

Bio-control of Root-Knot (*Meloidogyne incognita*) of Indian Spinach (*Basella alba* L.)

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Abstract Five treatments viz. BAU-Biofungicide (*Trichoderma* spp.) as side dressing and seed coating, nematicide (Carbofuran 10G) and allamanda leaf extract as side dressing along with a control were tested against root-knot of Indian spinach caused by *Meloidogyne incognita*. The findings of the study revealed that the number of galls and the number of egg masses in the roots were significantly reduced by the application of all the treatments over control. However, chemical carbofuran 10G gave superior result as it had increased shoot and root length, fresh weight of shoot and root as well as number of leaf per plant correspondingly with the lowest galling incidence and egg mass development in Indian Spinach. The interaction effects of the treatments and the varieties, the BARI Indian Spinach 1 as side dressing with carbofuran (V₁T₃) gave the highest performances on the plant growth characters and reduction of galls and egg masses development by 95.65% and 96.33% over control as compared to the local variety (69.55% and 64.31%). Whereas, BAU-biofungicide (V₁T₂) also showed considerable effect to increase the plant growth parameters in correspondence with the reducing of gall incidence and egg masses by 79.74% and 77.78% over control in BARI Indian Spinach 1 as compared to the local variety (67.83% and 49.95%).

Keywords Indian Spinach, Root-knot, Carbofuran, BAU-Biofungicide, Allamanda Leaf

1. Introduction

Indian Spinach (*Basella alba*) is a common and economically important leafy vegetables grown throughout all the season in Bangladesh. This vegetable is rich in calcium, magnesium, potassium and iron as well as several vitamins including vitamin A [1]. The soil and climate of Bangladesh are very favorable for the cultivation of Indian spinach and the average yield of BARI Spinach-1 was

recorded 40-46 ton/ha [2].

However, the yield of the vegetable is not satisfactory because of many constraints including the infection of several plant parasitic nematodes. Around 15 genera of plant parasitic nematodes are reported to associate with commercial crops grown in Bangladesh, where *Meloidogyne* spp. are found most abundant and widespread [3]. Among the nematode diseases, the root-knot caused by *Meloidogyne javanica* and *Meloidogyne incognita* are one of the most common and damaging nematodes on Indian spinach [4-5]. Furthermore, the root-knot disease causing by nematode predisposes the plants for further invasion by other micro-organisms [6]. Surprisingly, the nematode population in the soils of Bangladesh found to be increasing day by day [7].

The management of root-knot disease have been tried through integrated use of cultural, chemicals, biological and botanical means from very beginning. However, different chemicals like carbofuran, carbendazem, bavistin etc. are found to be effective for the controlling root-knot nematodes and vastly used commercially for the controlling of nematode diseases. However, the use of chemicals is too expensive, particularly, for poor farmers of Bangladesh as well as a difficult task to determine the precise dose of the chemical for its application to the field. In addition, non-judicial use of chemicals in agriculture causes air, soil, water pollution and health hazards [8].

As alternative of chemicals, bio-fungicides are included in the concept of biological control represents a natural and ecological approach to controlling diseases that reduces chemical inputs and their effects [9-10]. In biological control, living microorganisms act as antagonist, parasites and predators [11] and *Trichoderma* played a considerable role as biocontrol agent [12] which also stimulates the growth of the plant against plant pathogenic nematodes [13].

On the other hand, some plant leaves suppress plant parasitic nematodes when incorporated into the soil [14]. Various plant extracts and plant parts have recently been reported to have antinematodal properties [15-20]. Leaves,

bark, fruits, flowers and seeds of many plants are also found to be toxic to plant parasitic nematode, especially to root-knot nematode [21-24]. Plant grown in the cultivated land, road side may be used to prepare the solution or paste for application against root-knot disease of vegetables [22, 25].

Number of reports are available on the management of root-knot but application of different treatments viz. BAU-Biofungicide, nematicide and plant extract like allamanda leaf on the management of root-knot of Indian Spinach so far is not done in Bangladesh. Therefore, for the first time we want to observe the effect of BAU-Biofungicide, nematicide and allamanda leaf extract against root-knot of Indian spinach.

2. Materials and Methods

The experiment was conducted in the research field of the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Bangladesh, from June to August 2008.

2.1. Treatments and Layout of Experiments

BAU-Biofungicide, nematicide carbofuran and allamanda leaf extract were used in the following treatments: T₀: Control; T₁: Seed coating with biofungicide; T₂: Side dressing with biofungicide (after and before inoculation); T₃: side dressing with carbofuran; T₄: side dressing with allamanda leaf extract. Each treatment was replicated thrice followed by Randomized Complete Block Design (RCBD) with two varieties. The experimental plots were prepared by proper plowing and laddering with the requisite amount of manures and fertilizers.

2.2. Collection and Sterilization of Seeds

Seed samples of two Indian spinach varieties, V1: BARI Spinach-1 (Chitra) and V2: local variety were collected from Bangladesh Agricultural Research Institute, Gazipur and local market of Dinajpur, Bangladesh, respectively. Before sowing seeds, surface sterilization of the seeds were done with mercuric chloride solution (0.001%) for 1-2 minutes followed by three times washing with sterile distilled.

2.3. Collection and Application of BAU-Biofungicides

For seed treatment, BAU-biofungicide (*Trichoderma* sp.) was collected from Disease Resistance Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Seeds were treated with biofungicide @ 1:40 w/w [26] by moistening the seed with rice forth in sterilized petridishes. The seeds were thoroughly mixed with the biofungicide by shaking the petridishes and placed in a cool and dry place under shade for drying and seeds were sown in the field by maintaining proper moisture.

BAU-Biofungicides were applied as side dressing in two installments @ 500 mg per plant, around the root region of seedlings before and after 5 days of inoculation of seedlings.

2.4. Application of Carbofuran

Granular nematicide (carbofuran 10G) was collected from the local market of Dinajpur, Bangladesh and were used as side dressing in two installments @ 500 mg per plant.

2.5. Preparation and Application of Allamanda Leaf Extracts

Twenty five grams (25 g) of allamanda shade dried leaf powder were macerated separately with 50 ml of sterile distilled water using mortar and pestle and filtered through four layer of cheese cloth. The prepared solution was applied by side dressing @ 5 ml per plant.

2.6. Inoculation of Indian Spinach Plant

After 20 days of sowing, each Indian spinach plant was inoculated with the eight egg masses collected from brinjal plants. On each side of the plant, 4 egg masses were placed near the base (root-region) of the seedlings opening the soil.

2.7. Recording and Analysis of Data

After 50 days of inoculation, data on the length of shoot and root (cm), weight of shoot and root (g), number of leaf per plant, number of galls per g of root and number of egg masses per g of root were recorded.

All the recorded data were analyzed following MSTAT-C program and mean difference was evaluated by Duncan's Multiple Range Test (DMRT) at 0.05 levels.

3. Results

Our aim was to observe the effect of BAU-biofungicide and allamanda leaf extracts for the first time against root-knot disease of Indian Spinach.

3.1. Effect of Treatments on Plant Growth Parameters

The effect of BAU-biofungicide, nematicide (Carbofuran 10G) and allamanda leaf extract were recorded on shoot length, shoot weight, root length, root weight, no. of leaf/plant, number of galls/g of root, no. egg masses /g of root. The highest shoot length was obtained in T₃ when plant treated with carbofuran as side dressing (69.50 cm) followed by side dressing with BAU-biofungicide (T₂, 48.17 cm) whereas the lowest shoot length was obtained from control (38.17 cm). The maximum shoot weight (97.50 g) was found with carbofuran 10G as side dressing (T₃) followed by BAU-biofungicide (85.83 g) and allamanda leaf extract (82.83 g) and the minimum shoot weight was found from

control (68.50 g). Similar to the shoot length, the maximum root length was observed in carbofuran treated plant (T_3 , 52.50 cm) which is statistically similar with BAU-Biofungicide (48.00 cm) treating plants as side dressing (T_2) and least root length was found from control (26.17 cm). Likewise other parameters, the highest root weight was also obtained from side dressing with carbofuran (30.17 g) followed by BAU-biofungicide (25.47 g) and the lowest root weight was obtained from control (18.97 g). Since leaf of Indian Spinach is the consumable parts, we have also observe the effect of the treatments on leaf number per plant. The maximum leaf plant⁻¹ was observed in T_3 when plant treated with carbofuran (44.00) as side dressing followed by BAU-biofungicide (37.50) and the minimum

leaf plant⁻¹ was found from control plant (28.17) (Figure 1).

3.2. Effect of Treatments on Root-knot Parameters

Number of galls and the egg masses on roots are the two important parameters to evaluate the root-knot intensity in plants. The highest no. galls/g of root (21.50) was found in control plant whereas, the lowest no. galls/g root (6.67) was observed in carbofuran treating plant as side dressing (T_3) which is statistically similar with BAU-Biofungicide (9.17). Similarly, the highest no. egg masses /g root was found in T_0 (9.17) and the lowest no. egg masses /g root found in carbofuran (1.67) followed by BAU-Biofungicide treating plant (T_2 , 3.33) (Figure 1).

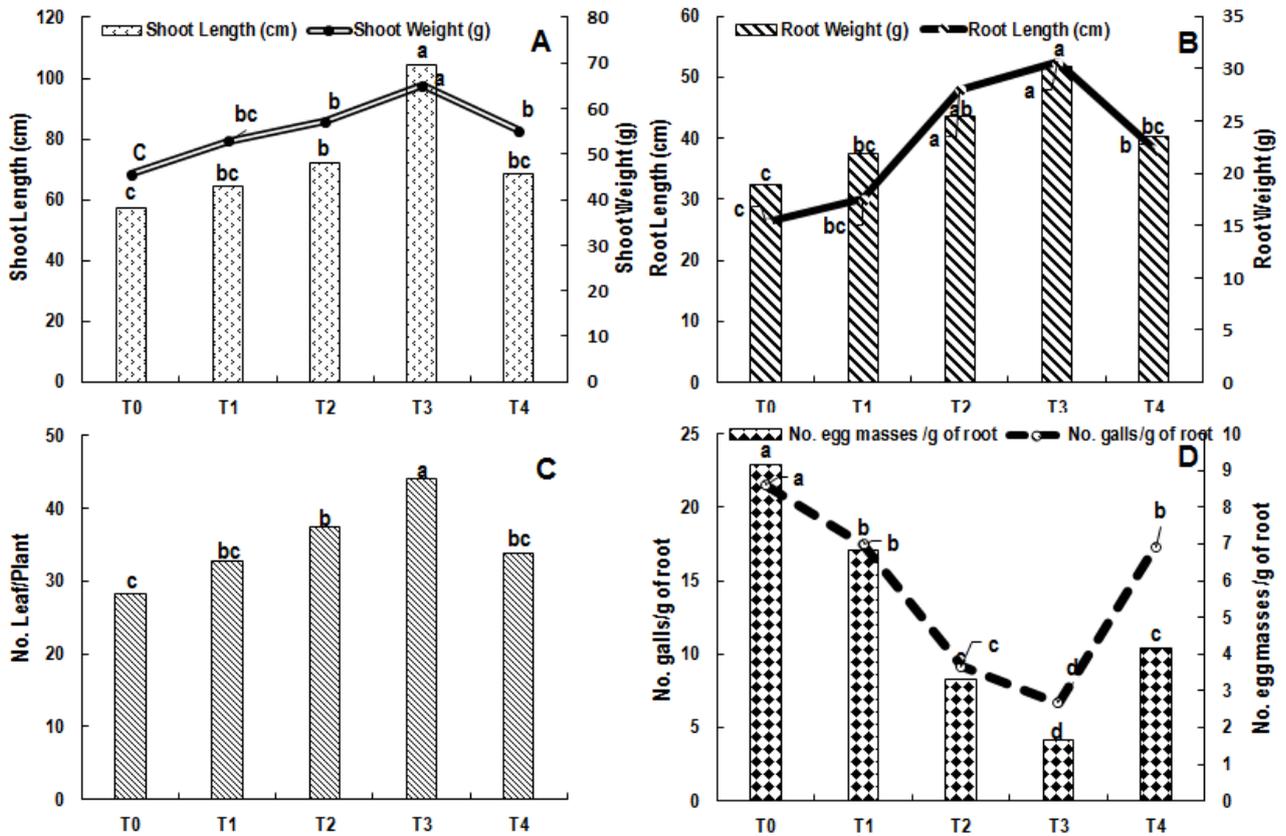


Figure 1. Effects of different treatments on the (A) Shoot length (cm) and weight (g), (B) Root length (cm) and weight (g), (C) No. of leaf/plant, (D) No. of galls/g of root and no. of egg masses/g of root of Indian Spinach after inoculated with *Meloidogine incognita*

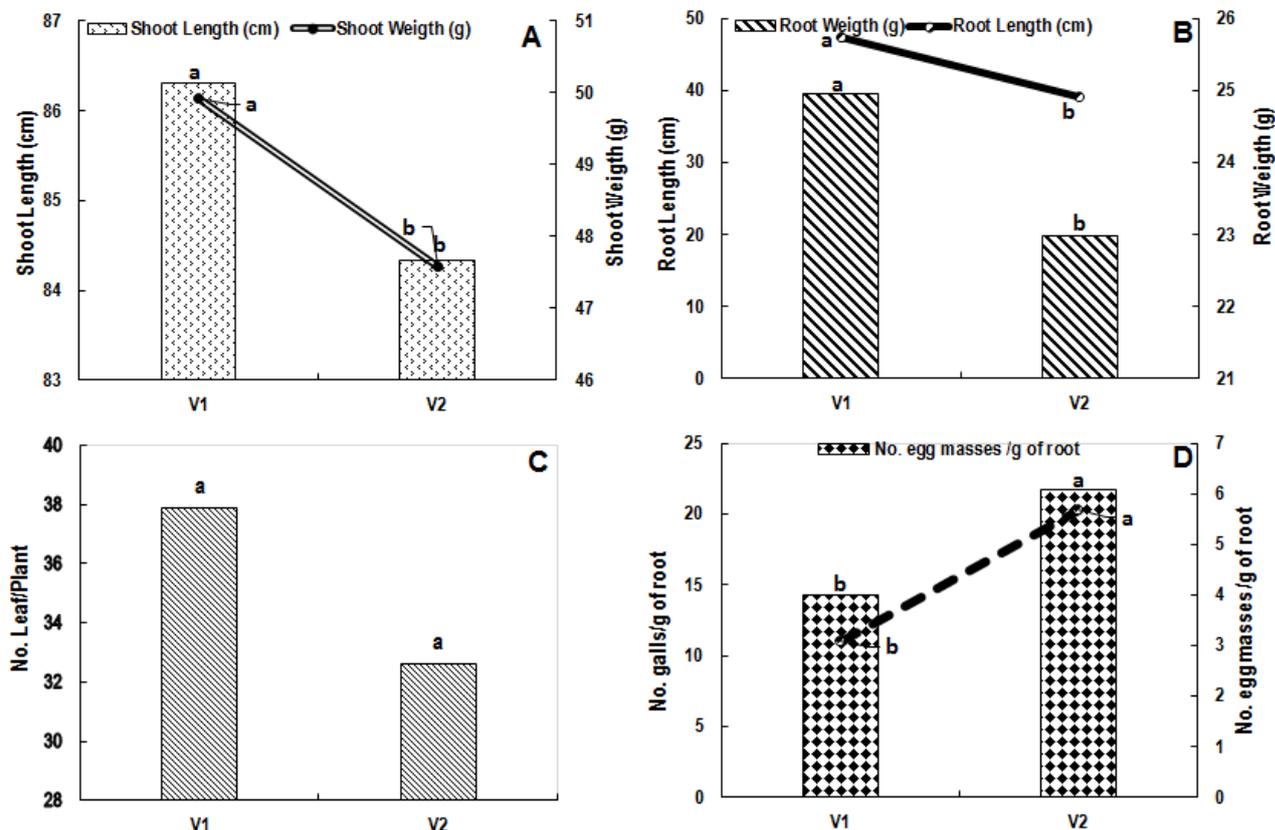


Figure 2. Effects of two Indian Spinach varieties on the (A) Shoot length (cm) and weight (g), (B) Root length (cm) and weight (g), (C) No. of leaf/plant, (D) No. of galls/g of root and no. of egg masses/g of root after inoculated with *Meloidogyne incognita*

3.3. Responses of the Two Indian Spinach Varieties on the Plant Growth Characters and Root-knot Parameters

We have used two varieties in this study and also observed their response against the treatments in regards to length of shoot and root, weight of shoot and root, number of leaf per plant, number of galls per g of root and number of egg masses per g of root (Figure 2). The BARI Indian Spinach 1 (V₁) gave significantly higher length of shoot and root, weight of shoot and root compared to the local variety (V₂), while no statistical significant variation was found in terms of number of leaf plant⁻¹. In regards with the root-knot intensity, the highest number of galls (20.27) and number of egg mass (6.07) per g of root were recorded with the local variety (V₂) in compare to the BARI Indian Spinach 1 (V₁).

3.4. Interaction Effects of the Treatments and Varieties on the Plant Growth Characters and Root-knot Parameters

We have also observed the interaction effect of the treatments and the varieties on the plant growth, galling incidence and development of egg masses (Figure 3). Statistical significant differences were found among the treatments with the individual variety in terms of shoot and root length, shoot and root weight and number of leaf per plant but no similar kind of responses were found in the interaction of the treatments with the two varieties (BARI Indian Spinach 1 and local variety). However, Interaction effects of the treatments and varieties was found to be statistically significant in respect of number of galls and egg masses per gram of root. In side dressing with carbofuran, the BARI Indian Spinach 1 (V₁T₃) gave significantly highest response to the number of galls/g of root (1.00) followed by BAU-Biofungicide (V₁T₂) (4.66) as compared to the local variety (V₂T₃, 11.67) and (V₂T₂, 12.33), respectively. The lowest no. of egg masses per g of root (0.33) were obtained from side dressing of carbofuran treating plants of BARI Indian Spinach 1 (V₁T₃) while the highest no. of egg masses per g of root was found in control (9.33) and (9.00) of both of the Indian Spinach varieties (V₂T₀ and V₁T₀). (Figure 3)

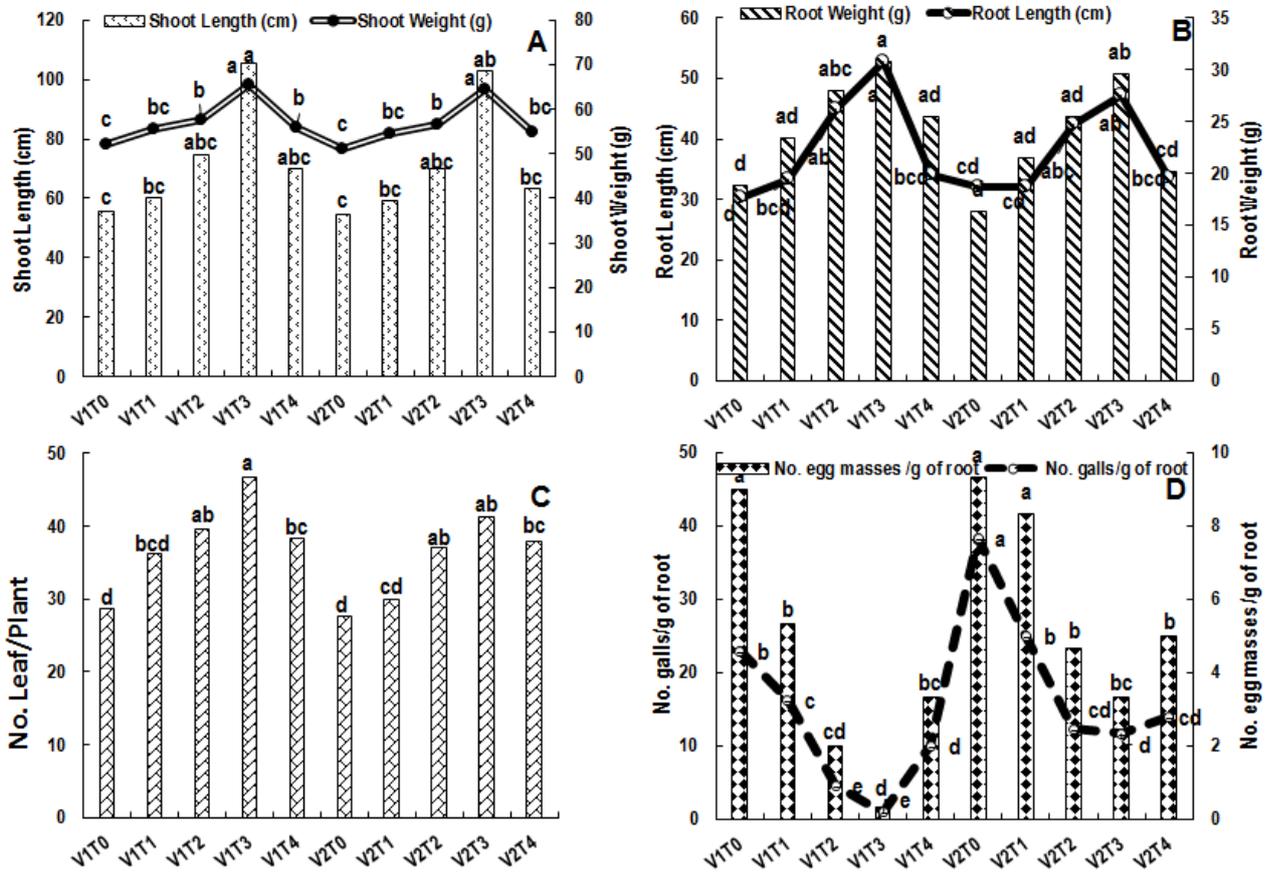


Figure 3. Interaction effects of different treatments and varieties of Indian Spinach on the plant growth parameters, galling incidence and development of egg masses after inoculated with *Meloidogyne incognita*

4. Discussion

For the first time we aimed to study the root-knot disease of Indian Spinach in Bangladesh. The effect of BAU-Biofungicide, allamanda leaf extract and nematicide (carbofuran 10G) against root-knot (*Meloidogyne incognita*) of Indian spinach was found significant in respect to plant growth characters and suppression of nematode development in two Indian Spinach varieties (BARI Indian Spinach 1 and local variety).

The results indicated that maximum length of shoot and root, fresh weight of shoot and root, number of leaf per plant were obtained with the treatment of carbofuran as side dressing followed by side dressing with BAU-Biofungicide, allamanda leaf extract and seed coating with BAU-Biofungicide (Figure 1). On the other hand, control treatment gave significant reduction in all plant growth characters correspondingly with maximum galling incidence and development of egg masses.

These results suggested that carbofuran and BAU-Biofungicide (*Trichoderma harzianum*) showed better responses with increased growth of shoot and root and higher weight of shoot and root with lower galling incidence as well as egg mass development as compared to control of both of the Indian Spinach varieties. Between the two varieties

BARI Indian spinach 1 showed better plant growth characters and suppressing the nematode activities in compare with the local variety (Figure 2). Similarly, the interaction effects of the treatments and varieties also revealed that both of the Indian Spinach varieties interacting with carbofuran and BAU-Biofungicide gave superior responses in increasing most of the plant growth characters and reducing the galling incidence followed by allamanda leaf extracts (Figure 3).

Carbofuran is systemic in nature; this chemical inhibits the growth and development of the nematode inside the plants by suppressing its activities. As a result, plant metabolism was less distributed and allowed better growth of the plant. Carbofuran treated cowpea plant roots inoculated with *Meloidogyne* spp. shows less gall formation [27]. Carbofuran can reduce the root-knot nematode (*Meloidogyne incognita*) incidence, severity rating, root-gall index, reproduction factor and the degree of resistance in Hybrid Yam (tuber) Varieties which alter the tuber production and also reduced root galls of *Meloidogyne incognita* in the soil as well as improved growth of infected tomato plants [28-29]. Both types of applications of furadan 5G and Miral 3G against root-knot of brinjal in granular and liquid forms gave superior results on plant growth characters with corresponding lower number of galls, adult females and

eggmasses [30]. The statements of all these authors are in agreement with the present findings.

The control of root-knot disease caused by *Meloidogyne incognita* with BAU-Biofungicide (*Trichoderma harzianum*) also gave better growth of shoot and root with lower galling incidence and eggmass development of two Indian Spinach varieties in this study. The effectiveness of *Trichoderma* spp. may be attributed to the fact that the fungi occupy the niche before nematode infection and thereby hinder the establishment of the nematode pathogen [31-33].

Biocontrol agents improve the health of plants and thus contribute to overall productivity. These agents are also self-propagating under favourable conditions, and therefore, may remain in the soil for a long period.

Since, chemical agents like carbofuran are efficient in controlling nematodes [28], their persistence may pose ecological problems [34]. Therefore, biocontrol is suggested to be a safer solution. Although chemical nematicide carbofuran appeared to be more effective in nematode control but their high costs, health and environmental hazards may discourage many farmers. On the other hand, BAU-Biofungicide showed better results on plant growth characters, suppression of galling incidence and egg mass development of Indian Spinach. Therefore it is recommended that control of root-knot disease of Indian Spinach may be reduced by the use of bio-agent like BAU-Biofungicide instead of chemical nematicide for eco-friendly management of this nematode disease. However, field trial is essential before any recommendation is made to the farmers.

5. Conclusions

All the treatments used in this study are found to reduce or suppressed the population of plant parasitic nematode in the infested soil. The highest plant growth characters of Indian Spinach in respect of length of shoot and root, fresh weight of shoot and root and reduced incidence of galling were found by the use of carbofuran. Application of BAU-Biofungicide as side dressing also found to be quite effective on increasing of growth of Indian Spinach with reduced galling incidence and nematode development like carbofuran. Allamanda leaf extract also showed significant effect on growth of the vegetable with reducing to root-knot as compared to control. However, to find out the most effective and eco-friendly management practices of root-knot disease, further in depth research with BAU-biofungicide and allamanda leaf extracts against Indian Spinach is necessary in field level.

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