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Effect of Bio-agents on growth and root-knot (*Meloidogyne javanica*) disease of soybean

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Abstract The effects of four treatments with BINA-Biofertilizer, BAU-Biofungicide, BINA-Biofertilizer + BAU-Biofungicide as bio-agents along with a control were tested against root-knot (*Meloidogyne javanica*) of three soybean varieties (ASOME-SEME, ACC-1222 and ASSET-93-19-3). The bio-agents were used as seed treatment. The bio-agents individually and combinedly increased significantly the plant growth in terms of 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. The plants treated with BAU-Biofungicide performed better than that of BINA-Biofertilizer followed by their combination. The highest number of nodules per plant was found with BINA-Biofertilizer. ASSET-93-19-3 appeared with higher plant growth characters, nodulation and yield with reduced galling compared to other varieties. Negative correlations were found between gall numbers and plant growth characters with nodulation and between gall numbers and yield components.
Key words: Soybean, Bio-agent, *Meloidogyne javanica*

Introduction

Soybean [(*Glycine max* L.) Merrill] belongs to the family *Leguminosae*. It is a well-recognized grain legume of the world. Now-a-days, soybean has become the most important oil seed crop all over the world. Soybean may be called the "Protein hope of future" because of its high nutritive value containing about 42-45% protein, 18-20% edible oil and 42-46% carbohydrate (Gowda and Kaul, 1982). On an average, about 8-10% of the protein intake in Bangladesh diet originates from animal sources (Begum, 1989) and the rest can be met from plant sources by increasing the consumption of vegetables and pulses including soybean. Soybean plants, like many other legumes can fix atmospheric nitrogen symbiotically and about 80-90% nitrogen demand could be supplied by soybean through symbiosis (Bieranvand *et al.,* 2003).

The low yield of soybean is discouraging in Bangladesh compared to other soybean producing countries. This is mainly due to the infection of different diseases. Among the diseases, root-knot is one of the constraints for soybean production (Mian, 1986). Valiente *et al.* (1990) stated that *Meloidogyne* spp. decreased soybean yield to a great extent. Ahmed and Srivastav (1996) observed significant reduction in all growth parameters of soybean due to the infection of *Meloidogyne incognita*. So, to increase the yield and quality of produce the effective, economic and eco-friendly disease management deserves priority. Bio-control, a modern approach of disease management can play a significant role here. *Trichoderma* based BAU-Biofungicide has been found promising to control root knot diseases of French bean (Rahman, 2005). Use of antagonist bacteria like *Rhizobium* and *Bradyrhizobium* also has significant effect in controlling root knot of mungbean (Khan *et al.,* 2006). Thus, the present study was undertaken to see the effect of BAU-Biofungicide (*Trichoderma harzianum*), BINA-Biofertilizer (*Bradyrhizobium* sp.) and their combination in controlling root knot disease caused by *Meloidogyne javanica*, and subsequent plant growth of soybean.

Materials and Methods

Sixty earthen pots (30 cm diameter) were filled with sterilized soil (5 kg soil per pot) composing loamy soil, sand and well decomposed cowdung at a ratio of 2:1:1 to raise soybean plants. Before filling with soil, the pots were sterilized with formalin with a view to avoiding any contaminations. Seeds of three varieties of soybean, ASOME-SEME (V_1), ACC-1222 (V_2) and ASSET-93-19-3 (V_3) were collected from the Germplasm Centre, Department of Genetics and Plant Breeding, BAU, Mymensingh. The peat based inoculant *Bradyrhizobium* sp. for soybean was collected from the division of Soil Science, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. BAU-Biofungicide was collected from the Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Four treatments used in the experiment are To = Control, T1 = BINA-Biofertilizer, T2= BAU-Biofungicide and T3= BINA-Biofertilizer + BAU-Biofungicide. Apparently healthy, mature and disease free soybean seeds were taken for the purpose of sowing. Before sowing, these seeds were treated with 10% chlorox solution for 30 seconds and were subsequently rinsed with sterilized distilled water for three times. For seed treatment, rice froth was added each in case to moisten the seed coat and then bio agents were thoroughly mixed with them. In case of T1 seeds were mixed thoroughly with BINA-Biofertilizer until proper coating of seeds. In case of T2 seeds were properly mixed with BAU-Biofungicide at a ratio of 1:40 w/w (Seed; BAU-Biofungicide) and in T3 i.e., seed were coated with BINA-Biofertilizer first and then again coated with BAU-Biofungicide. Soil in the pot was mulched properly and the seeds of ASOME-SEME (V_1), ACC-1222 (V_2) and ASSET-93-19-3 (V_3) were directly sown in the respective pots of treatment. Equal numbers of surface sterilized seeds were directly sown in few pots as control. It was done for all the varieties. Each pot received apparently three healthy and uniform sized seeds. The seeds were then thinly covered with soil. After germination of seeds, one healthy seedling was allowed to grow in each pot by removing the others. Weeding, irrigation, mulching etc. were done as and when necessary. Mature egg masses of root-knot nematode (*Meloidogyne javanica* L.) were collected from severely galled root system of brinjal (*Solanum melongena* L.) previously inoculated with single egg mass of *Meloidogyne javanica.* For inoculation, reddish brown mature egg masses were collected from infected roots with the help of fine forceps and then placed in a petridish containing 5 ml of sterile water to avoid desiccation. After 52 days of planting, each soybean plant was inoculated with eight egg masses collected from infected brinjal plants. On each side of the plant, 4 egg masses were placed on the exposed roots of the seedling by opening the soil at the stem base. Thus the experiment was designed in two factors Randomized Complete Block Design (RCBD) with five replications. After 75 days of inoculation, plants were carefully uprooted from the pots, washed with tap water gently carefully and different parameters were studied.

Results and Discussion

The three soybean varieties (ASOME-SEME, ACC-1222 and ASSET-19-3) showed significant differences based on their growth characteristics (length of shoot and root, fresh weight of shoot and root), nodulation, yield components (no. of pods plant^{-1}, weight of seeds plant^{-1}), galling incidence and number of egg masses plant^{-1} (Table 1). V_3 (ASSET-93-19-3) was the best variety as it showed better performance in most cases including the lowest incidence of galling per g of root. Responses of three varieties of soybean were found to be insignificant in respect of development of adult females, J2, J3 and J4 after inoculation with *Meloidogyne javanica* (Table 2). The effects of four different treatments on different plant growth characters such as nodulation, weight of seeds plant^{-1} and galling incidence were found to be significant ($P \geq 0.05$) (Table 3).T_2 (BAU-Biofungicide) performed the best in most cases compared to other three treatments followed by T_1 (BINA-Biofertilizer). Sharon *et al.* (2001) similarly observed increased fresh weight of shoot and reduction of galling incidence in tomato infected with *Meloidogyne javanica* following soil pretreatment with *Trichoderma* peat bran preparations. Senthilkumar and Rajendran (2004) reported that fungal agent *Trichoderma harzianum* appeared as egg parasitic or opportunistic to root-knot nematode. Yang-Xiu Juan *et al.* (2000) also reported that *Trichoderma harzianum* showed better control of *Meloidogyne javanica* in tomato. It is suggested that improved proteolytic activity of the antagonist may be important for the biological control of the nematode. Tverdyukev *et al.* (1994) observed that *Trichoderma* produces Trichodermin, which render its antagonistic activity against various diseases. Hoffmann-Hergarten *et al.* (1998) found that seed treatment with *Rhizobium* significantly decreased root galling and enhanced biomass. This is due to the fact that biofertilizer helps the Biological Nitrogen Fixation (BNF) through symbiotic relationship which enhances the plant growth. Similar results were found in this experiment where BINA-Biofertilizer (*Bradyrhizobium* sp.) gave maximum nodulation with comparatively 1ower galling incidence, adult females and juvenile growth stages than control. The treatments effects were also found to be significant compared to control on the population development of adult female nematodes in soybean varieties ASOME-SEME (V_1), ACC-1222 (V_2) and ASSET-93-19-3 (V_3) inoculated with *Meloidogyne javanica* (Table 4). Rahman (2005) also reported that seed treatment with BAU-Biofungicide reduced the number of adult females, J_2, J_3 and J_4 juveniles of *Meloidogyne javanica.* Li-Bin *et al.* (2005) similarly observed that rhizobacteria suppressed the root-knot nematode (*Meloidogyne javanica*). This rhizobacteria exhibited strong nematicidal activity by killing the second stage larvae of *Meloidogyne javanica*.

Table 1. Response on the growth, nodulation, yield, galling and egg masses in three soybean varieties inoculated with *Meloidogyne javanica*

Variety	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Number of nodules plant^{-1}	Number of pods plant^{-1}	Weight of seeds plant^{-1} (g)	Number of galls per g of root	Number of egg masses per g of root
ASOME-SEME (V_1)	47.27 b	23.91	14.57 c	2.45 b	3.25 b	8.95	10.68 a	2.15 a	1.60 b
ACC-1222 (V_2)	33.22 c	22.62	15.59 b	2.28 b	2.60 c	9.20	8.60 b	2.25 a	1.45 b
ASSET-93-19-3(V_3)	71.93 a	26.50	17.55 a	4.24 a	3.90 a	10.10	12.02 a	1.50 b	2.35 a
LSD ($P \geq 0.05$)	6.685	NS	0.506	1.018	0.311	NS	1.786	0.294	0.685

Each value is an average of five replications, NS = Not significant, Values in the column having same letter(s) do not differ significantly at $P \geq 0.05$ level by DMRT

Table 2. Response of three soybean varieties ASOME-SEME (V_1), ACC-1222 (V_2) and ASSET-93-19-3 (V_3) on the development of adult females and juveniles of *Meloidogyne javanica*

Variety	Number of adult females per 5 galls	Number of J_2 juveniles per 5 galls	Number of J_3 juveniles per 5 galls	Number of J_4 juveniles per 5 galls
ASOME-SEME (V_1)	0.85	0.85	0.75	0.65
ACC-1222 (V_2)	1.05	0.90	0.90	0.80
ASSET-93-19-3(V_3)	0.85	0.85	0.70	0.85
LSD ($P \geq 0.05$)	NS	NS	NS	NS

Table 3. Effect of different treatments on the plant growth, nodulation, yield, galling and egg masses in soybean varieties inoculated with *Meloidogyne javanica*

Treatments	Length of shoot (cm)	Length of root (cm)	Fresh wt. of shoot (g)	Fresh wt. of root with nodules (g)	No. of nodules plant^{-1}	No. of pods plant^{-1}	Wt. of seeds plant^{-1} (g)	No. of galls per g of root	No. of egg masses per g of root
T_0	45.06 b	18.16 c	12.01 e	3.25 ab	1.08 e	8.25	5.88 c	3.58 a	2.24
T_1	50.70 ab	24.28 ab	14.67 c	2.69 b	5.08 a	10.75	11.54 b	1.92 c	1.92
T_2	54.23 ab	28.18 a	17.33 b	2.51 b	3.25 c	9.67	11.47 b	1.17 d	1.50
T_3	46.15 b	21.14 bc	13.28 d	2.12 b	2.58 d	8.42	9.21 b	2.67 b	2.00
LSD ($P \geq 0.05$)	8.631	5.778	0.653	1.315	0.401	NS	2.306	0.379	NS

T_0 = Control, T_1 = BINA-Biofertilzer, T_2 = BAU-Biofungicie and T_3 = BAU-Biofungicie + BINA-Biofertilizer

Table 4. Effect of different treatments on the development of adult females and juveniles of *Meloidogyne javanica* in infected soybean varieties

Treatments	Number of adult females per 5 galls	Number of J_2 juveniles per 5 galls	Number of J_3 juveniles per 5 galls	Number of J_4 juveniles per 5 galls
T_0 (control)	1.33 a	1.25	0.92	1.00
T_1 (BINA-Biofertilzer)	0.83 ab	0.75	0.92	0.75
T_2 (BAU-Biofungicide)	0.67 b	0.75	0.58	0.58
T_3 (BINA-Biofertilizer+ BAU-Biofungicie)	1.17 ab	1.00	0.83	0.83
LSD ($P \geq 0.05$)	0.580	NS	NS	NS

Correlation study revealed significant and negative correlations between gall number and plant growth characters as well as gall number and yield components (Figures 1, 2, 3 and 4). It is evident from the study that control of *Meloidogyne javanica* with antagonistic bio-agents like BAU-Biofungicide (*Trichoderma harzianum*) and BINA-Biofertilizer (*Bradyrhizobium* sp.) used as seed treatments are quite effective. Therefore, control of root-knot disease of soybean caused by *Meloidogyne javanica* may be explored through use of BAU-Biofungicide and BINA-Biofertilizer for eco-friendly management of this nemic disease avoiding chemical nematicides. However, field trials are essential before recommendation for the farmers.

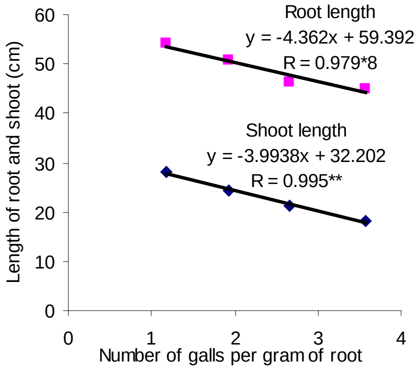

Fig. 1 Relationship between gall number and root and shoot length

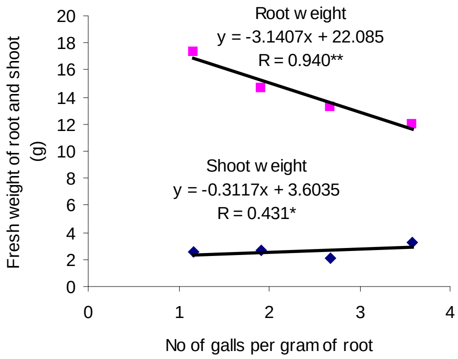

Fig. 2 Relationship between number of galls per gram of root and shoot fresh weight

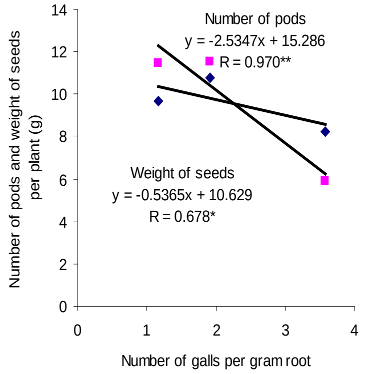

Fig. 3 Relationship between gall number and number of pods and weight ofseeds per plant (g)

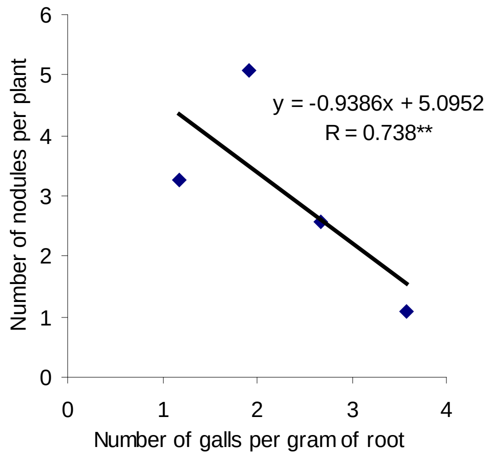

Fig. 4 Relationship between number of galls per gram of root and number of nodules per plant

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