

## Bio-control of root-knot (*Meloidogyne incognita*) of soybean

M. Islam, M.U. Ahmad, M.S. Khatun, M.O.H. Reza and M.G. Kibria<sup>1</sup>

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, <sup>1</sup>Bangladesh Agricultural Research Institute, Gazipur-1701.

**Abstract:** The experiment was conducted in the net house of the Seed Pathology Centre, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during 2011. BAU-Biofungicide, Allamanda tablet and Neem oil as bio-control agents including a control were tested against root-knot (*Meloidogyne incognita*) of three soybean varieties (BARI Soybean-5, BAU-S-80 and BAU-S-109). BAU-Biofungicide and Allamanda tablet were used as side drenching and Neem oil as seed treatment. BAU Bio-fungicide showed the best performance with the highest growth of shoot as well as weight of shoot, number of pods per plant, number of nodules per plant correspondingly with higher yield per plant as evident with higher weight of seeds consequently with decreased galling incidence and lower numbers of adult females, eggmasses, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles. Allamanda tablet also responded with increased length of shoot, number of pods per plant, number of nodules per plant and weight of seeds per plant correspondingly with lower galling incidence, lower development of adult females, J<sub>2</sub> and J<sub>3</sub> juveniles of *Meloidogyne incognita* compared to Neem oil and control treatments. BARI Soybean-5 appeared with maximum length of shoot and root, fresh weight of shoot and root with nodules, number of nodules per plant correspondingly with lower eggmasses. Varieties BAU-S-80 gave higher responses in case of number of pods and weight of seeds per plant simultaneously with higher number of galls. Negative correlations were found between gall numbers and plant growth characters as well as yield.

**Key words:** Soybean, BAU-Biofungicide, Allamanda tablet, neem oil, root-knot.

### Introduction

Soybean (*Glycine max*) is a very important and well-recognized oil seed and protein crop in the world with hundreds of food, feed and industrial uses. The crop grows well in tropical, sub-tropical and temperate regions. It contains high nutritive value about 40-45% protein, 18-20% edible oil and 20-26% carbohydrate. Soybean can meet up protein, unsaturated fatty acid, minerals like Ca and P including vitamins A, B, C, D and other different nutritional needs (Rahman, 1982). Like many other legumes, soybean plants can fix atmospheric nitrogen to an extent of 300 kg ha<sup>-1</sup> year<sup>-1</sup> and reduce the N requirement by 25-75% with *Bradyrhizobium japonicum* (Keyser and Li, 1992).

In Bangladesh, yield of soybean is unexpectedly low compare to than that of other soybean producing countries of the world. Many reasons are responsible for this low yield. Among these, diseases are the most important and the nematode disease root-knot is a major constraint for higher production of soybean (Mian, 1986). The ability of soybean plant to fix Nitrogen (N) symbiotically is substantially reduced after the infection of root-knot nematode *Meloidogyne incognita* (Coyne and Oyekanmi, 2007). The root-knot nematode (*Meloidogyne incognita*) is the most troublesome plant-parasitic nematode in the tropics (Adegbite, 2007). The root-knot nematodes (*Meloidogyne* spp.) can significantly reduce seed yield in soybean (Harris *et al.*, 2003). Santos *et al.* (2001) found that root-knot nematodes (*Meloidogyne* spp.) are the main pests of soybean in Brazil. The root-knot nematodes, *Meloidogyne* spp., are widely distributed throughout Bangladesh (Mian, 1986) as it 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. Different chemicals and bio-control agents including botanicals are used for controlling nematode diseases. But the majority of synthetic pesticide chemicals having a broad-spectrum activity pose well known risk for consumers as well as for the environment. Moreover, nematodes soon show resistance against these chemicals. Chemicals affect adversely on soil beneficial organisms and environment which finally collapse the ecosystem.

Also chemical nematicides are costly. On the other hand bio-control agents are less costly, safe for human as well as environment. BAU-Biofungicide, Allamanda tablet and Neem oil using as nematode controlling agents are new approaches as eco-friendly measures. *Trichoderma* spp., component of BAU-Biofungicide, act either as antagonists, parasites or predators (Rao *et al.*, 1997 and Reddy *et al.*, 1998). Allamanda tablet as a botanical fungicide has potential nematicidal effect and reduce nematode (*M. javanica*) population (Rasheduzzaman, 2005 and Shabiha, 2005). Neem oil gives positive response with increase fresh weight of modified root consequently with reduced galling and nematode development like chemical curaterr (Mahbuba, 2008).

There is an ample scope of increasing its cultivation by using different bio-control agents without any disturbance of the natural environment and beneficial microorganisms. Therefore, the present study was undertaken to assess the comparative efficacy of some biological means on the control of root-knot (*Meloidogyne incognita*) of Soybean.

### Materials and Methods

The experiment was conducted in the net house of the Seed Pathology Centre, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during 2011. Two factors factorial experiment with three soybean varieties viz. BARI Soybean - 5 (V<sub>1</sub>), BAU-S-80 (V<sub>2</sub>) and BAU-S-109 (V<sub>3</sub>) and four treatments viz. Control (T<sub>0</sub>), BAU-Biofungicide (T<sub>1</sub>), IPM Allamanda tablet (T<sub>2</sub>), and Neem oil (T<sub>3</sub>) was conducted in Randomized Complete Block Design with five replications. Due to absence of homogenous condition e.g. light intensity and rate of ventilation in net house, Randomized Complete Block Design was used instead of Completely Randomized Design. BAU-Biofungicide (2 % solution) and allamanda tablets (30 ml solution after dissolving in normal water at the ratio 1:8 for 15 minutes) were applied as side drench separately for each replicated plant of all three varieties after 10 days of inoculation with eggmasses. Neem oil was applied as seed treatment @ 5 ml/ 10 g seed before sowing.

For the experiment sandy loam soil, well decomposed cowdung and sand were taken at the ratio of 2: 1.5: 0.5 and mixed uniformly. Formalin @ 30 ml was dissolved in 1000 ml water for per cubic feet of soil for sterilization. Polythene sheet was used for covering the formalin treated soil and removed after 72 hours. The sterilized soil was exposed for air drying for 48 hours and to remove excess vapour of formalin. Sixty earthen pots (30 cm diameter) were taken and each was filled with 6 kg of sterilized and dried soil. For avoiding any contamination the pots were sterilized with formalin before filling with soil. For preparation of inoculum few brinjal plants were previously inoculated with single eggmass of *Meloidogyne incognita*. Severely galled root system of brinjal plants (*Solanum melongena*) was used for collecting mature eggmasses of root-knot nematode (*Meloidogyne incognita*). The brinjal plants were allowed to grow in large earthen pots (25 cm diameter) under glasshouse condition. Reddish brown mature eggmasses were collected from infected roots with the help of fine forceps for inoculation. A moist petridish was used for keeping eggmasses.

Six seeds were directly sown in the pots on 03 March 2011 and the seeds were thinly covered with soil. One healthy seedling was allowed to grow in each pot by removing the others after germination of seeds. Weeding, mulching, irrigation etc. were done as and when necessary. After 35 days of planting, each soybean plant was inoculated with six eggmasses collected from infected brinjal roots. On each side of the plant, 3 eggmasses were placed on the exposed roots of the seedling by opening the soil at the stem base. After 60 days of inoculation, the plants at mature stage were carefully uprooted from the pots and ten parameters were studied including length of shoot and root, fresh weight of shoot and root, number of pods, weight of seeds and number of nodules per plant, number of galls and eggmasses per g of root and number of adult females, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles in 10 galls/treatment.

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.

## Results and Discussion

**Effects of treatments:** Effects of four different treatments on the growth, yield, nodulation and galling incidence and development of adult females, eggmasses, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles of *Meloidogyne incognita* in the soybean varieties are presented in Table 1 and 2, respectively. Maximum length of shoot (55.20 cm), number of pods per plant (12.13), weight of seeds per plant (12.57 g) and number of nodules per plant (4.60) were obtained with the treatment BAU-Biofungicide. There appeared the lowest galling incidence (1.53 galls per g of root) in this treatment indicating its more suppressing effect on galling. The lower numbers of adult females, eggmasses, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles were found with the treatment BAU-Biofungicide. The effectiveness of *Trichoderma spp.*, component in BAU-Biofungicide may be attributed to the fact that the fungi occupy the niche before nematode infection and thereby hinder the establishment of the nematode pathogen. Tverdyukov *et al.* (1994) reported about an antibiotic substance Trichodermin produced by *Trichoderma* which showed antagonistic activity against various diseases. Similar kind of antibiotic substance might have been involved with the treatment of BAU-Biofungicide in the present investigation. Sarker (2009) reported that BAU-Biofungicide combination with Bio-nematicide 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 *Meloidogyne incognita* in soybean. Hasan (2004) and Mustarin (2004) similarly reported higher nodulation, higher yield, less number of galls, less adult females, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles in mungbean and soybean infected with *Meloidogyne javanica*.

**Table 1.** Effects of different treatments on the growth, yield, nodulation and galling in soybean inoculated with *Meloidogyne incognita*

Treatments	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Number of pods / plant	Weight of seeds/ plant (g)	Number of nodules / plant	Number of galls/ g of root
T <sub>0</sub> (Control)	42.22 d	16.36 d	12.30 c	2.78 c	8.53 c	7.49 d	1.60 c	3.60 a
T <sub>1</sub> (BAU -Biofungicide)	55.20 a	19.54 b	16.71 a	3.35 b	12.13 a	12.57 a	4.60 a	1.53 d
T <sub>2</sub> (Allamanda tablet)	53.34 b	20.65 a	16.99 a	3.98 a	10.67 b	11.50 b	3.53 b	2.13 c
T <sub>3</sub> (Neem oil)	45.85 c	17.53 c	13.38 b	3.32 b	8.33 c	8.86 c	3.13 b	2.53 b
LSD (P $\geq$ 0.05)	0.749	0.144	0.348	0.151	0.687	0.132	0.559	0.392

Values in the column having common letter(s) do not differ significantly at P $\geq$  0.05 level by DMRT.

In case of length of root, and fresh weight of root with nodules, the treatment Allamanda tablet gave maximum values (20.65 cm and 3.98 g, respectively). Lower development of adult females, J<sub>2</sub> and J<sub>3</sub> juveniles of the *Meloidogyne incognita* were also observed in the treatment with Allamanda tablet. Ahmmed (2009) similarly observed that Allamanda tablet showed

performance giving better length of shoot and root, fresh weight of root correspondingly with decreased number of galls, adult females, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles of *Meloidogyne incognita* in brinjal. Rasheduzzaman (2005) and Shabiha (2005) reported that extract of leaf, flower and stem bark of Allamanda and solution of Allamanda tablet gave more or less identical response in reducing gall, eggmasses and

nematode (*Meloidogyne javanica*) population in carrot and pea. Their reports are in agreement the present findings. The treatment Neem oil showed lower performance than the treatments with BAU-Biofungicide and Allamanda tablet and better performance compared to control treatment in plant growth characters, nodulation correspondingly with higher galling and nematode development. Mahbuba (2008) stated that Neem oil gave positive response with increased fresh weight of modified root consequently with reducing galling and nematode development. Neem products have been found to be effective against root-knot nematode *Meloidogyne incognita* on tomato, brinjal and chilli in reducing the root-knot index and healthier growth of plants (Agbenin *et al.*,

2004 and Sivakumar and Ramakrishan, 2005). Archana and Prasad (2003) reported that neem formulations gave the highest shoot length, fresh shoot weight, and fresh root weight of soybean correspondingly with lowest number of galls of *M. incognita*. All these reports are in consonance with the present findings. Control treatment with *M. incognita* alone was responsible for the significant reduction 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. Moreover, the highest galling incidence (3.60 galls per g of root) correspondingly with the lowest seed yield performance (7.49 g/plant) was observed with the control treatment.

**Table 2.** Effects of different treatments on the development of adult females, eggmasses and juveniles of *Meloidogyne incognita* in inoculated soybean

Treatments	Number of adult females/ 10 galls	Number of eggmasses/ g of root	Number of J <sub>2</sub> juveniles/ 10 galls	Number of J <sub>3</sub> juveniles/ 10 galls	Number of J <sub>4</sub> juveniles/ 10 galls
T <sub>0</sub> (Control)	4.20 a	2.53 a	2.07 a	2.67 a	3.80 a
T <sub>1</sub> (BAU -Biofungicide)	2.27 c	1.60 c	0.93 d	1.47 c	2.13 c
T <sub>2</sub> (Allamanda tablet)	2.60 c	1.80 bc	1.33 c	1.47 c	2.33 bc
T <sub>3</sub> (Neem oil)	3.07 b	2.13 b	1.67 b	2.13 b	2.53 b
LSD (P≥ 0.05)	0.344	0.340	0.303	0.374	0.323

**Table 3.** Responses on the growth, yield, nodulation and galling in three soybean varieties inoculated with *Meloidogyne incognita*

Variety	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Number of pods/plant	Weight of seeds/plant (g)	Number of nodules/plant	Number of galls/g of root
BARI Soybean - 5 (V <sub>1</sub> )	55.82 a	19.95 a	16.03 a	4.29 a	9.45 b	10.07 b	3.30 a	2.15 b
BAU-S-80 (V <sub>2</sub> )	47.53 b	18.25 b	14.35 b	2.93 b	10.50 a	10.43 a	2.80 b	2.55 a
BAU-S-109 (V <sub>3</sub> )	44.10 c	17.36 c	14.16 b	2.85 b	9.80 b	9.82 c	3.55 a	2.65 a
LSD (P≥ 0.05)	0.649	0.124	0.301	0.131	0.595	0.114	0.485	0.339

**Table 4.** Responses of three soybean varieties on the development of adult females, eggmasses and juveniles of *Meloidogyne incognita*

Variety	Number of adult females/ 10 galls	Number of eggmasses/ g of root	Number of J <sub>2</sub> juveniles/ 10 galls	Number of J <sub>3</sub> juveniles/ 10 galls	Number of J <sub>4</sub> juveniles/ 10 galls
BARI Soybean -5 (V <sub>1</sub> )	2.95	2.00 b	1.50	1.80	2.60
BAU-S-80 (V <sub>2</sub> )	3.00	1.65 c	1.45	2.00	2.70
BAU-S-109 (V <sub>3</sub> )	3.15	2.40 a	1.55	2.00	2.80
LSD (P≥ 0.05)	NS	0.295	NS	NS	NS

Values in the column having common letter (s) do not differ significantly at P≥0.05 level by DMRT. NS = Not significant

**Responses of soybean varieties:** Responses of the varieties with respect to the length of shoot and root, fresh weight of shoot and root with nodules, number of pods per plant, weight of seeds per plant, number of nodules per plant, number of galls per g of root and number of eggmasses per g of root were found to be significant and presented in Table 3 and 4. Among the three varieties of soybean, BARI Soybean-5 gave maximum length of shoot and root, fresh weight of shoot and root with nodules,

number of nodules per plant correspondingly with lower eggmasses. On the other hand, varieties BAU-S-80 and BAU-S-109 gave similar responses in case of fresh weight of shoot and root with nodules correspondingly with higher number of galls.

**Interaction effects of the treatments and varieties:** Interaction effects of the treatments and varieties on number of pods per plant and number of galls per g of root were found to be insignificant (Table 5). The interaction

effects of the treatments and varieties revealed that treatment T<sub>1</sub> (BAU-Biofungicide) interacting with the variety V<sub>1</sub> (BARI Soybean-5) gave significantly the highest length of shoot (64.25 cm), while the least responses were found with control treatment interacting with all the varieties. In case of length of root, fresh weight of shoot and root with nodules, promising response was obtained with treatment T<sub>2</sub> (Allamanda tablet) interacting with variety V<sub>1</sub> (BARI Soybean-5). In respect of weight of seeds per plant highest weight (13.30 g) was found with treatment T<sub>1</sub> (BAU-Biofungicide) interacting with the variety V<sub>2</sub> (BAU-S-80). In case of number of nodules per plant treatment T<sub>1</sub> (BAU-Biofungicide)

interacting with all the varieties appeared to give more or less promising response though no significant difference was found among the treatments other than T<sub>0</sub> (control) interacting with V<sub>1</sub> (BARI Soybean-5) and V<sub>2</sub> (BAU-S-80) with minimum effect having the lowest number of nodules per plant. Among the treatments, treatment T<sub>2</sub> (Allamanda tablet) appeared to give better effect in respect of length of shoot and weight of seeds interacting with all the varieties-compared to treatment T<sub>3</sub> (Neem oil). The interaction effects of the treatments and varieties of soybean on the development of adult females, eggmasses, J<sub>2</sub>, J<sub>3</sub> and J<sub>4</sub> juveniles of *Meloidogyne incognita* were found to be insignificant (Table 6).

**Table 5.** Interaction effects of treatments and varieties of soybean on the growth, yield, nodulation and galling

Varieties	Treatments	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Number of pods/ plant	Weight of seeds/ plant (g)	Number of nodules/ plant	Number of galls/ g of root
V <sub>1</sub>	T <sub>0</sub>	48.05 e	17.84 g	13.70 e	4.23 b	8.20	7.42 h	1.40 e	3.40
	T <sub>1</sub>	64.25 a	20.74 b	16.70 bc	3.88 c	10.80	12.11 bc	5.00 a	1.20
	T <sub>2</sub>	60.35 b	22.14 a	18.55 a	5.57 a	10.60	11.97 c	3.40 bc	1.60
	T <sub>3</sub>	50.62 d	19.08 e	15.17 d	3.49 d	8.20	8.78 f	3.40 bc	2.40
V <sub>2</sub>	T <sub>0</sub>	40.10 h	16.36 i	11.92 g	2.81 gh	8.80	7.56 h	1.20 e	3.60
	T <sub>1</sub>	53.70 c	19.42 d	17.15 b	3.01 efg	13.60	13.30 a	4.60 a	1.60
	T <sub>2</sub>	51.63 d	19.96 c	16.11 c	3.26 de	11.00	11.24 d	2.60 cd	2.40
	T <sub>3</sub>	44.71 f	17.24 h	12.20 fg	2.63 h	8.60	9.63 e	2.80 cd	2.60
V <sub>3</sub>	T <sub>0</sub>	38.50 i	14.88 j	11.29 h	2.91 fg	8.60	7.51 h	2.20 de	3.80
	T <sub>1</sub>	47.65 e	18.46 f	16.28 c	3.15 ef	12.00	12.30 b	4.20 ab	1.80
	T <sub>2</sub>	48.05 e	19.86 c	16.31 c	3.11 ef	10.40	11.29 d	4.60 a	2.40
	T <sub>3</sub>	42.22 g	16.28 i	12.77 f	2.21 i	8.20	8.18 g	3.20 bcd	2.60
LSD (P≥ 0.05)		1.299	0.246	0.602	0.261	NS	0.228	0.969	NS

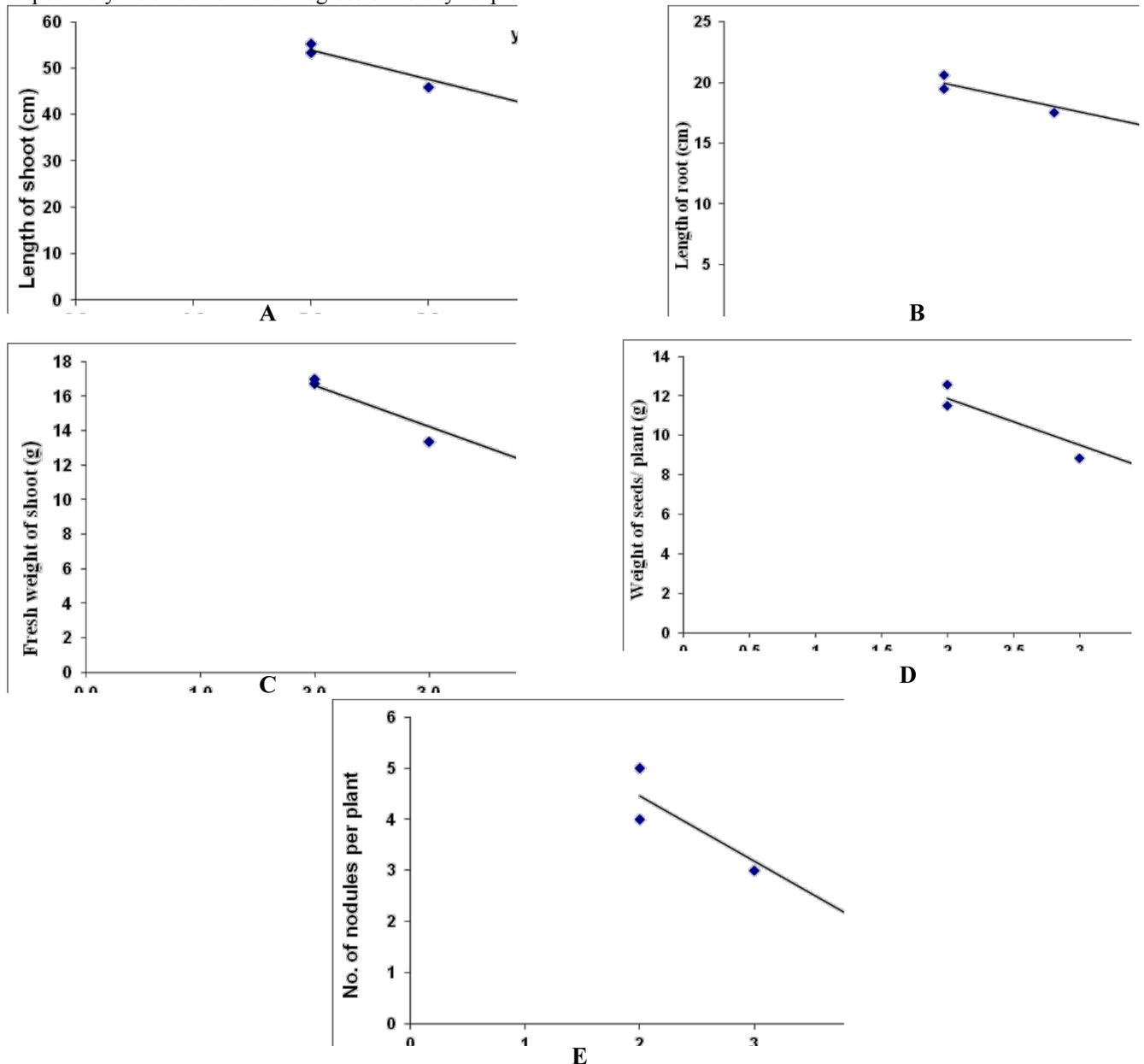
**Table 6.** Interaction effects of treatments and varieties of soybean in the development of adult females, eggmasses and juveniles of *Meloidogyne incognita*

Varieties	Treatments	Number of adult females/ 10 galls	Number of eggmasses/ g of root	Number of J <sub>2</sub> juveniles/ 10 galls	Number of J <sub>3</sub> juveniles/ 10 galls	Number of J <sub>4</sub> juveniles/ 10 galls
V <sub>1</sub>	T <sub>0</sub>	4.00	2.20	2.00	2.40	3.60
	T <sub>1</sub>	2.20	1.80	1.00	1.40	2.20
	T <sub>2</sub>	2.40	1.80	1.20	1.40	2.20
	T <sub>3</sub>	3.20	2.20	1.80	2.00	2.40
V <sub>2</sub>	T <sub>0</sub>	4.20	2.20	2.00	2.80	3.80
	T <sub>1</sub>	2.40	1.20	0.80	1.40	2.00
	T <sub>2</sub>	2.60	1.60	1.40	1.60	2.40
	T <sub>3</sub>	2.80	1.60	1.60	2.20	2.60
V <sub>3</sub>	T <sub>0</sub>	4.40	3.20	2.20	2.80	4.00
	T <sub>1</sub>	2.20	1.80	1.00	1.60	2.20
	T <sub>2</sub>	2.80	2.00	1.40	1.40	2.40
	T <sub>3</sub>	3.20	2.60	1.60	2.20	2.60
LSD (P≥ 0.05)		NS	NS	NS	NS	NS

T<sub>0</sub> = Control , T<sub>1</sub> = BAU-Biofungicide , T<sub>2</sub> = Allamanda tablet, T<sub>3</sub> = Neem oil, V<sub>1</sub> = BARI Soybean -5, V<sub>2</sub> = BAU-S-80, V<sub>3</sub> = BAU-S-109

**Correlation and regression study:** Correlation study was done to examine the relationship of gall number with length of shoot and root, gall number with fresh weight of shoot and root, gall number with number of pods per plant, gall number with weight of seeds per plant and gall number with number of nodules per plant. The study revealed significant and negative correlations between gall number and length of shoot and root (Figs. 1A and 1B), gall number and fresh weight of shoot (Fig. 1C), gall number and weight (g) of seeds per plant (Fig. 1D) as well as gall number and number of nodules per plant (Fig. 1E) and followed the regression equations  $y = -6.2427x + 66.32$  ( $r = -0.974^{**}$ ),  $y = -1.8909x + 23.7$  ( $r = -0.953^{*}$ ),  $y = -2.3836x + 21.4$  ( $r = -0.967^{**}$ ),  $y = -2.35x + 16.57$  ( $r = -0.964^{*}$ ),  $y = -1.2727x + 7$  ( $r = -0.944^{*}$ ), respectively. Correlation and regression study depicted

that treatments with BAU-Biofungicide, Allamanda tablet and Neem oil gave positive and higher response in increasing the growth characters of soybean with respect to length of shoot and root, fresh weight of shoot, weight of seeds and number of nodules suppressing the nematode activities as evident with lower galling incidence encountered with higher plant growth and yield performance. The reports given by Mustarin (2004), Sivakumar and Ramakrishan (2005) and Sultana *et al.* (2006) are also in support of these findings. Therefore, control of root-knot disease of soybean caused by *Meloidogyne incognita* may be explored through use of BAU-Biofungicide and Allamanda tablet for eco-friendly management of this nemic disease avoiding chemical nematicide.



**Fig. 1.** Relationship between (a) number of galls g<sup>-1</sup> of root and length of shoot (cm), (b) number of galls g<sup>-1</sup> of root and length of root (cm), (c) number of galls g<sup>-1</sup> of root and fresh weight of shoot (g), (d) number of galls g<sup>-1</sup> of root and weight of Seeds plant<sup>-1</sup> (g), (e) number of galls g<sup>-1</sup> of root and number of nodules plant<sup>-1</sup>

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