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RESEARCH ARTICLE

EFFECT OF BIO-NEMATICIDE AND BAU-BIOFUNGICIDE AGAINST ROOT-KNOT (MELOIDOGYNE SPP.) OF SOYBEAN

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ABSTRACT

Meloidogyne spp. considered highly dangerous on soybeans. It is very difficult to find the suitable method for controlling without affecting on the environment. Therefore, in this study we used Four treatments with a newly developed Bio-nematicide, BAU-Biofungicide, Bio-nematicide + BAU-Biofungicide including control were tested against root-knot (*Meloidogyne incognita*) of two soybean varities (Sohag and BARI Soybean-5). The bio-agents were used as side dressing. Bio-nematicide in combination with BAU-Bio-fungicide showed the best performance with the highest length of shoot and root, fresh weight of shoot and root with nodules, weight of seeds and number of nodules per plant correspondingly with decreased number of galls and adult females of the nematode. Bio-nematicide and BAU-Biofungicide showed better performance in plant growth characters, yield of seeds and nodulation resulting in reduced galling and nematode development. BARI soybean-5 appeared with higher plant growth characters, nodulation and yield with reduced galling compared to variety Sohag. Positive response was observed with Bio-nematicide interacting with all the varieties of soybean. Negative correlation was found between gall numbers and all plants growth, nodulation and yield components. The combination between biological control agents is useful for the supporting and succeeding the biocontrol of Meloidogyne incognita. Thus, it is leading to save the environment from the residue of pesticides.

KEYWORDS

soybean, Bio-nematicide, biocontrol, Trichoderma, *Meloidogyne*.

1. INTRODUCTION

The soybean [Glycine max (L.)] is a species of legume native to East Asia which is an important source of fats, minerals, proteins, vitamins and energy for human and livestock (Tamagno et al., 2018; Aslam et al., 2019). Like many other legumes it can fix atmospheric nitrogen symbiotically. Yield of soybean in Bangladesh is unexpectedly lower than other soybean producing countries of the world. Root-knot diseases is a major constraint for higher production of soybean. Root knot nematode *Meloidogyne* genus widely occurs all over the world with a large host range which is one of the most important pests limiting the productivity in agricultural field productivity (Ramzan et al., 2019). The soil and climatic conditions of Bangladesh has made her an ideal abode for nematodes. For controlling nematidic diseases different chemical and organic ingredients are used having problems like toxicity to wide range of soil organisms, and appearance of resistant strains among nematodes, though farmers are using those to control plant-parasitic nematodes broadly (Lafta and Kasim, 2019; El-Dabaa et al., 2019). Furthermore, chemical nematicides are so costly.

BAU-Biofungicide and Bio-nematicide using as nematode killing agents are new approaches as eco-friendly measures. Potentially antagonistic microorganisms in minimizing the crop damage by the soil borne pathogens has been reported (Uikey et al., 2019). *Trichoderma* species were evaluated for their efficiency in controlling soil borne plant pathogenic nematodes like *Meloidogyne spp* (Lafta and Kasim, 2019; El-Dabaa et al., 2019). It is reported that *Trichoderma* could decrease the pathogenic factors associated with root-knot nematodes and could be applied as controlling agents for *Meloidogyne* spp. Researchers found that All tested variants suppressed nematode reproduction and root galling and result in plant growth improvement compared to the control (Yankova et al., 2014). The lowest rate of infestation and the highest total yield were established in the combination BioAct WG and *Trichoderma viride* strain T6. Till now little attention has been given for controlling the disease by biological means without any disturbance of the natural environment and beneficial microorganism. The main objective of this study was undertaken for observing the effect of bio-control agents BAU-Biofungicide and a newly developed Bio-nematicide for controlling root-knot (*Meloidogyne spp.)* of Soybean.

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2. MATERIALS AND METHODS

2.1 Site Selection

The experiment was conducted in the Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh. Sandy loamy soil, sand and well decomposed cow dung were taken at the ratio of 2:1:1 and mixed uniformly. At the rate of 3% (V/V) formalin was used to sterilize for per cubic feet of soil. Polythene sheet was used for covering the formalin treated soil and allowed to stay for 72 hours without any disturbance. The polythene sheet was removed after 72hours and the sterilized soil was exposed for air drying for 48hours to remove excess vapor of formalin. Forty earthen pots (30cm diameter) were taken and each was provided with a small broken piece of brick on the bottom of the pore of earthen pot and filled with 5kg of sterilized and dried soil.

2.2 Seed Collection

Seeds of soybean variety BARI Soybean-5 were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

2.3 Collection of Trichoderma harzianum

BAU-Biofungicide was collected from the Disease Resistance laboratory, Department of Plant Pathology, BAU, Mymensingh.

2.4 Collection of Bio-nematicide

Bio-nematicide was collected from Department of Plant Pathology, BAU, Mymensingh.

2.5 Sowing of soybean seeds and after care of seedling

Pot soil was loosened properly and five seeds of variety BARI soybean-5 were directly sown in the respective pots of treatment. Equal number of surface sterilized seeds was directly sown in ten pots designated as control. One healthy seedling was allowed to grow in each pot by removing the others after germination of seeds. Necessary weeding, irrigation etc. were done when necessary.

2.6 Preparation of inoculum and inoculation of soybean plants

Few brinjal plants were previously inoculated with single egg mass of (*Meloidogyne incognita)* growing in pots with sterilized soil. Severely galled root system of brinjal was used for collecting mature egg masses of root-knot nematode. Reddish brown mature egg masses were collected from infected roots of these plants with the help of fine forceps for inoculation. A moist petri dish was used for keeping egg masses. After 20 days of planting, each soybean plant was inoculated with 6 egg masses collected from infected brinjal plants. On each side of the plant, 3 egg masses were placed on the exposed roots of the seedling by opening the soil at the stem base.

2.7 Preparation of Bio-nematicide

Neem seeds, leaf and stem bark dried. After drying all plant parts were blended with blender. Neem Plant powder were mixed with talc powder as 1:1 ratio to use in the soybean pot experiments.

2.8 Application of Bio-Nematicide and BAU-Biofungicide

Both Bio-nematicide and BAU-Biofungicide were used as seed coating during seed sowing at the rate of 6g per kg of soybean seeds and side dressing as secondary dose at 20days after germination. Total three secondary doses have been applied at the rate of 1g of Bio-nematicide and BAU-Biofungicide. Ten gram soybean seeds of BARI Soybean-5 variety mixed with Bio-nematicde, BAU-Biofungicide and Bio-nematicide + BAU-Biofungicide respectively. One milliliter (1ml) water added to mixing the inoculum with soybean seeds and left for 30minutes in cool and shady place for proper coating. Control treatment seeds were treated with one milliliter (1ml) water.

2.9 Different parameters studied

After 95 days of inoculation, the plants at mature stage were carefully uprooted from the pots and the following parameters in relation to plants and pathogen were studied:

2.10 Measurement of length and fresh weigh of shoot and root

Length of shoot was measured from the base of the stem up to the top most leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root apex. The shoot and root portion were blotted with fine tissue paper and fresh weights were measured by electrical balance before the materials could get desiccated.

2.11 Counting number of galls g^{-1} of root

Randomly 1g of fresh root was taken from the bulk to count the number of galls formed. Average number of galls g^{-1} of root was counted from five replicated plants. Then, the roots were preserved in 5% formalin solution.

2.12 Number of adult females, J_2, J_3 and J_4 juveniles in 5 galls/treatment

Number of adult females, J_2, J_3 and J_4 juveniles were counted and recorded after proper staining of the galls.

2.13 Statistical analysis of data

All data were analyzed following standard procedures for analysis of variance. Differences between means were evaluated for significant level following a modified Duncan's Multiple Range Test (DMRT). Linear correlation co-efficient and determinations of the slope and intercept values of linear equations were also performed following standard statistical methods. Except where otherwise stated, differences referred to, in the text were significant at $P \geq 0.05$ level of probability.

3. RESULTS

3.1 Effect of different treatments on the growth, yield, nodulation, galling incidence and egg mass development in soybean

In the present study, four treatments with Bio-nematicide, BAU-Biofungicide, Bio-nematicide + BAU-Biofungicide including control were used to assess their effect on different plant growth characters, yield, nodulation, galling incidence and development of adult females and juveniles of root knot nematode (*Meloidogyne incognita*) in soybean.

3.1.1 Length of shoot

Lengths of shoot were significantly influenced by the treatments. Mean length of shoot ranged from 31.32cm to 49.20cm. The highest shoot length was recorded with treatment T_3 (Bio-nematicide + BAU-Biofungicide) having 49.20cm followed by treatment T_2 (BAU-Fungicide) and T_1 (Bio-nematicide) having 44.51cm and 37.24cm, respectively. The control treatment T_0 gave the lowest response with minimum shoot length 31.20cm (Table 1; Plates 1a, 1b, 2a and 2b).

Table 1: Effects of different treatment on the growth, yield, nodulation, galling, egg masses in soybean after 95 days of inoculation

Treatments	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodule (g)	Number of pods per plant	Weight of pods per plant (g)	Number of seeds per plant	Weight of seeds per plant (g)	Number of nodules per plant	Number of galls per g of root	Number of egg masses per g of root
T_0	31.32d	13.16c	9.29b	1.31b	14.70d	7.39d	26.50d	5.97c	2.60c	3.65a	2.59a
T_1	37.24c	16.69b	10.31b	1.49b	21.80c	9.89c	37.70c	7.97b	8.00b	1.75c	0.91b

T_2	44.51b	17.11b	10.28b	1.50b	28.20b	11.04b	53.90b	8.50b	9.80b	2.99b	2.71a
T_3	49.20a	24.62a	14.31a	2.07a	36.00a	13.26a	66.30a	11.44a	11.10a	0.76d	0.72b
$S\bar{x}$	0.503	0.530	0.540	0.111	0.587	0.221	2.137	0.230	0.521	0.166	0.186

3.1.2 Length of root

Maximum length of root was recorded with treatments T_3 followed by T_2, T_1 and T_0 having 24.62cm, 17.11cm, 16.69cm and 13.16cm, respectively. T_2 and T_1 gave statistically identical response in root length. Control treatment T_0 appeared with minimum root length (Table 1; Plates 3a, 3b, 4a and 4b).

3.1.3 Fresh weight of shoot

Fresh weight of shoot ranged from 9.29g to 14.31g. Significantly, the highest shoot weight 14.31g was found in plants treated with T_3 followed by the plants treated with T_1, T_2 and T_0 having 10.31g, 10.28g and 9.29g, respectively. Statistically lower and significantly identical response was found among the treatments T_2, T_1 and T_0 (Table 1).

3.1.4 Fresh weight of root with nodules

Maximum fresh weight of root with nodules was noted with treatment T_3 having 2.07g. Significantly lower and statistically similar fresh weight of root with nodules were observed with the treatments T_2, T_1 and T_0 having 1.50g, 1.49g and 1.31g, respectively (Table 1).

3.1.5 Number of pods per plant

Maximum number 36.00 of pods per plant was found with the treatment T_3 followed by T_2, T_1 and T_0 having 28.20, 21.80 and 14.70, respectively (Table 1).

3.1.6 Weight of pods per plant

Maximum weight of 13.26g of pods per plant was observed with the treatment T_3 followed by T_2, T_1 and T_0 having 11.08g, 9.89g and 7.39g, respectively. Control treatment gave the minimum weight of pods per plant (Table 1).

3.1.7 Number of seeds per plant

Like that of number of weight of pods per plant, treatment T_3 gave the highest number of seeds 66.30 per plant followed by T_2, T_1 and T_0 having 53.90, 37.70 and 26.50 number of seeds per plant, respectively. Control treatment appeared with the minimum number of seeds (Table 1).

3.1.8 Weight of seeds per plant

The highest significant seed weight 11.44g per plant was found with the treatment T_3 followed by 8.51g, 7.97g and 5.97 g in the treatments T_2, T_1 and T_0, respectively. But the treatments T_2 and T_1 gave statistically identical response in seed weight (Table 1).

3.1.9 Number of nodules per plant

Maximum and statistically identical numbers of 11.10 and 9.80 of nodules per plant were recorded with treatments T_3 and T_2 followed by 9.80, 8.00 and 2.60 nodules in the treatments T_2, T_1 and T_0, respectively (Table 1).

3.1.10 Number of galls per g of root

The control treatment T_0 was found to have significantly the highest number of 3.65 of galls per g of root followed by T_2 T_1 and T_3 having 2.99, 1.75 and 0.76 galls/g of root, respectively. The minimum number of galls/g of root was recorded in the treatment T_3 (Bio-nematicide + BaU-Biofungicide) having 0.76 (Table 1; 3a, 3b, 4a and 4b).

3.1.11 Number of egg masses per g of root

Significantly higher and statistically identical numbers 2.71 and 2.59 of eggmasses were observed with the treatments T_2 and T_0, respectively. Lower significant and statistically similar numbers of 0.91 and 0.72 of eggmasses were found with the treatments T_1 and T_3, respectively (Table 1, plates 3a, 3b, 4a and 4b).

3.2 Effect of different treatments on the development of *Meloidogyne incognita* in the inoculated soybean plants

Effects of five different treatments on the development of adult females J_2, J_3 and J_4 juveniles of *Meloidogyne incognita* in the soybean varieties are presented in Table 2.

3.2.1 Adult female

Maximum number 3.20 of adult females of *M. incognita* was found with the treatment T_2 followed by T_0, T_1 and T_3 having 3.00, 1.50 and 1.20, respectively. Treatments T_0 and T_2 appeared to have statistically similar response on the growth of adult females. Same was true for treatments T_1 and T_3 (Table 2; Plates 7, 9 and 10).

3.2.2 J_2 juveniles

Maximum number of 3.30 of J_2 juveniles was found with the treatment T_0 followed by T_2, T_1 and T_3 having 3.20, 1.50 and 1.20, respectively. Statistically identical responded was found between the treatments T_0 and T_2 as well as between T_1 and T_3 (Table 2).

3.2.3 J_3 juveniles

In case of J_3 the highest significant number 3.20 of J_3 juveniles was recorded with the treatment T_0 followed 3.00, 2.30 and 1.60 in the treatments T_2, T_1 and T_3 respectively. But, there was no significant difference between the treatments T_1 and T_3 as well as among the treatments T_0, T_1 and T_2 with respect to J_3 juveniles (Table2).

3.2.4 J_4 juvenile

In case of J_4 juveniles maximum number of 3.00 was recorded with the treatment T_0 followed by T_2, T_1 and T_3 having 1.80, 1.70 and 1.70, respectively. Significantly lower and identical response was found with respect to J_3 population among the treatments T_1, T_2 and T_3 (Table 2).

Table 2: Effect of different treatments on the development of adult and juveniles of *Meloidogyne incognita* on soybean after 95days of inoculation

Treatments	Number of adult females per 5gals	Number of J_2 juveniles per 5galls	Number of J_3 juveniles per 5galls	Number of J_4 juveniles per 5galls
T_0	3.00a	3.30a	3.20a	3.00a
T_1	1.50b	1.30b	2.30ab	1.70b
T_2	3.20a	3.10a	3.00a	1.80b
T_3	1.20b	1.50b	1.60b	1.70b
$S\bar{x}$	0.343	0.324	0.504	0.361

4. Discussion

Maximum length of shoot and root, fresh weight of shoot and root with nodules, weight of seeds per plant were obtained with the treatment Bio-nematicide + BAU-Biofungicide. In case of number of egg masses per plant, the treatment BAU-Biofungicide gave higher value than Bio-nematicide. In respect of length of shoot and root, fresh weight of shoot and root with nodules, weight of seeds per plant, number of nodules per plant control treatment with *M. incognita* alone was responsible for the significant reduction. In addition, the highest galling incidence correspondingly with the lowest yield performance was observed with control treatment.

Increased growth of plant yield and nematode controlling capability of BAU-Biofungicide treatments observed in this experiment could be because of release of growth promoting secondary metabolites and toxic metabolites of *Trichoderma*. Some scientists also observed increased growth in tomato, soybean, tobacco and capsicum in pot and field experiments after using Trichoderma inoculum (Goswami and Mittal, 2004).

As bio-agents Bio-nematicde and BAU-Biofungicide showed better responses with higher growth of shoot and root as well as higher weights of shoot and root with nodules, number of nodules per plant correspondingly with higher yield per plant as evident with higher weight of seeds. There appeared significantly lower galling incidence in both Bio-nematicide and BAU-Biofungicide treated plants indicating their suppressing effect on galling as observed with their combined application. Researchers reported *Trichoderma viride* as egg parasitic against *Meloidogyne incognita* in the present study, reduced galling incidence along with reduction of adult females might have been resulted from the adverse influence on hatching of inoculated eggs and eggs produced after first and second generation *Meloidogyne incognita* as similarly stated by the above authors (Goswami and Mittal, 2004). In this present study Bio-nematicide showed better result than BAU-Biofungicide in controlling adult nematode. It also showed significant result in controlling J_2, J_3, and J_4 nematode population. Furthermore, Bio-nematicide showed better performance than BAU-Biofungicide in controlling egg masses. But it showed lower responses than combined application of Bio-nematicide and BAU-Biofungicide in every sphere of investigation.

Among the obtained results Bio-nematicide was most against eggmasses. Nemato-toxic compounds of the neem plant, especially the azadirachtins, are released through volatilization, exudation, leaching and decomposition. The modes of action of these compounds are complex, and a number of mechanisms in relation to nematode management are yet to be fully explored. Azadiractin is considered strong anti-feedant because of its effects on the insect's chemoreceptors, which deter the insects from consuming the plant. Moreover, azadiractin not only blocks peptide hormone release that cause molting abnormalities but also cause damage in insect's tissues, including muscle, fat and gut cells. Researchers reported inhibitions in most fungal plant pathogens by substances produced by Trichoderma spp. and suggested that controlling other soil-borne pathogenic fungi *Trichoderma harzianum* will further provide a better, enabling environment where plants will develop more vigorously without being suppressed by other microorganisms, which would otherwise affect or make it vulnerable to secondary infections pioneered by plant-parasitic nematodes (Izuogu et al., 2019).

The findings of the present study can be correlated with of Akhtar and Malik, where they have reported that phenols, amino acids, aldehydes and fatty acids are release from neem which is antagonistic to root knot nematodes (Akhtar and Malik, 2000). Our results are supported by the study of previous work (Ganai et al., 2014; Lal and Rana, 2012). They states that organic amendments of soil using dried poultry litter, municipal refuse, oil cakes of ground nut, neem mustard & neem products have been found effective in the control of *Meloidogyne incognita*. In present study thus it may be concluded that changes in protein after infection are related to defence action, because abnormal metabolites are produced in adjacent non-infected tissues. Such metabolites accumulated in infected tissues and are toxic to parasites and inhibit their growth and penetration.

The metabolites released from the chemical constituents of neem stimulated the plant cells to release abnormal metabolites which repel the nematodes from the uninfected cells of plant. So, the use of neem products stimulated and changes the physiology of plant cells and tissue to repel the nematode parasites. In this study the combined effect of Bio-nematicide and BAU-Biofungicide gave higher significant growth of shoot and root, significant amount of fresh weight of shoot and root, higher number of pods per plant and weight of pods per plant, weight of seeds per plant, higher number of nodules with lower galling incidence and reduced population of adult females of *M. incognita.*

From the overall study, it was revealed that the highest plant growth characters of soybean in respect of length of shoot and root, fresh weight of shoot and root with nodules, weight of seeds per plant, number of nodules per plant and reduced incidence of galling with lower development of adult females of *Meloidogyne incognita* were achieved by the side dressing with Bio-nematicide + BAU-Biofungicide as well as side dressing with Bio-nematicide compared to the control treatment. The efficacy of Bio-nematicide to control root-knot disease of soybean along with improved plant growth characters, nodulation and yield components were found almost equally good to combined application of BIO-nematicide with BAU-Biofungicide. It is also evident that control of *Meloidogyne incognita* with antagonistic bio-agents like Bio-nematicide and BAU-Biofungicide as side dressing components are quite effective.

5. Conclusion

Using chemical fungicide to control root-knot disease of soybean caused by *M. incognita* is so costly and harmful for our environment but this diseases may be controlled through use of Bio-fungicide for eco-friendly management. But, field trial is essential before any recommendation in made to the farmers. Considering the importance of soybean as a valuable crop and its greater yield loss due to the nemic root-knot disease, more attention has to be given for the control of the disease by biological means without disturbing the natural balance and the environment.

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