Efficacy of four cruciferous germinated grinded seed on adjusting Meloidogyne incognita infecting soybean plants

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Abstract

The influence of Brassica germinated grinded seeds cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl against M. incognita infecting soybean cv. Giza 22 were studied under glasshouse conditions (25±5°C) of the Unit of Nematology Research, Faculty of Agriculture, Mansoura university. Cabbage germinated grinded seeds (GGS) singly or integrated with oxamyl at half dose showed the maximum values in improving total plant fresh weight (113 and 67.0%), plant length (49.5 and 42.9%), shoot dry weight (105.4 and 42.3%) and number of leaves per plant (166.7 and 100.0%), as well as accomplished the best results in reducing number of galls, eggmasses and females on root system of soybean plants since their values were amounted to 80, 83.8 and 80.9% respectively. Likewise, similar trend was achieved as cabbage integrated with oxamyl at half dose with values of 68.9, 82.2 and 77.4% for number of galls, eggmasses and females comparing to nematode, respectively. The concentrations of nitrogen, phosphorus, potassium and the content of total phenol were gradually decreased, while the content of total chlorophyll clearly increased with the infection of nematode alone. Otherwise, all studied treatments solely recorded significant increase in the content of nitrogen, phosphorus, potassium and total contents of phenol and reduced the content of total chlorophyll with a negative value.

Keywords: Brassica, germinated, grinded, seeds, cabbage, broccoli, Meloidogyne incognita, control, oxamyl.

1. Introduction

Soybean, Glycine max (L.) Merrill., it containing about 42-45% of protein, 30-35% of carbohydrates, 20-22% is consider one of the more essential of vegetable oil, and high concentration of vitamins, amino acid, riboflavin, niacin, thiamin, rich of unsaturated fatty acids and free of cholesterol, phosphorus, calcium, and iron [1,2]. Regarding to [3], Egypt cultivated about 23800 fed this area produced about 35000 ton. While, the worldwide cultivation reached about 121.5 million ha this area produced about 334.9 million ton. Moreover, soybean contributes to an effective role in increasing soil fertility through the process of fixing air nitrogen with bacteria of the genus rhizobium. The growth and productions of different of agricultural crops in the tropical and sub-tropical were decreased due to plant parasitic nematodes [4-6]. The most harmful nematode pests on variety of crops in subtropical areas are root-knot nematodes, Meloidogyne spp. [7-11]. Lately, the root-knot nematode Meloidogyne spp. is considering one of the five nematode types with high population masses and associated with soybeans, peanuts, rapeseed plants during 2000 to 2003 seasons in ten governorates of Egypt [12-14]. Due to the decreases of growth and production indicated from the infection of root-knot nematodes soybean plants, it is too importance to diminish crop injury by searching for effective practices. Using of chemical nematicides maybe does not always show actual and commercial [15]. Moreover, using chemical nematicides causes serious human health problems and environmental pollution [16-19]. Thus, searching for substances environmentally friendly alternatives are required for decrease the population of nematode.

Though, combined nematode practice by using several control methods i.e. soil amendments, organic materials, oil cakes of certain seeds, powder of various parts of brassica crops as abiotic with trying to applied of nematicides usual great devotion between the nematologists as long as effective control actions against the target nematode, all these methods maybe decrease the numbers of nematode and prevents the environmental pollution. The objective of the current investigation was to clarify the efficacy of four Brassica germinated grinded seeds alone or combined with oxamyl on controlling Meloidogyne incognita contaminating soybean under glasshouse conditions (25±5°C).
2. Materials and Methods

2.1. Nematode stock culture, broadcasts and preparing nematode inoculum

To assemble and regulate the inoculate of Meloidogyne incognita eggs; M. incognita was formerly notorious affording to [18]. Under the Experimental Unit of Nematology Research, Faculty of Agriculture, Mansoura university, the root systems infested with heavy eggmasses of M. incognita of numerous growing coleus Coleus blumei plants, planted in plastic bags (25 cm width) and occupied with disinfected loamy sandy soil (1:1 v/v), this soil was washed by tap water and for 90 seconds with 1.0% of (NaOCl), shacked strongly, then rapidly pass NaOCl solution using nested 60-mesh sieve over a 400-mesh sieve to get free eggs. Then, quickly put the nested under watercourse for some minutes to eliminate the remaining NaOCl. Finally, the no. of eggs per unit bulk of water was calculated and inoculated plants with free eggs [20].

2.2. Germinated grinded seeds (GGS) preparation

About 5 gm of cabbage, cauliflower, broccoli and radish seeds that previously collected and botanically identified seeds were placed in Petri dishes separately, and then about 5 ml of distilled water were put, and then covered the dish. After 48 hours, the seeds of each plant were grinded in a blender separately and weighed the required amount regarding to the design of the experiment.

2.3. Impression of four Brassica germinated grinded seeds individually or combined with oxamyl on monitoring Meloidogyne incognita infecting soybean under greenhouse conditions (25±5°C).

To study the impact of four Brassica germinated grinded seeds i.e. cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl against M. incognita infecting soybean cv. Giza 22 was sown on 2018 and 2019 summer seasons on the first week of May under the conditions of greenhouse 25±5°C, fifty five plastic bags filled with one kilogram of loamy sandy soil (1:1 v/v) and hand planted with 3 seeds per plastic bags and then irrigated. After 15th days from sowing the plants were thinned into one seedling per plastic bag and the 50 plastic bags were separately inoculated with 2000 eggs of M. incognita and keep 5 seedlings plastic bags without infection with nematode eggs (as a control treatment). The tested plant application doses were separately added to five seedlings (bags) each after one week of nematode inoculation. All treatments were repeated five times and the treatments were as follows:

- Cabbage GGS (5 g/plant),
- Broccoli GGS (5 g/plant),
- Radish GGS (5 g/plant),
- Cauliflower GGS (5 g/plant),
- ½ Cabbage GGS (2.5 g/plant) + ½ oxamyl (0.15/plant),
- ½ Broccoli GGS (2.5 g/plant) + ½ oxamyl (0.15/plant),
- ½ Radish GGS (2.5 g/plant) + ½ oxamyl (0.15/plant),
- ½ Cauliflower GGS (2.5 g/plant) + ½ oxamyl (0.15/plant),
- oxamyl (0.3 g/plant),
- N alone (N= 2000 eggs of M. incognita).

2.4. Plant free of nematode and any treatment

All treatments were organized in a Randomized Complete Block Design (RCBD) with five replicates under the environmental of greenhouse (25±5°C) and the experimental units were irrigated as required. Plants uprooted after 45 days from the inoculation process, and plant growth characters i.e. shoot and root length and shoot fresh and dry weights and number of leaves per plant were recorded. Infested roots per replicate was separately washed, immobile in formalin at 4% and stain in lactic acid fuchsine 0.01 [21]. and then tested for recording nematode parameters i.e. no. of galls, developmental stages, females and eggmasses and recorded. Root gall index (RGi) and eggmasses index (EI) were estimated according to [18], as follows: 0= no galls, 1= 1-2 galls, 2= 3-10 galls, 3= 11-30 galls, 4= 31-100 galls and 5= more than100 galls. N, P and K concentrations, was determination using 0.2 gram of seedling dry weight. Total nitrogen content was estimated using the enhanced Kjeldahl method [22]. Phosphorus content was colorimetrically determined using the chlorostannous reduced, Molybdophosphoric Blue colour method, while potassium content was Flam photometry as mentioned by [23]. Chlorophyll content was spectrophotometrically determined using [24], then the chlorophyll content was calculated as mg/g of fresh weight according to [25]. Total phenols content was estimated using the method of [26]. The leaves were soaked in 2NHCl to extract tissues by using 10 ml of 2NHCl. After that, all crushed supplies were boiled using a water bath in a test-tube for half an hour. Then, it was filtered and the filtrate was put at room temperature over anhydrous CaCl₂ until dryness.
After that (0.5ml) of ethanol was added to the dried extracts and after five minutes 0.1ml of extract was taken to which 0.2 ml of Folin-Ciocaltes reagent (1.9 v/v) and 4.8 ml of distilled water were added. The tubes were shaken in an electric shaker for about ten minutes and saturated with a NaHCO₃ solution and it shaken again and incubated for 30 minutes at 25°C. Optical density was easimated at 660 nm and the content of total phenols as mg/g of fresh weight. Statistics were evaluated according to (ANOVA) as declared by [27], followed by Duncans multiple range test to compare means at P< 0.05 [28].

3. Results and Discussion

Data presented in Table (1) revealed the influence of four Brassica germinated grinded seeds cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl against M. incognita infecting soybean cv. Giza 22 under greenhouse conditions (25±5°C). Overall, the tested materials visibly ameliorated significantly soybean plant growth parameters to great extent. Amongst the tested components, cabbage GGS individually or combined with oxamyl at half dose showed the maximum values in improving total plant fresh weight (113and 67.0%), plant length (49.5 and 42.9%), shoot dry weight (105.4 and 42.3%) and number of leaves per plant (166.7and 100.0%). Meanwhile, radish GGs furnished the smallest values for the same plant growth measures that averaged 7.7, 24, 14.6 and 33.3%, respectively equating to nematode alone. Furthermore, oxamyl as a nematicide at the suggested dose ranked 2ed to that of leaves/plant for the former and less moderate values for the latter treatment that averaged 14.3, 40, 27.9 and 33.3%, parameters that averaged 31.9, 61.0, 87.2 and 33.3% for plant length, total plant fresh weight, shoot dry weight and number of leaves/plant for the former and less moderate values for the latter treatment that averaged 14.3, 40, 27.9 and 33.3%, respectively as compared with nematode alone. Moreover, the soil amendments with cauliflower and broccoli GGs showed absemiuous values of ameliorating plant parameters that averaged 31.9, 61.0, 87.2 and 33.3% for plant length, total plant fresh weight, shoot dry weight and number of leaves/plant for the former and less moderate values for the latter treatment that averaged 14.3, 40, 27.9 and 33.3%, respectively as compared with nematode alone. Furthermore, oxamyl as a nematicide at the suggested dose ranked 2ed to that of the dried leave powder of cabbage with percentage increase values of plant length, plant fresh weight and shoot dry weight and number of leaves per plant, that averaged 45.1, 88 and 69.2 and on par for the latter plant growth measures (166.7%), correspondingly compared with nematode alone.

Table 1. Impact of four Brassica germinated grinded seeds cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl against M. incognita infecting soybean cv. Giza 22 under greenhouse conditions as average over both summer seasons 2018 and 2019.

| Treatments | Length (cm) | Total plant length (cm)** Inc.% | No. of leaves ** Inc.% | Fresh weight (g) shoot root | Total plant F.Wt (g) ** Inc.% | Shoot dry weight (g) ** Inc.% |
|------------|-------------|--------------------------------|------------------------|----------------------------|-------------------------------|----------------------------|
| Cabbage GGS | 104.7 a 31.3 b | 136.1 a | 49.5 13.0 a 166.7 | 15.8 a 5.5 b | 21.3 a 113 2.67 a 105.4 |
| Cauliflower GGS | 90.1 c 30.3 c | 120.3 e | 31.9 8.0 bc 33.3 | 11.5 d 4.6 d | 16.1 d 61 2.46 b 89.2 |
| Broccoli GGs | 76.7 g 27.3 d | 104.3 g | 14.3 8.0 bc 33.3 | 10.5 f 3.5 h | 14.1 g 40 1.66 f 27.7 |
| Radish GGs | 75.3 h 23.1 d | 98.3 h | 7.7 8.0 bc 33.3 | 8.8 g 3.6 h | 12.4 i 24 1.49 g 14.6 |
| ½(Cabbage+oxamyl) | 100.3 a 30.1 b | 130.3 c | 42.9 12.0 a 100.0 | 12.3 c 4.4 e | 16.7 c 67 2.53 b 92.3 |
| ½(Cauliflower+oxamyl) | 88.0 f 28.3 c | 116.3 f | 27.5 8.0 bc 33.3 | 10.5 f 4.2 f | 14.7 f 47 1.86 e 46.2 |
| ½(Broccoli+oxamyl) | 95.3 d 33.1 a | 128.3 d | 40.7 9.0 b 50.0 | 11.1 e 4.0 g | 15.1 e 50 2.10 d 61.5 |
| ½(Radish+oxamyl) | 76.7 c 27.3 d | 104.3 g | 14.3 8.0 bc 33.3 | 10.5 f 3.5 h | 14.1 g 40 1.66 f 27.7 |
| Oxamyl + N | 98.6 e 34.0 a | 132.6 b | 45.1 13.0 a 166.7 | 12.8 b 6.1 a | 18.9 b 87.1 2.23 c 69.2 |
| N alone | 70.3 i 21.1 e | 91.3 j | 6.0 d | 7.5 i 2.5 i | 10.1 j ---- 1.30 h ---- |
| Plant free of any treatment | 65.1 j 30.3 b | 95.3 i | 4.4 7.1 c 16.6 | 8.1 h 5.1 c | 13.1 h 30 1.53 g 15.4 |
| L.S.D at 0.05 % | 1.04 1.07 0.98 | 0.98 ---- | 0.12 0.11 0.13 | ---- 0.07 ---- |

N= 2000 eggs of M. incognita
GGS= Germinated grinded seeds
* Data followed by the same letter are not significantly did not differ at p<0.05.
** Increase % = Treatment - N alone (Untreated) × 100
3.1. N alone (Untreated)

Statistics presented in Table (2) reveal that germinated grinded seeds cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl against M. incognita were significantly effective in reducing numbers of galls, eggmasses and females on roots of soybean cv. Giza 22 comparing to check under greenhouse conditions at 25±3°C. It is interesting to observe that among the tested plant products cabbage GGS accomplished the best results in reducing number of galls, eggmasses and females on root system of soybean plants since their values were amounted to 80, 83.8 and 80.9% respectively comparing to nematode alone. Likewise, similar trend was achieved as cabbage integrated with oxamyl at half dose with values of 88.9, 82.2 and 77.4% for number of galls, eggmasses and females comparing to nematode, respectively. In addition, results of cauliflower integrated with oxamyl at half dose treatment for these nematode criteria that averaged 68.4, 76.7 and 80.0%, were on par with that of radish with oxamyl at half dose treatment. However, radish GGs treatment showed the least values of number of galls (41.2%), eggmasses (59.8%) and females (52.5%), whereas cauliflower and broccoli GGs treatment gave the moderately values of the nematode criteria that averaged 62.7, 78.9 and 75.8% for the former and 51.4, 73.9 and 68.30% for the latter treatment, comparing to nematode alone, respectively. Moreover, it is also worthy to note that all tested treatments gave equal values of both nematode criteria i.e. root gall and eggmasses indices that averaged 4 each Vs 5 for nematode alone, respectively (Table 2).

| Treatments | No. of galls | R% | RGI | No. of female | R% | No. of eggmasses | R% | E.I |
|------------|--------------|----|-----|--------------|----|-----------------|----|-----|
| Cabbage GGS | 41.7 i        | 80.0 | 4   | 36.3 i       | 80.9 | 30.3 i          | 83.8 | 4   |
| Cauliflower GGS | 79.1 f       | 62.7 | 4   | 46.1 f       | 75.8 | 38.6 f          | 78.9 | 4   |
| Broccoli GGS | 103.1 c      | 51.4 | 4   | 68.3 c       | 64.1 | 48.3 c          | 73.9 | 4   |
| Radish GGs | 124.7 b      | 41.2 | 4   | 90.3 b       | 52.5 | 74.3 b          | 59.8 | 4   |
| ½(Cabbage + ox) | 66.1 h      | 68.9 | 4   | 43.1 h       | 77.4 | 33.3 h          | 82.2 | 4   |
| ½(Cauliflower + ox) | 67.1 g      | 68.4 | 4   | 45.1 g       | 76.3 | 37.3 g          | 80.0 | 4   |
| ½(Broccoli + ox) | 87.1 d     | 58.9 | 4   | 50.1 d       | 73.7 | 45.3 d          | 75.7 | 4   |
| ½(Radish + ox) | 67.1 g     | 68.4 | 4   | 45.1 g       | 76.3 | 37.3 g          | 80.0 | 4   |
| Oxamyl + N | 85.1 e      | 59.9 | 4   | 49.1 e       | 74.2 | 43.0 e          | 76.8 | 4   |
| N alone | 212.3 a    | --- | 5   | 190.3 a      | --- | 185.3 a         | --- | 5   |
| L.S.D at 0.05% | 0.36   | --- | --- | 0.35         | --- | 0.99            | --- | --- |

* Nematode inoculum = 2000 eggs of M. incognita; Individually value is a mean of five replicates.
* Data followed by the same letter are not significantly did not differ at p<0.05.

Statistics presented in Table (3) revealed the influence of four germinated grinded seeds i.e. cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl on nitrogen (N), phosphorous (P) and potassium (K) concentrations; and total phenol content as well as total chlorophyll content in leaves of soybean cv. Giza 22 infected by M. incognita under greenhouse conditions (25±3°C). It was evident that N, P, and K concentrations and total phenol were significantly diminished, while that of total chlorophyll content obviously increased by nematode infection alone. On the other hand, all tested components solely showed positive increase in N, P, and K percentage and total phenol content, while, reduced total chlorophyll content with a negative value, comparing to nematode alone (Table 3). The tested brassica products treatments, cabbage GGS ranked first and gave the maximum value of total phenol content (28.1%), followed by that cauliflower (24.5%), then broccoli (whole plant powder) (21.9%), whereas radish GGs treatment achieved the least values of total phenol content (7.5%), comparing to nematode alone, respectively. However, opposite trend was recorded regarding the percentage increase values of N, P, and K percentages, since their values could be arranged in descending orders as follows , 13.2, 14.4 and 14.3% for radish GGs, 6.5, 8.8 and 9.3% for broccoli GGs; 3.7, 6.1 and 5.0% for cauliflower GGs; and 1.6, 3.2 and 3.2% for cabbage GGS that represented the minimum values in this respect, respectively. Moreover, among the integrated materials, cabbage with oxamyl surpassed other treatments in...
the increment increase values of total phenol content (17.6%), followed by cauliflower with oxamyl (11.8%) and broccoli with oxamyl gave minimum value of this parameters that averaged 3.9%. Oxamyl as a systemic nematicide surpassed all tested biotic and abiotic factors in percentage increase values of N (17.2%), P (20.0%), K (20.0%) and total phenol content (30.1%), respectively comparing to nematode alone (Table 10). Meanwhile, plant receiving non of the tested soybean material showed better results in percentage increase values of N (19.7%), P (23.2%) and K (24.4%) than the tested materials except in the case of total phenol content with a negative value of 4.2%, respectively, comparing to nematode alone. These results are in good line with those recorded by [26,29].

Table 3. Nitrogen, phosphorus and potassium concentrations as well as chlorophyll and total phenol in fresh shoot of soybean cv. Giza 22 infected by Meloidogyne incognita treated with four Brassica germinated grinded seeds singly or integrated with oxamyl under greenhouse conditions as average over both summer seasons 2018 and 2019.

| Treatments               | * N  Mg/g | Inc. % | * P  Mg/g | Inc. % | * K  Mg/g | Inc. % | * Chlorophyll content | Total chlorophyll Mg/g | Dec. % | Total phenol Mg/g | Inc.% |
|--------------------------|-----------|--------|-----------|--------|-----------|--------|----------------------|------------------------|--------|------------------|-------|
| Cabbage GGS              | 4.32 i    | 1.6    | 0.387i    | 3.2    | 2.88i     | 3.2    | 0.353g               | 0.229h                 | 0.582g | -12.2            | 0.392b | 28.1             |
| Cauliflower GGS         | 4.41 h    | 3.7    | 0.398h    | 6.1    | 2.93h     | 5.0    | 0.353g               | 0.229h                 | 0.582g | -12.2            | 0.381c | 24.5             |
| Broccoli GGS            | 4.53 g    | 6.5    | 0.408g    | 8.8    | 3.05g     | 9.3    | 0.361f               | 0.237g                 | 0.598f | -9.8             | 0.373d | 21.9             |
| Radish GGS              | 4.81 d    | 13.2   | 0.429d    | 14.4   | 3.19d     | 14.3   | 0.386c               | 0.252d                 | 0.638c | -3.8             | 0.329g | 7.5              |
| ½(Cabbage+ox)           | 4.60 f    | 8.2    | 0.416f    | 10.9   | 3.08f     | 10.4   | 0.365e               | 0.240f                 | 0.605c | -8.7             | 0.360e | 17.6             |
| ½(Cauliflower+ox)       | 4.69 e    | 10.4   | 0.423e    | 12.8   | 3.16e     | 13.3   | 0.376d               | 0.246e                 | 0.622d | -6.2             | 0.342f | 11.8             |
| ½(Broccoli+ox)          | 4.89 c    | 15.1   | 0.443c    | 18.1   | 3.29c     | 17.9   | 0.393b               | 0.259c                 | 0.652b | -1.7             | 0.318h | 3.9              |
| ½(Radish+ox)            | 4.60 f    | 8.2    | 0.423e    | 12.8   | 3.19d     | 14.3   | 0.386c               | 0.252d                 | 0.638c | -3.8             | 0.329g | 7.5              |
| Oxamyl                   | 4.98 b    | 17.2   | 0.450b    | 20.0   | 3.35b     | 20.0   | 0.291h               | 0.198i                 | 0.489h | -26.2            | 0.398a | 30.1             |
| N alone                  | 4.25 j    | ---    | 0.375j    | ---    | 2.79j     | ---    | 0.398a               | 0.265b                 | 0.663a | --               | 0.306i | --               |
| Plant free of any treatment | 5.09 a    | 19.7   | 0.462a    | -2.3   | 3.47a     | 24.4   | 0.205i               | 0.271a                 | 0.376i | -43.3            | 0.293j | -4.2             |
| L.S.D at 0.05            | 0.03      | -----  | 0.001     | -----   | 0.012-----| 9.83  | 0.001                | ------                 | 0.001 | ------           | 0.001 | ------           |

* N = 2000 M. incognita eggs; Each value is a mean of five replicates.;
** Data followed by the same letter are not significantly did not differ at p<0.05.

 Apparently, in the present study, four brassica germinated grinded seeds i.e. cabbage, cauliflower, broccoli and radish seeds singly or integrated with oxamyl in comparison with oxamyl showed nematicial properties against the target pest, M. incognita infecting soybean cv. Giza 22, since all tested materials obviously caused ameliorating plant growth of both host plants and suppressed nematode criteria as well, respectively. These findings emphasized that the potential brassica seeds products i.e. cabbage, cauliflower and broccoli that seemed to have higher toxic action much more than tested microbial agents but not as much as oxamyl (10% G) which overwhelmed all other tested treatments in some cases.
The nematicidal activity of tested brassica seeds for example cabbage product is due to their contents of some substances i.e. glucosinolates and myrosinase found in diverse portions of the cell come into contact [30,31]. In addition, these substances lead to the formation of bioactive volatile isothiocynates [32,33]. Evidently, isothiocynates are consider the central components of the pest-pathogen-and weed-suppressive effects observed after soil incorporation of brassica tissue [34], and their inhibitory effect on fungi, bacteria, insects and nematodes and weeds has been amply demonstrated in in-vitro experiments [35,36]. These various tested materials maybe play a role in defense mechanisms of tested host plants i.e. soybean cv. Giza 22 against involving target nematodes. However, there was negatively associations among the individually applications of the tested components regarding the decline of chlorophyll content in the current study comparing to nematode alone [37].

Conclusion
It could be concluded that cabbage germinated grinded seeds (GGS) singly or integrated with oxamyl at half dose showed the maximum values in improving total plant fresh weight, plant length, shoot dry weight and number of leaves per plant as well as accomplished the best results in reducing number of galls, eggmasses and females on root system of soybean plants. Likewise, similar trend was achieved as cabbage integrated with oxamyl at half dose for number of galls, eggmasses and females comparing to nematode, respectively. The concentrations of N, P, and K and total phenol were significantly diminished, while that of total chlorophyll content obviously increased by nematode infection alone. On the other hand, all tested components solely showed significant increase in N, P, and K concentrations and total phenol content and reduced total chlorophyll content with a negative value.

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