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Comparative study of susceptibility, growth & yield of two varietiessoybean [*Glycine max* (L.) Merrill] varieties Shohag and AGS-334 against development of root-knot nematode (*Meloidogyne javanica*) with treatment BINA-Biofertilizer, BAU-Biofungicide, a chemical Nematocide.

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ABSTRACT

The study was carried out with four different treatments covering BINA-Biofertilizer, BAU-Biofungicide, a chemical nematicide (Curaterr) and a control to see Comparative study of susceptibility, growth & yield of two varieties soybean [Glycine max (L.) Merrill] varieties **Shohag** and **AGS-334** against development of root-knot nematode (Meloidogyne javanica) with treatment BINA-Biofertilizer, BAU-Biofungicide, a chemical Nematocide. Among the treatments, BINA-Biofertilizer and BAU-Biofungicide were used as seed treatment and nematicide Curaterr was used as side-dressing to see their effect against the root-knot nematode (Meloidogyne javanica) infecting two soybean varieties**Shohag** and **AGS-334**. All the treatments with BAU-Biofungicide, BINA-Biofertilizer and Curaterr gave significantly higher response in plant growth characters like length of shoot and root, fresh weight of shoot, fresh weight of root with nodules, weight of pods per plant, number of nodules per plant, weight and number of seeds per plant compared to the control treatment. Simultaneously lower galling incidence, and egg masses as well as lower development of adult females, J2, J3 and J4 juveniles of M. *javanica* were observed with those agents. **Varieties Shohag** gave betterfresh weight of shoot and nodulation, compared to the variety **AGS-334** other than higher J2 population in the variety Shohag, no significant differences were found among these two varieties**Shohag** and **AGS-334** on the development of adult females, J3 and J4 juveniles of M. *javanica*. Interaction effects of the treatments and varieties were found significant in respect of length of shoot and root, fresh weight of

shoot, number of nodules, number of seeds per plant and in cases of adult females and J2 population. In most of the cases positive responses were observed with the treatments with BINA-Biofertilizer, BAU-Biofungicide and Curaterr interacting with both of the varieties. Other than length and fresh weight of root with nodules, number and weight of seeds and nematode population the nematicidial treatment Curaterr gave more or less similar responses like that of BINA-Biofertilizer and BAU-Biofungicide.

INTRODUCTION

The soybean [Glycine max (L.) Merrill], a native of Eastern Asia, is an ancient crop with hundreds of food, feed and industrial uses. It is an introduced crop in Bangladesh. The soybean is a worldwide economic crop and the most important cultivated legume. The crop can be grown in tropical, sub-tropical as well as the temperate regions. Total production of soybean in Bangladesh during 2018 was 152 thousand tons from an area of 80 thousand hectares with an average yield of 1.5-2.3 tons per hector (BBS, 2018).

Soybean contains higher amounts of protein and oil than any other legume crops. Its seed contains about 40-45% protein and 18-22% oil. It provides around 60% of the world supply of vegetable protein and 33% of the edible oil. For these reasons soybean today is recognized as one of the leading agricultural crop in the world (Kaul and Das, 1986). Soybean is a good source of protein, lecithin, unsaturated fatty acids, minerals like Ca and P including vitamin A, B, C and D (Rahman, 1982). Soybean plants like many other legumes are capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with Rhizobium bacteria at the root system of the crop. This crop thus improves soil fertility and economizes crop production not only for itself but also for the next cereals and other non-legume crops grown in rotation and thereby, minimizing the regular rate of nitrogen fertilizer. There are many factors behind the low yield of soybean of which diseases pose great threat to its cultivation through yield reduction. Among the diseases, root-knot is a major constraint for soybean production. Santos et al. (2001) found that root-knot nematodes (Meloidogyne spp.) are the main pest of soybean in Brazil, M. javanica being one of the major economic important species. The root-knot nematodes, Meloidogyne spp., are widely distributed throughout the country (Mian, 1986) as Bangladesh lies in the sub-tropical region having hot and humid climate. The soil and climatic condition of Bangladesh has made her an ideal abode for nematodes. The nematode population in the soil of Bangladesh is increasing day by day (Chowdhury, 1976). The common species of root-knot nematode attack wide varieties of fruits, vegetables and field crops including soybean. Considerable damage to these crops may occur independently or in association with fungal or bacterial wilts and root rots (Sing, 1973). Root-knot nematodes due to their frequency of occurrence, cause high level of infestation along with other pathogens and these have been recognized as a major limiting factor in food production. In certain crops, the loss is heavy because root-knot predisposes the plants to invasion by other pathogens.

In Bangladesh different varieties of soybean cultivation is going on and nemic disease hamper the growth and yield of soybean. To control nemic diseases, different chemical ingredients are

used. In array of formulation, the majority of these chemicals having a broad-spectrum activity with well-known risk for consumers and to the environment. Besides, development of resistance of the pathogens against chemicals create problems to control them. Moreover, beneficial organisms are indiscriminately destroyed, environment is polluted, and finally collapse the ecosystem making control of pathogens increasingly difficult. Chemical nematicides are also costly. Use of BINA-Biofertilizer, BAU-Biofungicide in the control of root-knot nematode is a new approach as an eco-friendly measure. The management of nemic diseases can also be safely done using disease resistant cultivars, but hardly the resistant cultivars of the crops are available cultivars in Bangladesh which are resistant to root-knot diseases. Use of potentially antagonistic microorganisms in minimizing the crop damage by the soil borne pathogens has been reported (Lewis et al., 1995 and Lewis and Larkin, 1997). In biological control, microorganisms like Trichoderma spp., Paecilomyces lilacinus, Pasturia penetrans and Pseudomonas aeruginosa act either as antagonist, parasites or predators (Rao et al., 1997, Reddy et al., 1998 and Siddique et al., 1999). In biological control, living micro-organisms such as bacteria, viruses or fungi are employed as either antagonists, parasites or predator (Kwok et al., 1987). The use of antagonistic bacteria (Bradyrhizobium sp.) as biological control means may provide a great alternative for plant pathologist. In addition, Bradyrhizobium sp. could enrich soil nitrogen level through BNF (Biological Nitrogen Fixation) and crop plants could be benefited out of it. Khan et al. (1998) reported prominent antagonistic effect of Rhizobium against foot and root rot pathogens (Fusarium oxysporum and Sclerotium rolfsii) of lentil. They obtained higher seed yield and biomass production of lentil by using Biofertilizer in micro-plot trials.

Trichoderma has been found as effective bio-control agent of soil borne plant pathogenic nematodes like Meloidogyne spp. Devi et al. (2002) reported that treatment with Trichoderma improved plant height, shoot weight and root length and weight, and reduced nematode population. Sharon et al. (2001) evaluated Trichoderma harzianum for its potential to control the root-knot nematode Meloidogyne javanica and found that root galling was reduced and top fresh weight increased in nematode infested tomatoes following soil pretreatment with Trichoderma peat-bran preparations.

Different varieties of soybean has different level susceptibility against nemic disease and little attention has been given for controlling the disease by biological means without disturbing the natural balance and the environment. As an attempt to control the root-knot disease biologically without disturbing the environment the present study was undertaken with the objective of comparative study of susceptibility, growth & yield of two varieties soybean [Glycine max (L.) Merrill] varieties Shohag and AGS-334 against development of root-knot nematode (Meloidogyne javanica) with treatment BINA-Biofertilizer, BAU-Biofungicide, a chemical Nematocide

MATERIALS AND METHODS

The experiment was conducted in the glasshouse of the Seed Pathology centre, Bangladesh Agricultural University (BAU), Mymensingh. . The duration of the experiment ranged from the 2nd August 2005 to the 21st November 2005.

Preparation of soil and potting

Sandy loam soil, sand and well decomposed cowdung were taken at the ratio of 2:1:1 and mixed uniformly. After sterilized the soil with formalin, soil was covered by polythene sheet and allowed to stay for 72 hours without disturbance. After 72 hours, the polythene sheet was removed and the sterilized soil was exposed to air drying for 48 hours in order to remove excess vapor of formalin. Fifty earthen pots (30 cm diameter) were taken and each was provided with a small broken piece of earthen pot on the bottom pore and filled with 5 kg sterilized and dried soil.

Collection of Soybean seeds,BINA-Biofertilizer, & BAU-Biofungicide and chemical Nematocide.

Two varieties of soybean seeds, **Sohag (V1)** and **AGS-334 (V2)** were collected from BARI (Bangladesh Agricultural Research Institute), Joydebpur, Gazipur. The peat based inoculant *Bradyrhizobiumsp* for soybean was collected from the division of Soil Science, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. And BAU-Biofungicide was collected from Plant Pathologylaboratory, Bangladesh Agricultural University, Mymensingh. Chemical nematicide Curaterr was collected from a pesticide shop at Notonbazar , Mymensingh.

Surface sterilization of soybean seeds

Healthy, mature and disease free soybean seeds were taken for the purpose. Before sowing, these seeds were treated with 0.001% mercuric chloride solution for 30 seconds and were subsequently rinsed with sterilized distilled water for three times.

Seed coating with BAU-Biofungicide

Separate petridishes were taken the seeds of **Sohag (V1)** and **AGS-334 (V2)**. The petridishes were surface sterilized with methylated spirit before placing seeds inside them for treating with BAU-Biofungicide. Seeds were then treated with BAU-Biofungicide @ 1:40 w/w (BAU-Biofungicide: seed) following the method of Hossain (2003) by moistening the seeds with rice froth. The seeds were thoroughly mixed with the Biofungicide by shaking the petridishes. The coating of seeds was done and the coated seeds were placed in a cool and dry place under shade for drying and the seeds were ready for sowing in the pot on the same day in the afternoon.

Seed coating with BINA-Biofertilizer

Soybean seeds of varieties **Shohag (V1)** and **AGS-334 (V2)** were taken in separate petridishes. The petridishes were surface sterilized with methylated spirit before placing seeds inside them for treating with BINA-Biofertilizer. Seeds were then treated with Rhizobial inoculants by moistening the seeds with rice froth. The seeds were thoroughly mixed with the inoculant by shaking the petridishes. The coating of seeds were done and the coated seeds were placed in a cool and dry place under shade for drying and the seeds were ready for sowing in the pot on the same day in the afternoon.

Sowing of soybean seeds

Soil in the pot was mulched properly and the seeds of **Shohag (V1)** and **AGS-334 (V2)**were directly sown in the pots. Each pot received one apparently healthy and uniform sized seed. The seeds were then thinly covered with soil.

After care of seedlings

Weeding, irrigation, mulching etc. were done as and when necessary. The soil of the pot around the base of the plant was loosened with the help of khurpi from time to time up to the second month of growth to facilitate aeration and proper root growth. Since there was no incidence of any disease or insect attack, no fungicide or insecticide was applied to the crop.

Preparation of inoculum

Mature eggmasses of root-knot nematode *(Meloidogyne javanica L.*) were collected from severely galled root-system of brinjal (*Solanummelongena L.*). The brinjal plants were previously inoculated with single eggmass of *Meloidogyne javanica*. The brinjal plants were allowed to grow in large earthen pots (25 cm dia) under glasshouse condition of the Seed Pathology Centre. Bangladesh Agricultural University, Mymensingh. For inoculation, reddish brown mature eggmasses were collected from infected root with fine forceps. The egmasses were placed in a moist petridish.

Inoculation of soybean plants

After 20 days of planting, each soybean plant was inoculated with the eight egg masses collected from brinjal plants. On each side of the plant, 4 eggmasses were placed on the exposed roots of the seedling by opening the soil at the stem base.

Application of Curaterr (Carbofuran)

Granular nematicide Curraterr (carbofuran) was applied as side dressing in two instalments @ 500 mg around the root region of seedlings after 10 days of inoculation of seedlings.

Design of experiment

The two factors factorial experiment with two varieties and 4 treatments was conducted in Randomized Complete Block Design (RCBD). Each treatment was replicated five times. All the pots were arranged randomly. The two factors were as follows:

Factor A : Treatment - $\mathbf{T_0}$= Control ,$\mathbf{T_1}$ = BINA-Biofertilizer, $\mathbf{T_2}$ = BAU-Biofungicide ,$\mathbf{T_3}$ = Curaterr

Factor B : Variety-**Variety $\mathbf{V_1}$** : Shohag , **Variety $\mathbf{V_2}$** : AGS-334

Different parameters studied

After 83 days of inoculation, plants at mature stage were carefully uprooted from the pots and the following parameters in relation to plant and pathogen were studied: ***I)****Length of shoot (cm)* ***ii)****Length of root (cm)****iii)****Fresh weight of shoot (g)* ***iv)****Fresh weight of root with nodule (g)* ***v)****Weight of pods (g)/plant* ***vi)****Number of nodules/plant* ***vii)****Number of seeds/plant* ***viii)****Seed weight (g)/plant* ***ix)****Number of galls/g of root* ***x)****Number of egg masses/g of root* ***xi)****Number of adult females, J2, J3 and J4 juveniles/10 galls*

Measurement of length of shoot and root, fresh weight ofshoot and root

At first, the soil of pot was watered to make it moist for easy uprooting of the plant. Then the whole plant along with soil attached to its roots and nodules was lifted from the pot dipping in a bucket of water. Then with gradual and slow movement of roots in water, the soils were washed out from the roots. The roots were further cleaned under gentle running tap water and carefully washed. The root portion was separated from shoot portion with a sharp knife. Length of shoot was measured from the base of the stem up to the topmost 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 portions were blotted with fine tissue paper and fresh weights were measured by electrical balance before the materials could get desiccated.

Counting the number of nodules, galls, pods/plant, seed weight (g)/plant, egg masses/g of root

The number of nodules per plant was counted and recorded, the roots systems were examined under a hand lens and the number of galls/g root was counted for each treatment. The number of pods per plant were counted and recorded. Grain weight per plant in gram was measured by an electrical balance. The root systems of the plants were evaluated under a hand lens and number of egg masses per (g) of root were counted.

Staining of roots

For staining the galls, the following steps were followed:

i) Preparation of lactophenol: At first, the lactophenol solution was prepared by liquid phenol (500 ml), lactic acid (500 ml), glycerin (100 ml) and distilled water (500 ml) were poured orderly on to the two liter capacity conical flask and shaken thoroughly for mixing them well. To stain the prepared solution, cotton blue was added to the whole lot at the rate of 5 ml of 1% stock solution of cotton blue per 100 ml lactophenol.

ii) 150-170 ml lactophelol cotton blue was taken in a 250 ml beaker

iii) The beaker with the content was heated to the boiling point

iv) All the galls of each treatment were taken randomly in a special type of cloth-bag whose end was loosely woven with a lace. At the time of boiling of the liquid of the beaker, the cloth-bag having galls was dipped for about three minutes. Then, the bag was lifted from the beaker and galls of the bag washed with gentle running tap water to remove excess blue color.

v) Fresh lactophenol was poured on to 8 petridishes (for each variety) where galls of the respective treatments were retained for 5 days for destining.

vi) Ten galls per treatment were crushed one after another with fine pointed needles. Adult females, J2, J3 and J4 juveniles, if any, of M. javanica, found under stereo-binocular microscope were counted accordingly. Similarly, five counts were made and the average was calculated.

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.

RESULTS

Table 1. Effect of different treatments on the growth, nodulation, galling, eggmasses and yield of soybean

Treatments	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot	Fresh weight of	Weight of pods (g)	Number of nod	Number of seeds Plant^{-1}	Seed weight (g)	Number of galls g^{-1} of	Number of eggmasses
T_0	55.13c	22.61	11.19c	2.57	9.76b	0.40	39.80c	5.68b	4.80a	4.20a
T_1	98.72b	31.94	21.16	1.60	11.54	3.60	69.00	7.84b	1.50bc	1.20b
T_2	107.30	30.55	22.20a	1.22	15.24a	7.60	82.30	7.69b	2.60b	0.90b
T_3	103.20	37.82	23.80a	3.24	16.96a	8.50	98.90a	11.90	0.50c	0.50b
Level of	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Each value is an average of five replications

Values in the column having common letter(s) do not differ significantly at p≥ 0.05 level by DMRT.

Four treatments with BINA-Biofertilizer, BAU-Biofungicide and a chemical nematicide Curaterr including control were used to assess their effect on different plant growth characters, galling incidence, yield and development of adult females and juveniles of root knot nematode (Meloidogyne javanica) in soybean.

Data on the growth parameters viz. length of shoot and root, fresh weight of shoot and root with nodules, weight of pods per plant, number of nodules per plant, number of seeds per plant, weight of seeds per plant, number of galls per g of root and number of eggmasses per g of root of two soybean varieties Shohag (V1) and AGS-334 (V2) are presented in Table 1.

Effect on shoot length: Mean length of shoots were significantly influenced by the treatments. Mean length of shoot ranged from 55.13 to 107.3 cm. The highest shoot length (107.3 cm) was recorded in plants treated with BAU-Biofungicide followed by plants treated with Curaterr (103.20 cm). However the lowest length of shoot (55.13) was recorded in plants in control treatment followed by plants treated with BINA-Biofertilizer (T1).

Effect on root length: Mean length of roots ranged from 22.61 to 37.82 cm. Plants treated with the chemical Curaterr (T3) produced the highest root length 37.82 cm followed by lower significant and statistically identical root lengths 31.94 cm and 30.55 cm in treatments T1 (BINA-Biofertilizer) and T2 (BAU-Biofungicide), respectively. The lowest root length 22.61 cm was recorded in control (T0). (Table 1)

Effect on fresh weight of shoot: Mean fresh weights of shoot ranged from 11.19 g to 23.80 g. The

highest significant shoot weight (23.80 g) was found in plants treated with the Curaterr followed by the plants treated with BAU-Biofungicide (22.20 g) and BINA-Biofertilizer (21.16 g). But, the treatments effect of Curaterr and BAU-Biofungicide as well as BINA-Biofertilizer were found to be statistically identical. The control treatment (T0) gave the lowest shoot weight (11.19 g) (Table 1).

Effect on fresh weight of root with nodules: The highest significant fresh weight of root with nodules was recorded with treatment T3 having 3.24 g, while the lowest significant and statistically identical effects were found with the treatments T1 and T2 having 1.60 g and 1.22 g respectively. The control treatment T0 gave significantly higher fresh weight of root with nodules having 2.57 g (Table 1).

Effect on the weight of pods per plant: Significantly the highest and statistically identical weights of pods per plant were recorded with treatments T3 and T2 having 16.96 g and 15.24 g, respectively lower significant and statistically identical weights 11.54 g and 9.76 g of pods per plant were observed with the treatments T1 and T0, respectively (Table 1)

Effect on number of nodules per plant :The highest significant and statistically identical number 8.50 and 7.60 of nodules per plant were recorded with treatments T3 and T2, respectively lower significant number 3.60 of nodules was found with the treatment T1 followed by T0 having the lowest number 0.40 of nodules per plant (Table 1).

Effect on number of seeds per plant: Significantly the highest number 98.90 of seeds per plant was recorded with treatments T3 followed by lower significant and statistically identical numbers 82.30 and 69.00 were observed with the treatments T2 and T1, respectively. The lowest number 39.80 seeds per plant was found with the control treatment T0 (Table 1).

Effect on seed weight per plant: The highest significant seed weight 11.89 g, per plant was observed with the treatment T3 followed by lower significant and statistically identical seed weights 7.84, 7.65 and 5.68 g in the treatments T1, T2 and T0, respectively (Table 1).

Effect on the galling : The control treatment T0 was found to have significantly the highest number 4.80 of galls per g of root followed by T2, T1 and T3 having 2.60, 1.50 and 0.50 galls per g of root, respectively. But, there were no significant differences in respect of number of galls with the treatments T2 and T1 as well as T1 and T3 (Table 1).

Effect on the number of eggmasses per plant :The highest significant number 4.20 of eggmasses per g of root was noted with the control treatment T0 followed by T1, T2 and T3 having lower significant and statistically identical numbers 1.20, 0.90 and 0.50 of eggmasses per g of root, respectively (Table 1).

Table 2. Responses on the growth, galling and yield of the two soybean varieties Shohag (V_1) and AGS-334 (V_2) inoculated with *Meloidogyne javanica*

Variety	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules	Weight of pods (g) plant^{-1}	Number of nodules plant^{-1}	Number of seeds plant^{-1}	Seed weight (g) plant^{-1}	Number of galls g^{-1} of root	Number of egg masses g^{-1} of root

Shohag (V_1)	92.04a	30.27a	20.60a	2.37a	13.38a	7.80a	72.50a	8.46a	2.52a	1.75
AGS-334 (V_2)	90.09a	31.18a	18.56b	1.94a	13.37a	2.25b	72.50a	8.09a	2.45a	1.65
Level of signific	NS	NS	0.05	NS	NS	0.01	NS	NS	NS	NS

Each value is an average of five replications
NS = Not significant
Values in the column having common letter(s) do not differ significantly at p> 0.05 level by DMRT.

With four treatment responses of two varieties of soybean **Shohag** and **AGS-334** inoculated with M. *javanica* on the length of shoot and root, fresh weight of shoot and root with nodules, weight of pods per plant, number of nodules per plant, number of seeds per plant, weight of seeds per plant, number of galls per g of root and number of eggmasses per g of root are presented in (Table 2). Responses of both the varieties **Shohag** and **AGS-334** with respect to the fresh weight of shoot and number of nodules per plant were found to be significant. Among the two varieties, **Shohag (V1)** appeared to give significantly higher response in respect of fresh weight of shoot and number of nodules per plant. In cases of length of shoot and root, fresh weight of root with nodules, weight of pods per plant, number of seeds per plant, weight of seeds per plant, number of galls per g of root, number of egg masses per g of root the response were found to be non-significant (Table 2).

Table 3. Responses of two soybean varieties Shohag (V_1) and AGS-334 (V_2) on the development of adult females and juveniles of *Meloidogyne javanica*

Variety	Number of adult females/10 galls	Number of J_2 juveniles/10 galls	Number of J_3 juveniles/10 galls	Number of J_4 juveniles/10 galls
Shohag (V_1)	5.900a	4.900a	6.700a	5.250a
AGS-334 (V_2)	5.700a	4.00b	6.900a	4.700a
Level of significance	NS	0.05	NS	NS

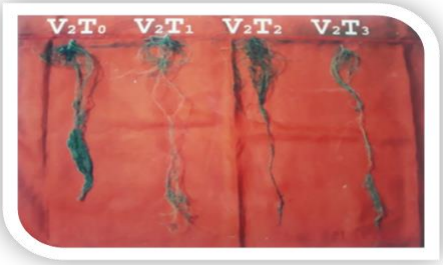
Each value is an average of five replications, NS = Not significant

Responses of the two varieties were found to be insignificant in respect of development of adult females, J3 and J4 juveniles, while the higher significant effect on the number of J2 juveniles was recorded in the variety **Shohag (V1)** compared to variety **AGS-334 (V2)** having 4.90 and 4.00 J2 juveniles, respectively (Table 3).

Photograph-1. *Comparative growth of variety* ***Shohag*** *(**V1**) under Treatments T_0,T_1 and T_3 after 55 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer,T_2= BAU-Biofungicide and T_3 =Curaterr)*

Photograph-2. *Comparative growth of variety* ***AGS-334*** *(**V2**) under Treatments T_0,T_1 and T_3 after 55 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer,T_2= BAU-Biofungicide and T_3 =Curaterr)*

Photograph-3. *Growth and galling incidence in root system of variety* *Shohag* *(**V1**) under Treatments T_0,T_1 and T_3 after 83 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer,T_2= BAU-Biofungicide and T_3 =Curaterr)*



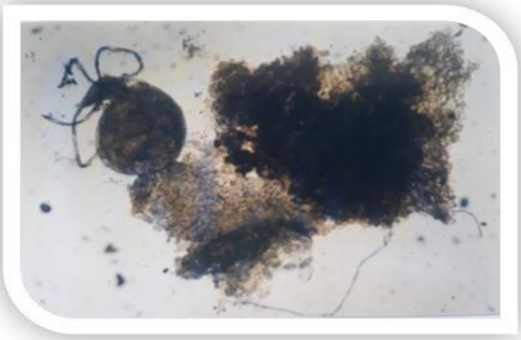
Photograph-4. *Growth and galling incidence in root system of variety AGS-**334** (**V2**) under Treatments T_0, T1 and T_3 after 83 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer, T2= BAU-Biofungicide and T_3 =Curaterr)*

Photograph-5..*Comparative growth and galling with treatments T_0, and $T_3$of variety Shohag (V_1)(T_0= Control, and T_3 =Curaterr)*

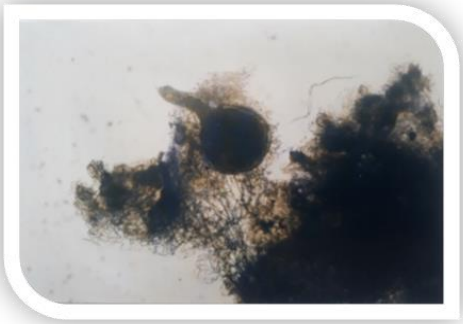
Photograph-6..*Comparative growth and galling with treatments T_0, and T_2 of variety* ***AGS-334*** *(V_2)(T_0= Control, and T2= BAU-Biofungicide)*

Photograph-7. *Comparative yield of pod of variety Shohag (V_1 underTreatments T_0,T_1 and T_3 after 55 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer,T_2= BAU-Biofungicide and T_3 =Curaterr)*

Photograph-8. *Comparative yield of pod of variety* ***AGS-334*** *(V_1 underTreatments T_0,T_1 and T_3 after 55 days of inoculation (T_0= Control, T_1= BINA-Biofertilizer,T_2= BAU-Biofungicide and T_3 =Curaterr)*



Photograph-9. *An adult female of Meloidogyne javanica with a broken eggmass in a crushed gall in the control (**T_0**)*



Photograph-10. *An isolated J_4 Juvenile of Meloidogyne javanica in a crushhe gall in the treatment with BINA-Biofertilizer(**T_1**)*

Photograph-11. *An isolated J_4 Juvenile of Meloidogyne javanica in a crushhe gall in the treatment with BAU- Biofungicide (**T_2**)*

Table 4. Interaction effects of treatments and varieties of soybean on the growth, nodulation, galling, eggmasses and yield

Treatment × Variety	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Weight of pods(g) plant^{-1}	Number of nodules plant^{-1}	Number of seeds plant^{-1}	Seed weight (g) plant^{-1}	Number of galls g^{-1} of root	Number of eggmasses g^{-1} of root
$T_0 \times V_1$	59.68d	18.22d	14.19d	2.598	9.47	0.60cd	34.60e	6.19	4.60	4.60
$T_0 \times V_2$	93.68c	28.72bc	18.83c	1.906	9.78	4.80b	58.60cd	6.79	1.60	0.80
$T_1 \times V_1$	109.18a	30.32bc	24.76a	1.152	17.49	12.20a	84.60b	8.12	2.20	1.00
$T_1 \times V_2$	105.50ab	47.00a	26.19a	2.538	17.04	10.20a	85.00b	10.17	1.00	0.80
$T_2 \times V_1$	105.64ab	43.82a	24.63a	3.822	16.78	13.60a	112.20a	12.75	0.60	0.600
$T_2 \times V_2$	103.76ab	35.16b	23.47a	1.306	13.30	2.40bcd	79.40bc	8.88	1.40	1.60
$T_3 \times V_1$	105.32ab	30.78bc	19.62bc	1.29	12.98	3.00bcd	80.00bc	7.25	3.00	0.80
$T_3 \times V_2$	100.72bc	31.81bc	22.96ab	2.65	17.140	3.40bc	85.60b	11.04	0.40	0.40
Level of significance	0.05	0.01	0.01	NS	NS	0.01	0.05	NS	NS	NS

Each value is an average of five replications

NS = Not significant T_0 = Control T_1 = BINA-Biofertilizer T_2 = BAU-Biofungicide T_3 = Curaterr. V_2 = AGS-334, V_1 = Shohag

Interaction effects of the treatments and varieties on the growth, weight of pods, nodulation, number and weight of seeds, galling, egg masses and yield are presented in (Table 3).

Interaction effects of the treatments and varieties on the length of shoot and root, fresh weight of shoot, number of nodules and seeds per plant were found to be significant. The interaction effects of the treatments and varieties revealed that treatments BINA-Biofertilizer and BAU-Biofungicide gave better responses in respect of length of shoot and root, fresh weight of shoot, number of nodules as well as number of seeds per plant interacting withthe varieties Shohag and AGS-334 compared to the treatment Curaterr. The lowest significant interaction effects were recorded with

treatment T_0interacting with both the varieties in most of the growth characters (Table 4).

Table 5. Interaction effects of treatments and varieties of soybean on the development of adult females and juveniles of *Meloidogyne javanica*

Treatment × Variety	Number of adult females/10 galls	Number of J_2 juveniles/ 10 galls	Number of J_3 juveniles/10 galls	Number of J_4 juveniles/10 galls
$T_0 \times V_1$	12.10a	11.00a	13.80	9.80
$T_0 \times V_2$	13.40a	8.40b	15.00	9.60
$T_1 \times V_1$	3.00cde	2.60de	4.20	4.00
$T_1 \times V_2$	5.40b	3.60cd	3.80	2.00
$T_2 \times V_1$	3.00cde	2.80de	4.20	4.00
$T_2 \times V_2$	3.00cde	2.40de	3.40	3.00
$T_3 \times V_1$	1.80e	1.60e	1.80	1.80
$T_3 \times V_2$	1.60e	1.60e	2.20	1.80
Level of significance	0.05	0.05	NS	NS

Each value is an average of five replications

NS = Not significant T_0 = Control T_1 = BINA-Biofertilizer T_2 = BAU-Biofungicide T_3 = Curaterr. V_2 = AGS-334, V_1 = Shohag

The interaction effects of the treatments and varieties on the development of adult females and J2 juveniles of Meloidogyne javanica were found to be significant. The highest interaction effects were observed with the treatments control and Shohag as well as control and AGS-334 in case of adult females having 12.10 and 13.40, respectively. Similarly, higher interaction effect was observed with control and Shohag in case of J2 juveniles with 11.00 followed by control interacting with AGS-334 having 8.40. Lower significant effects were observed in treatments BAU-Biofungicide and Curaterr interacting with both the varieties in respect of adult females and J2 juveniles (Table 5).

DISCUSSION

The study was carried out with four different treatments covering BINA-Biofertilizer, BAU-Biofungicide, a chemical nematicide (Curaterr) and a control to see comparative study of susceptibility, growth & yield of two varieties soybean [Glycine max (L.) Merrill] varieties Shohag and AGS-334 against development of root-knot nematode (*Meloidogyne javanica*) with treatment BINA-Biofertilizer, BAU-Biofungicide, a chemical Nematocide.

The results revealed that maximum length of shoot and root, fresh weight of shoot and root with nodules, weight of pods per plant, number of nodules per plant, number of seeds and weight of seeds

per plant were obtained with the nematicidial treatment (T3) in both the soybean varietiesShohag and AGS-334. On the other hand, control treatment T_0 with M. *javanica* alone was responsible for the significant reduction in respect length of shoot and root, fresh weight of shoot and root with nodules, weight of pods per plant, number of nodules per plant, number and weight of seeds per plant in both soybean varieties. Moreover, the highest galling incidence correspondingly with the lowest yield performance were observed with this treatment.

BAU-Biofungicide and BINA-Biofertilizer as bio-agent gave better response 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 pods number and weight of seeds. There appeared significantly lower galling incidence in both BAU-Biofungicide and BINA-Biofertilizer treated plants indicating similar suppressing effect on galling as observed with chemical nematicide Curaterr. Moreover, the lower number of egg masses, adult females and different juvenile stages J2, J3 and J4 were found with these treatments of the bio-agents somewhat similar to that of chemical Curaterr.

Trichodermin produced by Trichoderma which showed antagonistic activity against various diseases. Similar kind of antibiotic substance might have been involved with the treatment BAU-Biofungicide to hinder the activity of M. javanica as observed with higher growth characters as yield of plants along with lower galling incidence and development nematodes in the present investigation. Sharon et al. (2001) similarly observed increased fresh weight of shoot and reduction of galling incidence in tomato infected with M. javanica following soil pretreatment with Trichoderma peat bran preparations. It is suggested that improved proteolytic activity of the antagonist may be important for the biological control of the nematode. A good number of researchers (Rao et al., 1998; Goswami et al., 1998; Reddy et al., 1998; Devi and Hassan, 2002) also recorded effectiveness of Trichoderma spp. to reduce gall formation on different crops including soybean caused by Meloidogyne incognita and Meloidogyne javanica with corresponding increase of plant growth. Goswami et al. (1998) ,also reported that Trichodermaharzianum improved growth and higher yield of M. javanica infected plants and decreased the root galling index and the number of eggs per g of root. Treatment with Trichoderma improved plant height, shoot weight, root length and weight and reduced nematode population as stated by Devi et al. (2002). Davila et al. (1999) stated that Trichodermaharzianum has great potential as nematode bio-control agents associated with eggs, larvae and females of Melodigyne spp.

BINA-Biofertilizer as a bio-agent which also gave good response with higher growth of shoot and root as well as higher weight of shoot and root with nodules, number of nodules per plant and number and weight of seeds per plant correspondingly with lower galling incidence, lower development of egg masses, adult females and J2, J3 and J4 juveniles in the M. javanica inoculated soybean.

Abid et al. (1992) stated complete control of M. incognita in mungbean grown in 3% w/w cotton cake mixed with Bradyrhizobium sp. Siddiqui and Shaukat (2003) also observed that bacterial inoculants reduced M. incognita galling on tomato, brinjal, mungbean and soybean roots. Chahal and Chahal (1987) described that Rhizobium strain caused the greatest increase in plant growth but infection with nematode reduced this significantly. M. incognita multiplied at a greater rate when the seedlings were inoculated with Rhizobium, it was suggested that this was due to the better development of the plant in a supply of fixed nitrogen. Through BNF activities of Bioferilizer atmospheric N fix in soil,

which enhances the vigour of the plants and protects the plant from nematode vital injury. Antagonistic effect of Rhizobium sp. and Bradyrhizobiumjaponicum against various fungi attacking crops including soybean had been reported by Ghaffar (1993). The antagonistic effect of BINA-Biofertilizer (Bradyrhizobiumspp) in this study with a leguminous crop like soybean inoculated with M. javanica cannot be overruled as stated by previous authors.

Tiyagi and Alam (1988) observed that plant weight were adversely affected by the nematode M. incognita in Rhizobium treated as well as in untreated plants. Similar observations also made by Dalal and Bhatti (1996) and Ali et al. (1981). Sharma and Tiyagi (1990) showed that nematode in association with Rhizobium caused significant reduction in plant growth but the effect was less significant when rhizobium already established before the introduction of the nematode. In the present study, BINA-biofertilizer (Bradyrhiozbiumspp) mixed with seeds were sown in pots 10 days earlier of nematode inoculation and comparatively better responses were found in plant growth characters as well as reduction of nematode development.

In this study chemical nematicide Curaterr gave higher significant growth of shoot and root higher number of nodules, pods and seeds correspondingly with lower galling incidence, eggmasses and reduced population of adult females and juveniles of M. javanica in two varieties of soybean.

Lambert et al. (1993) stated that granular nematicide carbofuran controlled M. javanica of tomato and increased yield. Hassain (1995) reported that application of Furadan 5G brinjal plant gave superior response in plant growth characters with corresponding lower number of galls, adult females and eggmasses. Nanjegowda et al. (1998) similarly observed in case plant growth characters with reduced galling incidence eggmasses development and larval populations. Fazal et al. (2001) reported that carbofuran significantly reduced the number of galls and M. incognita larvae and significantly increased soybean yield. Curaterr being a carbofuran had similarly been found to give positive response in plant growth characters with negative impulse to the nematode as observed also in the present study with soybean. Similar observations on yield and lower galling incidence with suppressed nematode development were made by Fateam (2003), Khatun (2004), Hasseeb and Shukla (2004).

Interaction effects of the treatments and varieties were found significant in respect of length of shoot and root, fresh weight of shoot, number of nodules, number of seeds per plant and incase of adult females and J2 populations in most of the cases. Positive responses were observed with the treatments with BINA-Biofertilizer, BAU-Biofuncicide and Curaterr interacting with both of the varietiesShohag and AGS-334. Other than length and fresh weight of root with nodules, number and weight of seeds and nematode population. The nematicidal treatment Curaterr gave more or less similar responses like that of BINA-Biofertilizer and BAU-Biofungicide.

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, fresh weight of root with nodules, weight of pods per plant, number and weight of seeds per plant and reduced incidence of galling and egg masses with lower development of adult females, J2, J3 and J4 juveniles of Meloidogyne javanica were achieved by the seed treatment with BAU-Biofungicide and BINA-Biofertilizer as well as side dressing nematicide Curaterr compared to the control treatment. The efficacy of BAU-Biofungicide and BINA-Biofertilizer to control root-knot disease of soybean along with improved growth characters and yield components found were almost equally good to Curaterr.

Summary

This experiment was carried out to determine the effectiveness of BAU-Biofungicide, BINA-Biofertilizer as bio-agents and Curaterr as a chemical agent against root-knot (Meloidogyne javanica) of two soybean varieties Shohag and AGS-334 in four different treatments including control. The bio-agents were used as seed treatments and Curaterr was used as side dressing.

All the treatments with BAU-Biofungicide, BINA-Biofertilizer and Curaterr gave significantly higher response in plant growth characters like length of shoot and root, fresh weight of shoot, fresh weight of root with nodules, weight of pods per plant, number of nodules per plant, weight and number of seeds per plant compared to the control treatment. Simultaneously lower galling incidence, and eggmasses as well as lower development of adult females, J2, J3 and J4 juveniles of M. javanica were observed with those agents. Varieties Shohag gave better fresh weight of shoot and nodulation, compared to the variety AGS-334. Other than higher J2 population in the variety Shohag, no significant differences were found among these two varieties on the development of adult females, J3 and J4 juveniles of M. javanica. Interaction effects of the treatments and varieties were found significant in respect of length of shoot and root, fresh weight of shoot, number of nodules, number of seeds per plant and in cases of adult females and J2 population. In most of the cases positive responses were observed with the treatments with BINA-Biofertilizer, BAU-Biofungicide and Curaterr interacting with both of the varieties. Other than length and fresh weight of root with nodules, number and weight of seeds and nematode population the nematicidial treatment Curaterr gave more or less similar responses like that of BINA-Biofertilizer and BAU-Biofungicide.
The correlation studies also revealed significant and negative correlations between galling incidence and plant growth characters had positive response on which indicated that the treatments had positive response on plant growth and yield.

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