critical fruit characteristics were estimated. The average fruit weight, for example, differs dramatically between treatments and controls. Additionally, no significant differences in fruit diameter and length characteristics exist between biofumigant and nematicide application treatments.

3.1.3. Yield and Its Components

Figure 4 shows that early and total yield as number and weight per plant differed significantly across all treatments. Despite the relevance, no significant variations were identified following the integration of broccoli and radish plantlets. Both treatments led to a large quantity of fruits and weight in the early and overall yield for cucumber. As a result, the yield values after plantlets integration were twice as high as the control values. Early yields with cultivation after broccoli plantlets incorporation were 0.696 and 0.720 kg/plant, respectively, compared to the control, which had 0.302 and 0.324 kg/plant in two seasons. In general, improved production was associated with the usage of *Brassica* crops as bio-fumigants.

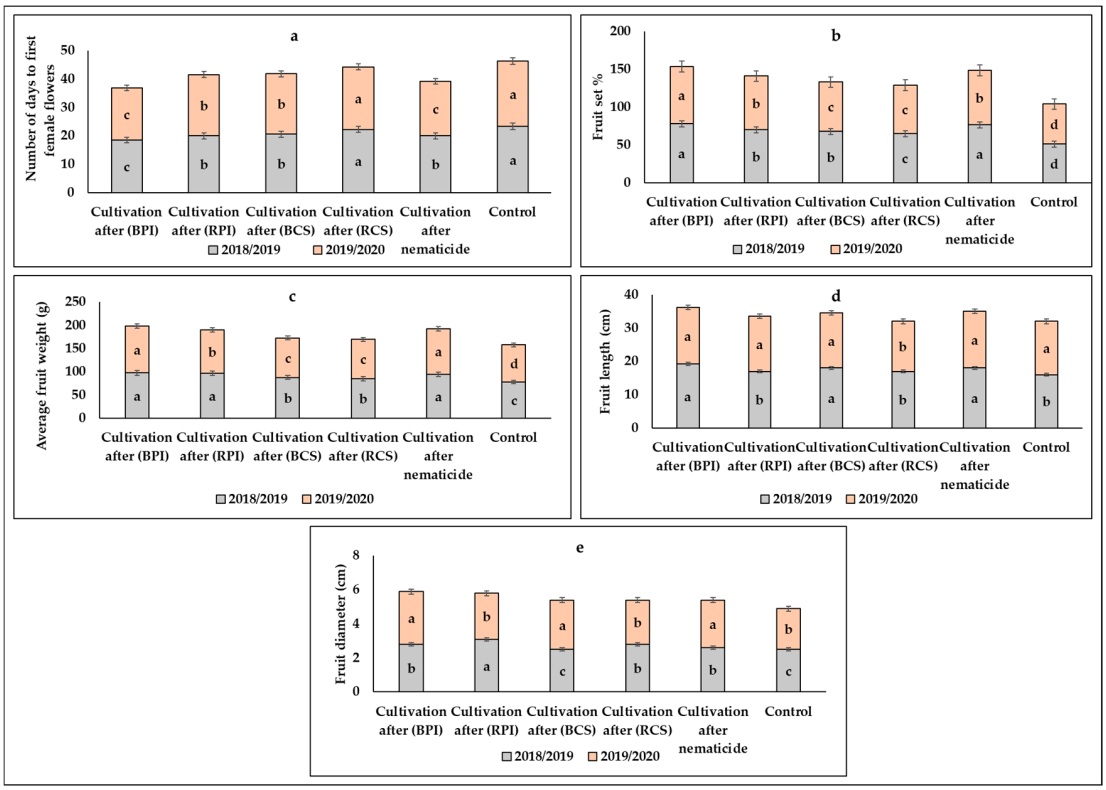

Figure 3. The effects of biofumigation, crop sequence, nematicide treatments compared with control on cucumber flowering and fruit characteristics, in two seasons 2018/2019 and 2019/2020. ((**a**) Number of days to first female flower, (**b**) fruit set %, (**c**) average fruit weight (g), (**d**) fruit length (cm), and (**e**) fruit diameter (cm)). Means (±SE) followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

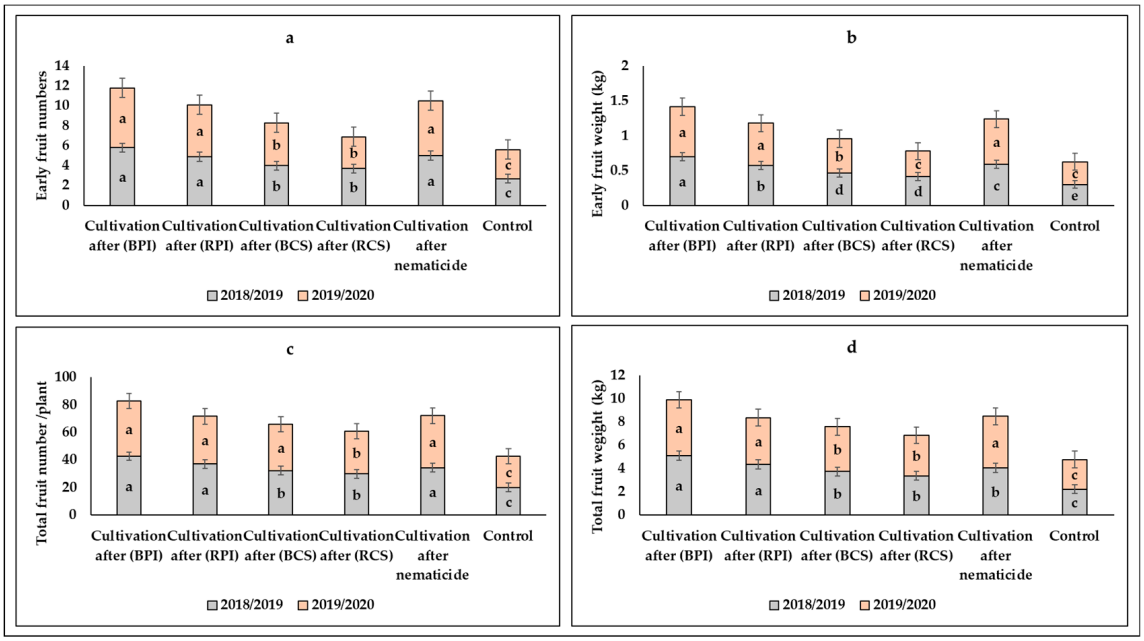

Figure 4. The effects of biofumigation, crop sequence, nematicide treatments compared with control on cucumber yield and its component, in two seasons 2018/2019 and 2019/2020. ((**a**) Early fruit numbers, (**b**) early fruit weight (kg), (**c**) total fruit numbers, (**d**) total fruit weight (kg)). Means (±SE) followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

3.2. The Effects of Bio-Fumigants and Crop Sequence Treatments on the Management of Root-Knot Nematode, Meloidogyne spp.

Figures 5 and 6 show significant differences in all nematode parameters with different treatments compared to the control. Control cucumber plants had the most infected roots, resulting in the greatest gall numbers of 245 and 197/plant root system during the first and second seasons, respectively. Gall number reduction was seen with all biofumigant and crop sequence treatments, particularly with cultivation following broccoli plantlets integration, where cucumber plants had the fewest gall counts of 52 and 48 galls/plant root system in two seasons, respectively. In regard to root-knot nematode control, there were no significant differences between nematicide administration and cultivation after BPI in any of the nematode parameters. For two seasons, nematicide application had the lowest egg mass numbers of control, biofumigant, and crop sequence. As for biofumigant treatments, superiority for cultivation after broccoli plantlets' inclusion was noticed. The findings revealed that biofumigant treatments reduced fecundity and that egg counts declined with cultivation after biofumigant treatments. In two seasons, egg deposition in control plants was 2925 and 2592 eggs/plant root system, respectively. The initial population numbers were 12,350/250 g soil for the first season and 11,700/250 g soil for the second season. The final population values for all treatments were substantially different. All treatments and control showed significant variations in nematode reproduction parameters. Cucumber cultivation after BPI had the lowest reproduction factor values compared to other biofumigants, crop sequence, and control treatments while there were no significant differences between BPI treatment and nematicide application treatment.

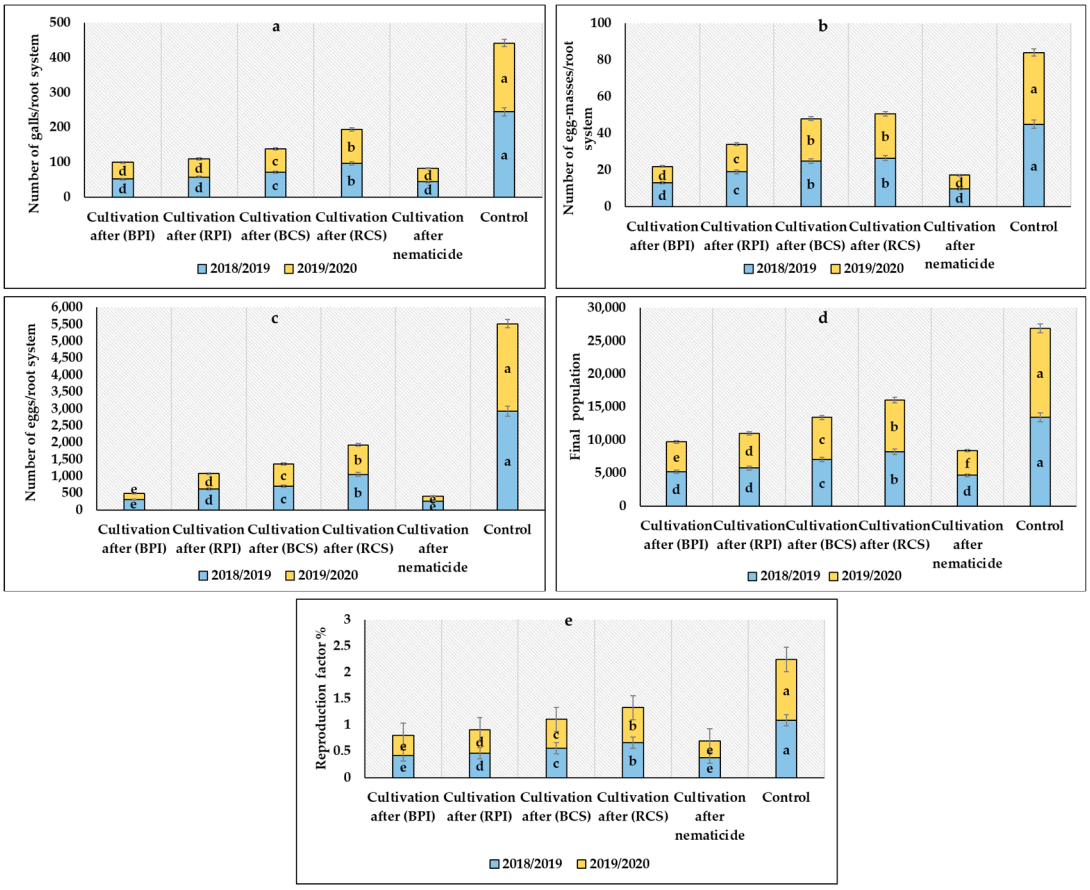

Figure 5. The effects of biofumigation, crop sequence, nematicide treatments compared with control on cucumber root systems in two seasons 2018/2019 and 2019/2020. ((**a**) the number of nematode galls, (**b**) egg masses, (**c**) eggs, (**d**) final population, (**e**) reproduction factor). Means (±SE) followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

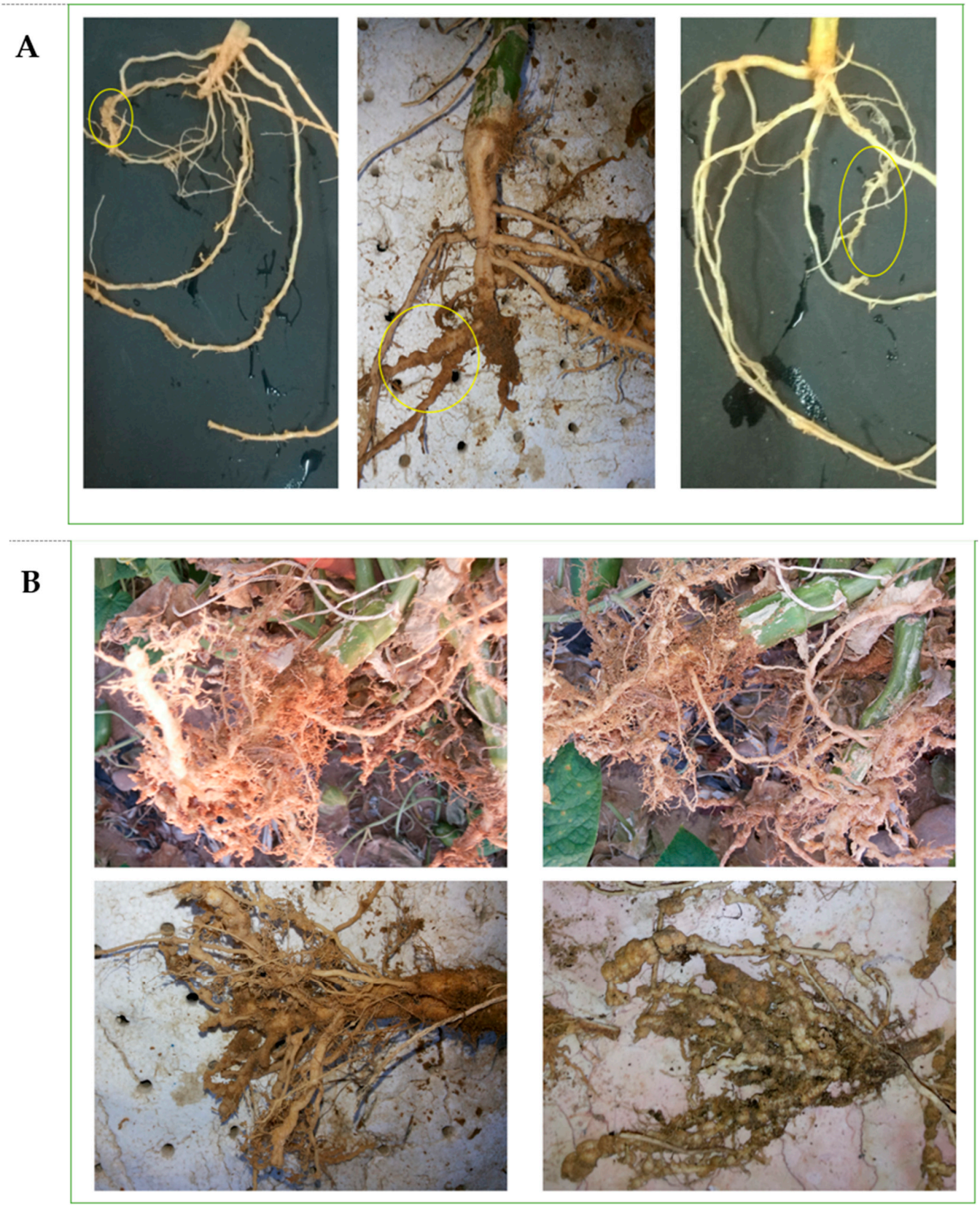

Figure 6. The effects of the incorporation of broccoli plantlets treatment on cucumber root systems (**A**) and the cucumber root systems in control treatment (**B**).

The data in Figure 7 demonstrated a reduction in galls, egg masses, and eggs with various biofumigant and crop rotation treatments used in addition to nematicide. All parameters were verified, while the gall, egg mass, and egg number reduction percentages increased to 77.37, 73.80, and 91.11%, respectively, with cultivation after broccoli plantlets inclusion. These results illustrate the effectiveness of a biofumigant approach for recurrent nematode control. Gall number decrease was seen with all biofumigant and crop sequence treatments but was most pronounced with cultivation after broccoli plantlets integration, where plants had the fewest galls (52 and 48 galls, respectively) in two seasons.

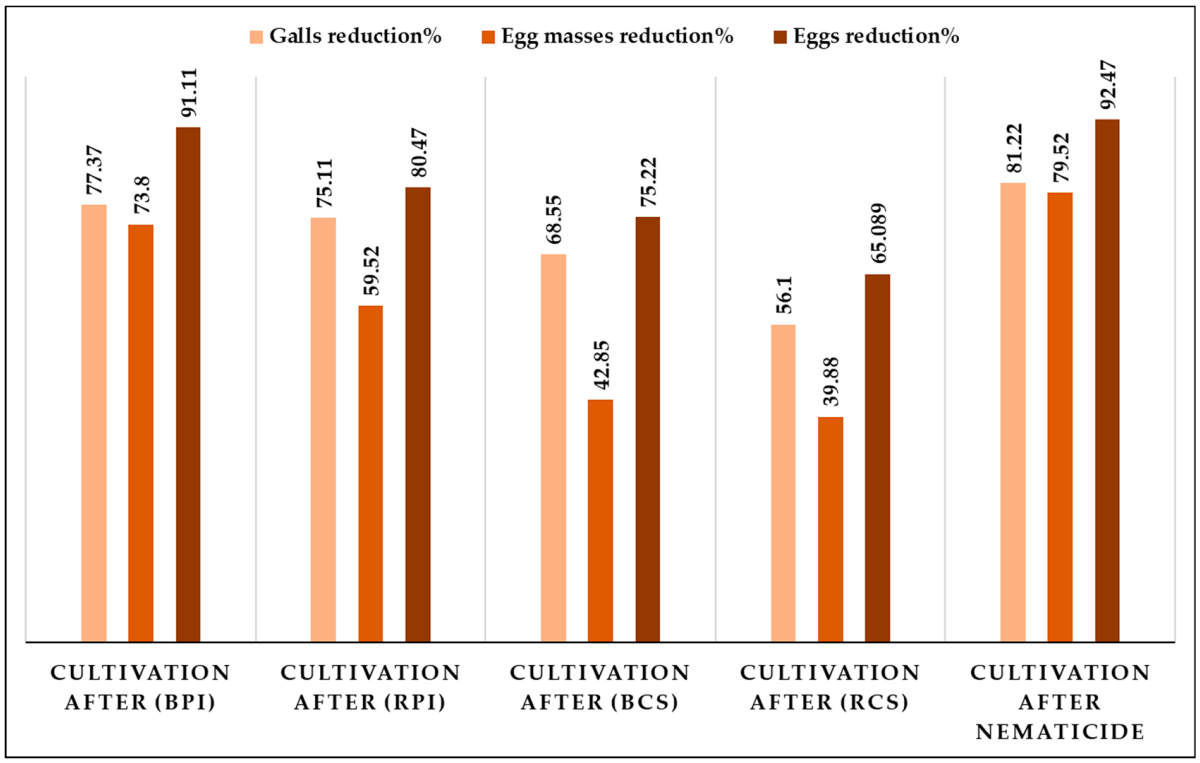

Figure 7. The effects of biofumigant, crop sequence, and nematicide treatments on gall, egg-mass, and egg numbers reduction in cucumber roots. Level of reduction of treatments was estimated according to reduction of nematode parameters compared with mean of control plants.

3.3. The Relationship between Biofumigant Crops as a Yield Enhancer and Nematode Suppressor and Their Total Phenols, Glucosinolates, Isothiocyanate Contents, and Myrosinase Activity

According to the data in Figure 8, broccoli plantlets had the highest levels of total phenols, GSLs, isothiocyanates, and myrosinase activity, followed by radish plantlets. The results showed that broccoli plantlets had the highest phenol content, followed by radish plantlets, which had a negative effect on nematode reproduction. When the previous chemical contents in all treatments were compared, it was discovered that the concentrations in plantlets were superior to adult roots. Figure 6 shows that plantlets of broccoli have the highest amount of isothiocyanate compared to adult roots in all biofumigant and crop sequence treatments. In both seasons, broccoli plantlets had the highest levels of isothiocyanate, while radish plant roots had the lowest levels. Isothiocyanate activity levels in plantlets were greater than those in mature plant roots. For two seasons, data showed that broccoli plantlets contained more isothiocyanates (44.8 and 40.7 g/g FW) than adult plant roots (30.5 and 29.9 g/g FW). In two seasons, radish plantlets produced more isothiocyanates (37.6 and 35.2 g/g FW) than adult roots (26.4 and 22.9 g/g FW), respectively. The concentration and composition of GSLs varied between cover crop roots and shoots. In terms of GSLs concentrations, a broccoli seedling had the highest concentrations com-

pared to a radish seedling and adult roots for either broccoli or radish. This concentration motivates nematode suppression in the cucumber crop immediately following.

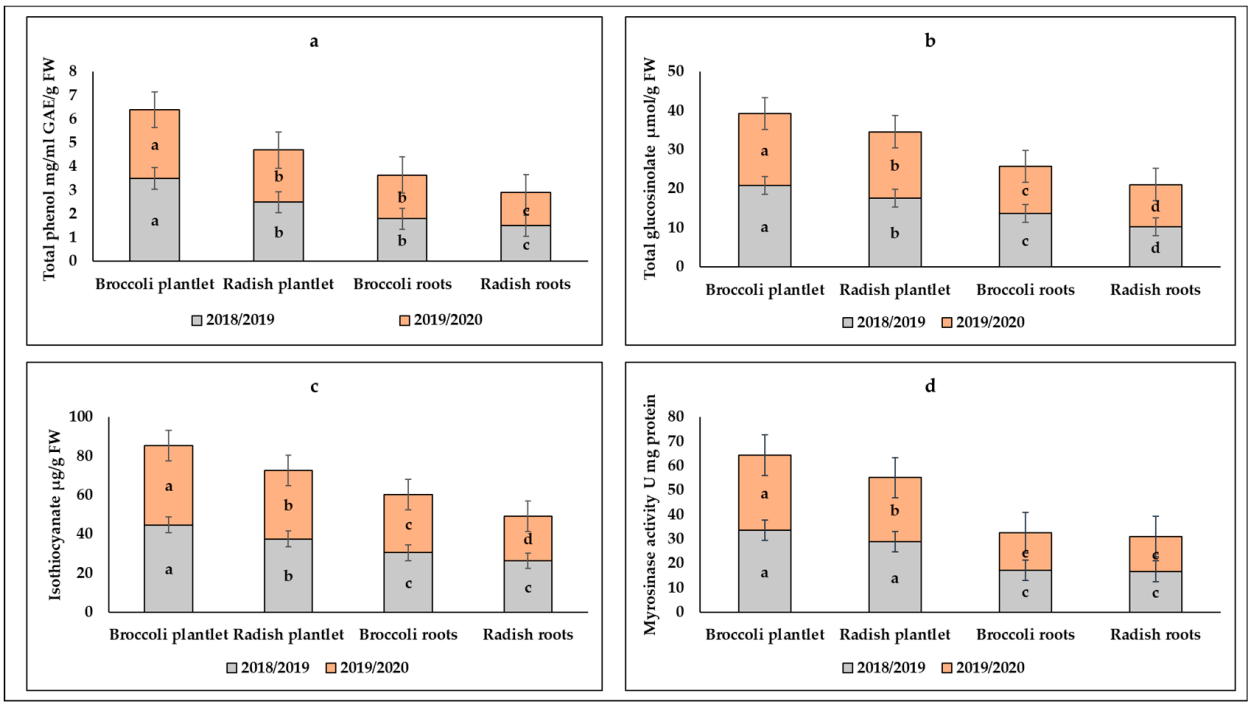

Figure 8. The concentrations of total phenol, total glucosinolates, isothiocyanates, and myrosinase activity contents according to various treatments in two seasons 2018/2019 and 2019/2020. ((**a**) total phenol, (**b**) total glucosinolates, (**c**) isothiocyanates, and (**d**) myrosinase activity contents). Means (±SE) followed by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

4. Discussion

This research was divided into two effective methods for cucumber crop production improvement and root-knot nematode reduction. According to the data, the bio-fumigation method was the most successful tactic. The efficacy of utilizing biofumigant crops before cucumber cultivation may be related to increased biomass production. There are several advantages to increasing organic matter, including improved soil structure, increased infiltration, increased water-holding capacity, and more effective long-term nutrient storage [22]. Furthermore, any increase in soil organic matter may result in better soil quality and productivity, which is reflected in crop growth and yield [23]. These improvements were reflected in the all-vegetative, fruit, and yield traits, where the cultivation after the broccoli plantlet incorporation method improved the vegetative cucumber growth, main stem length, plant fresh weight, and leaf area on cucumber, celery, zucchini, and tomato growth after broccoli incorporation [24–29]. The finding for leaf area was consistent with Anita [26] and Zhang et al. [30]. In the same context, Ploeg and Stapleton [31] discovered that cultivation in crop rotation was a particularly successful approach, especially following broccoli. Furthermore, there were no significant variations in flowering and fruit characteristics between nematicide administration and cultivation following broccoli plantlet inclusion. This finding may be attributed to plantlet integration impacts on nutrient efficiency in the soil, as discovered by Zhang et al. [30], who discovered the soil's nutritional condition improved following biofumigant application. Bio-fumigation is an excellent strategy for improving fruit quality and productivity [29,30].

Furthermore, yield and its components did not change across cultivations following the integration of broccoli and radish plantlets. Both of them had large fruit counts and weights in both early and total yield, with values that were double the control values. This finding was similar to that found by Anita [26] on celery yield following biofumigants.

According to Lazzeri et al. [9], zucchini yields increased overall yield by 14%. A similar increase was discovered in strawberry commercial yield [30]. Furthermore, the application of broccoli plant leftovers generates a considerable boost in melon output [29,31]. The mode of action of bio-fumigation is based on volatile molecules with pesticide properties that are released into the soil during plant degradation. Tissue maceration is required in brassica tissues to activate myrosinase and the glucosinolate system, resulting in the production of bioactive isothiocyanates (ITC).

Soil tillage is necessary for the tissues to come into contact with the nematodes, and black plastic mulch is required to protect ITC from volatilization loss, in order to enhance the biofumigation impact on *Meloidogyne* spp., as shown by Waisen [28]. The poisonous effects of glucosinolates or chemicals generated during the breakdown of green manures of *Brassica* species may inhibit nematode populations. The *Brassicaceae* biocidal activity appears to be related to the presence of the glucosinolate–myrosinase system and its ability to produce biologically active compounds such as isothiocyanates after cell lesion or destruction, as demonstrated in the current study [32–35]. *Brassica* tissues have been shown to suppress root-knot nematodes, *Meloidogyne* spp. in soil [36]. Glucosinolates stored in the vacuoles are digested by the enzyme myrosinase and subsequently transformed into poisonous isothiocyanates (ITCs) as a result of plant tissue damage [37,38]. Angus et al. [39] demonstrated the benefits of *brassica* as incorporating cover crops rich in GSL to induce ITC production in sustainable agriculture systems. The results revealed no significant differences between nematicide application and cultivation after broccoli plantlets incorporation in all nematode parameters. Monfort et al. [25] obtained the same result when they evaluated *Brassica* species as an alternative to methyl bromide for managing root-knot nematode populations in vegetable crops. They discovered that cover crop treatments had the lowest level of root-knot nematode populations when compared to methyl bromide. These findings proved that biofumigant treatments inhibited or had a negative impact on fecundity, as egg counts fell with cultivation after biofumigant treatments compared to controls [27]. There were significant changes in nematode reproduction factors (RF) between all treatments and the control [31]. The observed reduction is connected to the amount of chopped biomass that integrated into the soil and the synthesis of glucosinolates [40]. Significant effects were observed based on crop type and age for glucosinolate content in this study [41]. Broccoli was shown to be the least vulnerable biofumigant crop among four brassica crops by Youssef [5].

Another strategy used in this research was the cropping sequence, which is dependent on the allelopathy process. Allelopathy is a widespread biological phenomenon in which one organism produces biochemicals that impact the growth, survival, development, and reproduction of other species. These root exudates are known as allelochemicals, and they may be beneficial or detrimental to certain species. Use of allelopathy has risen in sustainable agriculture as a pesticide alternative to control negative environmental consequences since it is low-cost, environmentally benign, possible below the soil and in the plant system, and improves crop yield [42]. Allelochemicals' mode of action includes effects on cell structure, division and elongation, membrane permeability, oxidative and antioxidant systems, growth regulation systems, respiration, enzyme synthesis and metabolism, photosynthesis, mineral ion uptake, and protein and nucleic acid synthesis in pathogens. The results proved that crop sequences efficiently reduced nematodes and slowed their life cycles via allelochemicals secreted by roots [42].

The crop sequence was very successful in contrast to the control, since Curto et al. [43] noted that the nematode life cycle was generally slower in brassica crops than in tomatoes. According to El-Sherbiny and Awd Allah [29], the reduction of nematode parameters may be owing to various bio-nematocidal chemicals generated by *brassica* crops. Some studies indicate the release of naturally nematocidal compounds (such as isothiocyanates, glucosinolates, cyanogenic glycosides, alkaloids, fatty acids and their derivatives, sesquiterpenoids, steroids, diterpenoids, triterpenoids, and phenolic compounds) during the biodegradation of organic plant materials [44,45]. The isothiocyanates and other phytochemicals contained

in brassicaceous plants, such as phenols and ascorbic acids, may work synergistically with GLSs and are very poisonous to plant-parasitic nematodes. [7,46,47]. The biocidal content concentrations in plantlets were higher than the concentrations in adult roots [48]. Furthermore, sprouts of some crucifer cultivars have substantially greater quantities (10–100 times) of glucosinolates than mature plants [49,50]. The findings indicated that broccoli plantlets had the highest phenol concentration, followed by radish plantlets. These are the most frequent allelochemicals, which include phenols, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids, and quinones [51]. Phenols may have a deleterious impact on nematode reproduction [52]. Furthermore, the broccoli plantlets were particularly successful in terms of GLSs content, which was 10–15 times greater than Chinese cabbage, mustard, and radish [53]. Sang [53] discovered varied glucosinolate concentrations in the seeds, roots, and leaves of five cruciferous plants: cabbage, mustard, radish, rapeseed, and swede. *Brassica* roots release glucosinolates thatstimulate plant defenses [37].

5. Conclusions

According to the findings, the bio-fumigation approach was the most successful and demonstrated the highest vegetative, flowering, and yield characteristics values for cucumber crop production in naturally infested fields by root-knot nematodes. It is worth noting that cultivation after broccoli plantlets incorporation was the preferable approach because of the large quantity of GSLs produced, particularly isothiocyanates, which had a direct detrimental effect on nematode compared to radish plantlets. Another efficient approach used was the crop sequence system, which produced promising results based on allelopathy phenomenon in *brassica* roots. Finally, this study was a genuine and effective endeavor to develop a safe alternative to nematicide to tackle a severe issue in Egyptian soils, which were suffering from a high nematode infestation, resulting in massive cucumber crop losses. These findings suggest that the *brassica* crop be used as a biofumigant or put into a crop sequence to assist in managing root-knot nematode. In addition to generating a high yield, the eco-friendly approach of soil enhancement aids in integrated pest control in sustainable agriculture.

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