Bio-control of root-knot (*Meloidogyne incognita*) of mungbean

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Abstract: The experiment was conducted in the net house of the Seed Pathology Centre, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during 2011. The efficacy of BAU-Biofungicide, IPM Lab Biopesticide and marigold leaf extract including control were tested against root-knot nematode (*Meloidogyne incognita*) of three mungbean varieties viz. BARI Mungbean-3, BINAmoog-8 and Local. All the treatments were used as side drenching. Among the treatments, BAU-Biofungicide gave the best performance with increased length of shoot and root, fresh weight of shoot and root with nodules, number of pods and nodules per plant with higher weight of pods consequently with the lowest galling incidence. Simultaneously, lower numbers of adult females, eggmasses, J_2 and J_4 juveniles were also observed with this treatment. IPM Lab biopesticide showed better response in increasing growth, nodulation and yield characters of mungbean with decreased galling incidence and nematode population. Marigold leaf extract showed lower response in all the above characters compared to other bio-control agents. Control treatment showed the lowest effect in all the above growth and yield characters with higher galling incidence and nematode populations. BARI Mungbean-3 responded with higher growth and yield and lower nematode development followed by BINAmoog-8 and Local variety.
Key words: Mungbean, biofungicide, biopesticide, marigold.

Introduction

Mungbean *(Vigna radiata*) belonging to the family Leguminosae is one of the important pulse crop grown in Bangladesh. It grows throughout the country in both summer and winter seasons. The economic product of mungbean is its seed, which contains 22-24% of protein (Malik, 1994). Moreover, mungbean also contains high amount of Vitamins A, B, C and niacin, and minerals such as potassium, phosphorus and calcium, which are necessary for human body (Rattanawongsa, 1993). Mungbean contributes 20.94 % of total pulse production in the country. Mungbean is cultivated about 1,63,000 ha in every year in Bangladesh and production is about 1,50,000 ton , with average per ha yield 0.92 ton (AIS, 2012).

The average yield of mungbean in Bangladesh is very low compared to the other pulse growing countries. Among the constraints, responsible for this lower yield of mungbean, diseases are considered to be the most important (Bakr and Rahman, 1998). Mungbean is susceptible to many diseases caused by pathogens like fungi, bacteria, viruses, mycoplasma and nematodes (Talukder, 1974; Hoque and Choudhury, 1980). Plant parasitic nematodes are recognized as the causes of serious yield losses on a wide range of crops (Javad *et al.,* 2006). Mainly two species of root-knot nematodes, *Meloidogyne javanica* and *Meloidogyne incognita* are considered to be the major pests of mungbean (Mian, 1986).

It is difficult to control nematodes because of their wide host range and high rate of reproduction, with females capable of producing upto thousand eggs/female (Natarajan *et al.,* 2006). Different chemicals and bio-control agents including botanicals are used for controlling nemic diseases. But chemical control is expensive and create a potential hazard to the environment and human health (Tsay *et al.,* 2004). Now-a-days biological control agents are being used by many developed countries for combating the nemic diseases with the aim of increasing food and vegetable productions. Biological control agents are employed as antagonists, parasites or predators (Kwok *et al*., 1987). *Trichoderma* based BAU-Biofungicide and IPM Lab Biopesticide are being used as nematode controlling agents. BAU-Biofungicide significantly increased the root and shoot growth, nodulation and yield per plant of mungbean suppression of nematode (Hasan *et al*., 2005). *Trichoderma* produces Trichodermin which shows its antagonistic activity against various diseases (Tverdyukev *et al.*, 1994). Plant extracts have been reported to have antinematode properties (Mahmood *et al.*, 1982). Marigold has nematicidal properties against plant parasitic nematode. Marigold can suppress 14 genera of plant-parasitic nematodes root-knot nematodes *Meloidogyne* spp. (Suatmadji, 1969). As a pulse crop, mungbean plays important role in food stuff and economy, so attention should be given for 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 study the comparative efficacy of BAU-Biofungicide, IPM Lab biopesticide and Marigold leaf extract as bio-control agents against root-knot (*Meloidogyne incognita*) of mungbean.

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 mungbean varieties viz. BARI Mungbean - 3 (V_1) and BINA moog-8 (V_2) and Local (V_3) and four treatments viz. Control (T_0), BAU-Biofungicide (T_1), IPM Lab Biopesticide (T_2), and Marigold leaf extract (T_3) 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), IPM Lab Biopesticide (2 % solution) and Marigold leaf extract (10 ml solution after blending 25 g marigold leaves in 1000 ml water) were applied as side drench separately for each replicated plant of all three varieties after 10 days of inoculation with eggmasses.

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.* After 2-3 months, 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.

Healthy, mature and disease free mungbean seeds were treated with 10% chlorox solution for 30 seconds and were subsequently rinsed with sterilized distilled water for three times before sowing. Six seeds were directly sown in the pots on 06 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 and weight of pods and number of nodules per plant, number of galls and eggmasses per g of root and number of adult females, J_2, J_3 and J_4 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_2, J_3 and J_4 juveniles of *Meloidogyne incognita* in the mungbean varieties are presented in Table 1 and 2, respectively. Maximum length of shoot (25.33 cm) and root (20.95 cm), fresh weight of shoot (19.45 g) and root with nodules (9.16 g), number (10.27) and weight (11.07 g) of pods per plant and number of nodules per plant (4.60) were obtained consequently with the lowest galling incidence (1.53 per g of root) and numbers of adult females (1.20), eggmasses (1.60), J_2(1.07), J_3 (1.33) and J_4 (1.00) juveniles with treatment BAU-Biofungicide followed by IPM Lab biopesticide and Marigold leaf extract. Control treatment appeared with the significant reduced length of shoot (16.94 cm) and root (7.54 cm), fresh weight of shoot (11.40 g) and root with nodules (3.13 g) as well as number (3.99) and weight of pods per plant (4.59 g) and number of nodules per plant (2.27).

Table 1. Effects of different treatments on the growth, yield, nodulation and galling in mungbean 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 pods/ plant (g)	Number of nodules/ plant	Number of galls/ g of root
T_0 (Control)	16.94 d	7.54 d	11.40 d	3.13 d	3.99 d	4.59 d	2.27 c	7.46 a
T_1 (BAU -Biofungicide)	25.33 a	20.95 a	19.45 a	9.16 a	10.27 a	11.07 a	4.60 a	1.53 d
T_2 (IPM Lab biopesticide)	22.14 b	17.37 b	16.70 b	7.78 b	7.20 b	9.87 b	3.73 b	2.93 c
T_3 (Merigold leaf extract)	19.20 c	16.12 c	14.31 c	6.88 c	6.07 c	7.19 c	3.40 b	3.93 b
LSD ($P \geq 0.05$)	1.93	0.89	0.93	0.48	0.42	0.40	0.69	0.39

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

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

Treatments	Number of adult females/ 10 galls	Number of eggmassess/ g of root	Number of J_2 juveniles/ 10 galls	Number of J_3 juveniles/ 10 galls	Number of J_4 juveniles/ 10 galls
T_0 (Control)	4.07 a	6.80 a	3.07 a	3.13	3.13 a
T_1 (BAU -Biofungicide)	1.20 d	1.60 d	1.07 d	1.33	1.00 d
T_2 (IPM Lab biopesticide)	2.20 c	3.80 c	1.47 c	1.80	1.47 c
T_3 (Marigold leaf extract)	2.93 b	5.07 b	2.00 b	2.33	2.13 b
LSD ($P \geq 0.05$)	0.36	0.86	0.28	NS	0.38

NS = Not significant

The highest galling incidence (7.46 per g of root) correspondingly with the lowest yield performance was also observed with the control treatment.

BAU-Biofungicide and IPM Lab Biopesticide both being *Trichoderma* based formulation was found very effective to increase plant growth and yield and also showed suppressive effect on growth and development of root-knot nematode (*Meloidogyne incognita*). The effectiveness of *Trichoderma spp*. may be attributed to the fact that the fungi occupy the niche before nematode infection and thereby hinder the establishment of the nematode pathogen. Many researchers also recorded effectiveness of *Trichoderma spp.* to reduce gall formation and reducing final nematode population on different crops caused by *Meloidogyne incognita* and *Meloidogyne javanica* with corresponding increase of plant growth and yield. Akhtar *et al.* (2005) observed Trichoderma *harzianum* increase the growth and yield parameters and suppress nematode reproduction. *Trichoderma* was found to be antagonistic to *Meloidogyne spp.* (Faruk *et al.*, 1999). Hasan (2004) reported higher nodulation, higher yield, less number of galls, less adult females, J_2, J_3 and J_4 juveniles in mungbean infected with *Meloidogyne javanica*. Tverdyukev *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 and IPM Lab biopesticide as both are formulation of *Trichoderma* sp. Formulated *Trichoderma* effectively controlled soil-borne diseases including root-knot nematode in different crops (Meah, 2010). In the present study, reduced galling incidence along with reduction of adult females and juveniles might have been resulted from the adverse influence on hatching of inoculated eggs and eggs produced after first and second generation of *M. incognita* as similarly stated by the above authors.

The treatment Marigold leaf extract showed better performance in plant growth characters with higher number and weight of pods and nodulation correspondingly with lower galling and nematode development compared to control treatment. Weimin *et al.* (2008) reported that African marigold (*Tagetes erecta*) and French marigold (*T. patula*) were effective in reducing the most common root-knot nematode populations *Meloidogyne incognita* and *M. javanica*. Marigold extract showed lethal to the juveniles in the mortality test (Hassan *et al.*, 2003). Leaf and stem extracts showed the best effect on most of the plant growth characters by suppressing the galling incidence (Nargis *et al*., 2005). Marigold cultivars of *Tagetes patula*, *T. erecta*, *T. signata*, and a *Tagetes* hybrid all reduced galling and numbers of second-stage juveniles in tomato (Ploeg, 1999). Marigold roots release the chemical alpha-terthienyl, one of the most toxic naturally occurring compounds which is nematicidal, insecticidal (Marles *et al*., 1992). Polythinyles isolated from marigold plants (*Tagetes spp.*) may alter nematode behaviour and development, serve as nematicides, disrupt molting, hatching and other hormonally regulated processes (Youssef, 2008).

Table 3. Responses on the growth, yield, nodulation and galling in three mungbean 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 pods/ plant (g)	Number of nodules/ plant	Number of galls/ g of root
BARI Mungbean-3 (V_1)	22.27 a	16.82 a	16.28 a	6.98 a	8.34 a	8.72 a	3.80 a	3.30 c
BINAmoog-8 (V_2)	20.93 ab	15.82 a	15.46 b	6.86 a	7.10 b	8.03 b	3.15 b	3.95 b
Local (V_3)	19.51 b	14.36 b	14.66 b	6.33 b	5.20 c	7.80 b	3.55 ab	4.65 a
LSD ($P\geq 0.05$)	1.67	0.77	0.80	0.41	0.37	0.35	0.61	0.34

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

Variety	Number of adult females/ 10 galls	Number of eggmassess/ g of root	Number of J_2 juveniles/ 10 galls	Number of J_3 juveniles/ 10 galls	Number of J_4 juveniles/ 10 galls
BARI Mungbean-3(V_1)	2.30 b	3.55 b	1.80	2.00 b	1.55 b
BINAmoog-8 (V_2)	2.60 ab	4.65 a	1.85	2.05 b	2.10 a
Local (V_3)	2.90 a	4.75 a	2.05	2.40 a	2.15 a
LSD ($P\geq 0.05$)	0.31	0.74	NS	0.30	0.32

Responses of mungbean varieties: Responses of the varieties with respect to all characters except number of J_2 juveniles per 10 galls were found to be significant (Table 3 and 4). Among the three varieties of mungbean, BARI Mungbean-3 gave maximum fresh weight of shoot (16.28 g), number of pods per plant (8.34), weight of pods per plant (8.72 g) correspondingly with the lowest number of galls (3.30 per g of root), number of adult females (2.30), eggmasses (3.55) and J_4 (1.55) juveniles. BARI Mungbean-3 and BINAmoog-8 gave similar response in case of length of root, fresh weight of root with nodules, number of nodules per plant as well as number of J_3 juveniles.

Interaction effects of the treatments and varieties: Interaction effects of the treatments and varieties on the fresh weight of root with nodules, number of pods per plant, weight of pods per plant and number of nodules per plant were found to be significant (Table 5). The treatment T_1 (BAU-Biofungicide) interacting with the variety V_1 (BARI Mungbean-3) gave significantly the highest fresh weight of root with nodules (9.74 g), number of pods (12.00), weight of pods (11.42 g), number of nodules per plants (5.00). Higher responses in fresh weight of root with nodules, weight of fruit, number of pods per plant, number of nodules per plants were also observed with treatment T_1 interacting with variety V_2 (BINA Mung-8). Comparatively better effect was found with treatment T_1 interacting with V_3 (Local variety) with respect to fresh weight of root with nodules, weight of pods, number of pods per plant and number of nodules. Among the treatments, treatment T_2 (IPM Lab Biopesticide) appeared to give more or less better effect in respect of length of shoot and weight of seeds interacting with all the varieties compared to treatment T_3 (Marigold leaf extract).

Table 5. Interaction effects of treatments and varieties of mungbean 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 pods/ plant (g)	Number of nodules/ plant	Number of galls/ g of root
V_1	T_0	17.68	7.52	11.53	2.56 h	4.59 g	5.97 e	3.40 bc	6.40
	T_1	27.06	22.30	20.15	9.74 a	12.00 a	11.42 a	5.00 a	1.20
	T_2	23.92	18.30	18.19	8.15 cd	9.40 c	10.17 bc	3.40 bc	2.40
	T_3	20.40	17.08	15.22	7.46 de	7.40 e	7.30 d	3.40 bc	3.20
V_2	T_0	17.10	8.10	12.09	3.54 g	5.00 fg	4.10 f	1.20 d	7.40
	T_1	25.60	21.70	19.32	9.25 ab	10.40 b	11.01 a	4.60 ab	1.60
	T_2	21.72	17.28	16.29	7.92 cd	7.60 e	9.87 c	3.20 bc	2.80
	T_3	19.30	16.28	14.12	6.86 ef	5.40 f	7.13 d	3.60 b	4.00
V_3	T_0	16.04	7.00	10.57	3.29 gh	2.40 h	3.71 f	2.20 cd	8.60
	T_1	23.32	18.84	18.88	8.50 bc	8.40 d	10.79 ab	4.20 ab	1.80
	T_2	20.78	16.60	15.59	7.26 de	4.60 g	9.57 c	4.60 ab	3.60
	T_3	17.90	15.00	13.59	6.31 f	5.40 f	7.13 d	3.20 bc	4.60
LSD ($P \geq 0.05$)		NS	NS	NS	0.82	0.73	0.69	1.21	NS

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

Varieties	Treatments	Number of adult females/ 10 galls	Number of eggmassess/ g of root	Number of J_2 juveniles/ 10 galls	Number of J_3 juveniles/ 10 galls	Number of J_4 juveniles/ 10 galls
V_1	T_0	3.40	5.60	3.00	3.13 a	2.20
	T_1	1.00	1.80	1.00	1.33 e	0.80
	T_2	2.00	2.80	1.40	1.80 d	1.20
	T_3	2.80	4.00	1.80	2.33 bc	2.00
V_2	T_0	4.20	7.60	3.00	0.12 f	3.60
	T_1	1.20	1.20	1.00	1.20 e	1.00
	T_2	2.20	4.20	1.40	1.80 d	1.60
	T_3	2.80	5.60	2.00	2.00 cd	2.20
V_3	T_0	4.60	7.20	3.20	3.00 a	3.60
	T_1	1.40	1.80	1.20	1.40 e	1.20
	T_2	2.40	4.40	1.60	1.40 e	1.60
	T_3	3.20	5.60	2.20	2.40 b	2.20
LSD ($P \geq 0.05$)		NS	NS	NS	0.35	NS

T_0 = Control, T_1 = BAU-Biofungicide, T_2 = IPM Lab biopesticide, T_3 = Marigold leaf extract, V_1 = BARI Mungbean – 3, V_2 = BINAmoog-8, V_3 = Local

Interaction effects of the treatments and varieties of mungbean on the development of J_3 juveniles of *M. incogita* was found to be significant and presented in Table 6. Treatment T_1 (BAU Biofungicide) interacting with all the varieties resulted minimum development of J_3 juveniles. Treatment T_2 (IPM Lab Biopesticide) appeared to give better effect interacting with all the varieties compared to treatment T_1 BAU Biofungicide. Lower effect in suppressing the development of J_3 observed with T_3 interacting with all the varieties in the development of J_3 juveniles. The interaction effects of the treatments and varieties of mungbean on the development of adult females, eggmasses, J_2, and J_4 juveniles of *M. incognita* were found to be insignificant.

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 pods 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. 1 and 2), gall number and fresh weight of shoot and root (Figs. 3 and 4), gall number and number of pods per plant (Fig.5), gall number and weight (g) of pods per plant (Fig. 6) as well as gall number and number of nodules per plant (Fig.7) and followed the regression equations y= -1.571x + 27.16 (r= -0.933*), y= -2.621x + 25.96 (r= -0.995***), y = -1.529x +21.58 (r= -0.963**), y= - 1.194x + 11.51 (r= -0.999***), y= -1.071x + 11.04(r= -0.926**), y=-1.303x+13.391(r=-0.973**) and y=-0.571x+5.79 (r=-0.956*), respectively. It indicated that the galling incidence hampered the crop growth in terms of shoot and root length along with fresh weight of shoot, weight of seeds per plant, number of nodules per plant. In other words, treatments gave positive responses increasing the growth characters as well as suppressing the nematode activities as evident with decreased galling incidence. The report given by Sivakumar and Ramakrishan (2005) supports these findings.

From the experiment, it was conceived that control of *Meloidogynae incognita* with BAU-Biofungicide and IPM Lab biopesticide as side drenching is quite effective for eco-friendly management of this nemic disease.

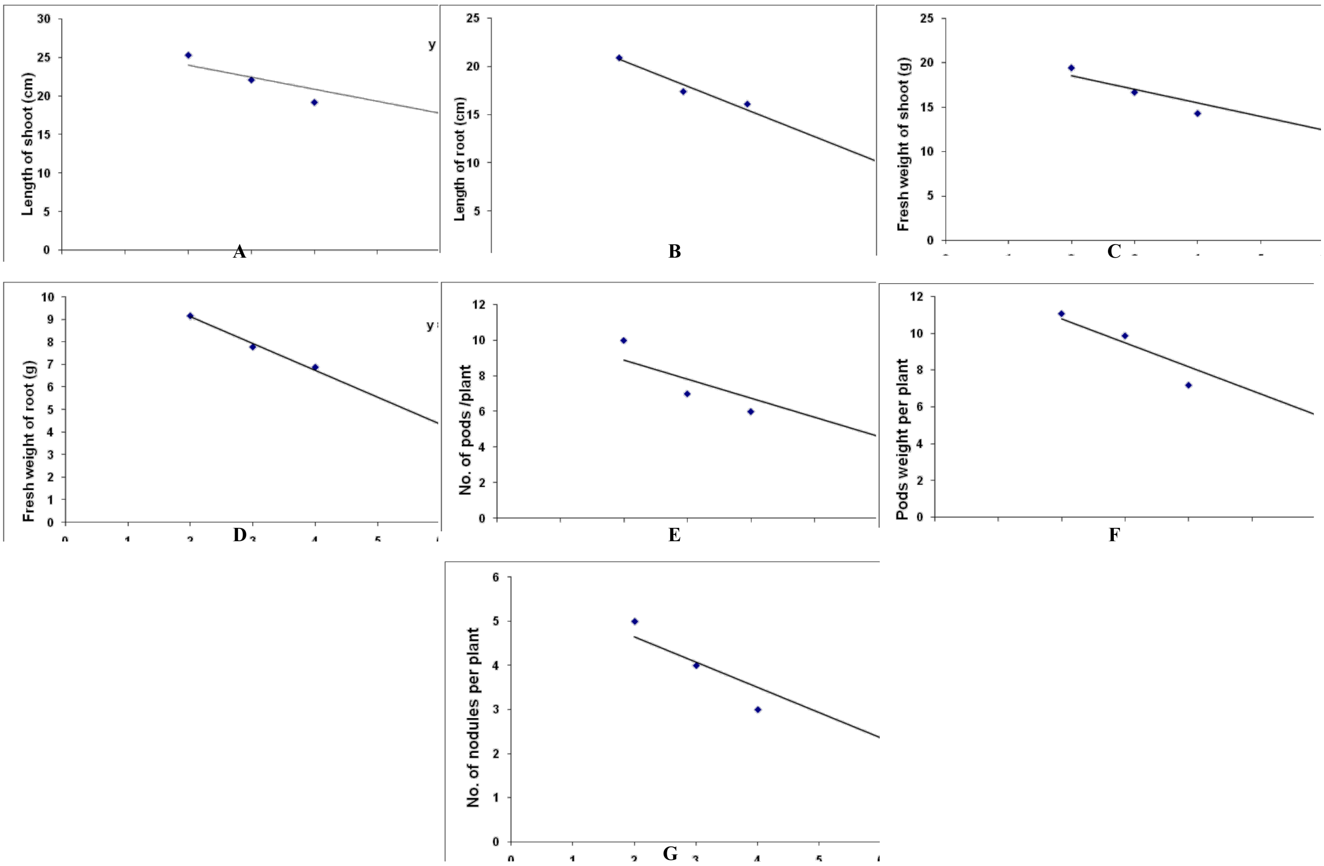

Fig.1. Relationship between (A) number of galls g^{-1} of root and length of shoot (cm), (B) number of galls g^{-1} of root and length of root (cm), (C) number of galls g^{-1} of root and fresh weight of shoot (g), (D) number of galls g^{-1} of root and fresh weight of root with nodules (g), (E) number of galls g^{-1} of root and number of pods per plant, (F) number of galls g^{-1} of root and weight of pods per plant (g), (G) number of galls g^{-1} of root and number of nodules per plant

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