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Effect of Inocula Level of *Meloidogyne Javanica* and *Sclerotium rolfsii* on the Growth, Yield and Galling Incidence of Groundnut

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Abstract: Mixed inocula of *Meloidogyne javanica* and *Sclerotium rolfsii* in five different treatments including control were tested for the growth, yield, galling incidence and development of the nematode in groundnut. Progressively higher galling incidence and higher number of adult females and juvenile populations of *M. javanica* correspondingly with lower plant growth, nodulation and yield per plant were recorded from lower to higher levels of inocula ranging from 4–10 egg masses of *M. javanica* with 0.025 - 0.2% w/w of *S. rolfsii* . Galling incidence was negatively correlated with plant growth, nodulation and yield of groundnut.

Key words: Inocula levels, *Meloidogyne javanica*, *Sclerotium rolfsii*, galling incidence, groundnut

Introduction

Groundnut (*Arachis hypogaea* L.) is the important and well-recognized grain legume, vegetable oil and protein crop in many countries of the world. In Bangladesh, groundnut is the second important oil seed crop after rape and mustard on the basis of annual production, but it ranks third on the area basis. Groundnut produces the highest oil and protein per unit area (Ahlawal and Chahal, 1986). Its calorie value is 2 to 2.5 times more than that of cereal crops. Groundnut contains 20-30% protein. Groundnut is subjected to attack by many diseases caused by fungi, bacteria, viruses, mycoplasma and nematodes (Ahmed and Hossain, 1985; Mian, 1986). Mian (1986) reported that 17 genera of nematodes attack different crops along with groundnut in Bangladesh where *Meloidogyne* sp. are predominant. The common species of root-knot nematode attack wide varieties of fruits, vegetables and field crops including groundnut. The root-knot disease is of economic importance to the growers. *Sclerotium rolfsii* is a soil borne pathogen and it causes wilt disease in Bangladesh. (Ashrafuzzaman, 1986). It is often found that *Sclerotium rolfsii* and root-knot nematodes *Meloidogyne* sp. remain closely associated with plants in soil and cause disease complex (Powell *et al.,* 1971). The individual effect as well as combined effect of these organisms in disease complex is not yet thoroughly studied in Bangladesh, although this type of study has already been done in other countries (Khan and Saxena, 1969; Lanjewar and Shukla, 1985). So, the present research work was undertaken to determine the effect of different inocula levels of *Meloidogyne javanica* and *Sclerotium rolfsii* on the plant growth, nodulation, galling incidence and yield of groundnut and to determine the combined effect of *Meloidogyne javanica* and *Sclerotium rolfsii* on the development of nematode populations in groundnut.

Materials and Methods

The experiment was conducted both in the laboratory and glasshouse of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during the period of March to July, 2001. Sandy loam soil was uniformly mixed with air dried cowdung and sand at the ratio of 2:2:1. The soil was treated with 3% formalin solution for sterilization and covered with polythene sheet. After 72 h the treated soil was exposed and air-dried for 48 h in order to remove excess vapour of formalin. Earthen pots (30 cm diameter) were filled with 5 kg sterilized and dried soil. A 20 cm earthen plate was placed below each pot to retain excess water. Healthy, mature and disease free seeds of groundnut (var. Dhaka-1) were collected from the Seed Foundation of Trisal thana and Madina seed store of Mymensingh, Bangladesh. Before sowing, seeds were treated with 0.001% mercuric chloride solution for 1 min and were subsequently rinsed with sterilized distilled water for three times. Three seeds of groundnut were sown per pot. Only one healthy seedling per pot was allowed to grow. Sowing of seeds in the inoculated set was done after seven days of soil inoculation with the nematode and fungal pathogens. Seeds were grown in the control pots in the same manner without any inoculation. The pots were watered twice a day regularly. The potted soil around the base of the plant was loosened from time to time with the help of khurpi.

The experiment was set up in the glasshouse. Five treatments including control were used with five replications. All the pots were arranged in completely randomized design (CRD) with the following treatments:

T_0 = Control (without *Meloidogyne javanica* and *Sclerotium rolfsii*),
T_1 = *M. javanica* (10 egg masses) + *S. rolfsii* 0.2% (w/w),
T_2 = *M. javanica* (8 egg masses) + *S. rolfsii* 0.1% (w/w),
T_3 = *M. javanica* (6 egg masses) + *S. rolfsii* 0.05% (w/w) and
T_4 = *M. javanica* (4 egg masses) + *S. rolfsii* 0.025% (w/w).

The fungus *Sclerotium rolfsii* was sub-cultured in PDA medium in petridishes. Oat seeds were washed and soaked in water for 48 h. About 50 gm of soaked seeds were taken in 250 ml. Erlenmeyer flask plugged tightly with cotton and then autoclaved for 20 min under 15 lbs pressure at 120^{0}C. After sterilization the sterilized oat seeds in the flask were inoculated with the small agar blocks containing *Sclerotium rolfsii* from pure culture plate and incubated at 28±2^{o}C for seven days for the proper mycelial growth of the fungi. The ground oat cultures were stored in refrigerator and used for the purpose of inoculating the soils. The potted soils were inoculated with the inoculum of *Sclerotium rolfsii*. Four different levels of inoculum of the pathogen were used. The levels were 0.2, 0.1, 0.05 and 0.025 % weight by weight of dry soil. The inoculated soil were incubated for seven days and watered regularly in order to allow the fungus to grow uniformly in the soil. Egg masses of *Meloidogyne javanica* were collected from the roots of brinjal plants cv. "Singnath" which were previously inoculated with a single egg mass of *Meloidogyne javanica* obtained from diseased brinjal plant. Surface sterilization of the collected egg masses were done with 0.001% solution of mercuric chloride for about one minute followed by subsequent washing with water. Groundnut seedlings of 25 days age grown in pot soil were inoculated with sterilized egg masses of *Meloidogyne javanica*.

The data was collected from uprooted groundnut plants from pots after 70 days of inoculation for length of shoot (cm), length of root (cm), fresh weight of shoot (g), fresh weight of root with nodule (g), number of pods per plant, number of nodules per plant, number of galls g^{-1} root and yield per plant (g), and number of adult females of nematodes, J_2, J_3 and J_4 stages. The collected data was analyzed statistically to find out the level of significance. The means for all the treatments were counted and the analysis of variance was studied by F-test for the treatment means and replication means. The mean differences were evaluated for their significant level by Duncan's new multiple range test (DMRT).

Results and Discussion

Results of different inocula levels of *M. javanica* and *S. rolfsii* on the plant growth, yield and galling incidence of groundnut are shown in Table 1. The highest (44.50 cm) shoot length was observed with the treatment T_0 (control) while the lowest was recorded with treatment T_1 having 15.02 cm long shoot. The second highest shoot length was recorded with treatment T_3 (35.70 cm) followed by T_2 and T_4 having 31.90 and 31.60 cm, respectively. But the effect of T_2 and T_4 was found to be statistically similar with the highest levels of inocula of *M. javanica and S. rolfsii*. Non inoculated treatment T_0 produced the highest 31.19 cm root length followed by the treatments T_3, T_4 and T_2 having 25.99, 24.79 and 23.73 cm, respectively (Table 1). But statistically their effect was found to be identical. Significantly lower root length 14.29 cm was observed with treatment T_1. The highest significant shoot weight was recorded with the control treatment T_0 followed by the treatments T_2, T_3 and T_4 having 30.46, 32.08 and 30.28 g, respectively. But no statistical difference was found among the last three treatments. The treatment T_1 gave significantly the lowest shoot weight 17.32 g. The effect of different treatments in respect of fresh weight of root with nodules was found to be significant. The highest fresh weight of root with nodules 5.164 g was observed with the control treatment T_0, while the lowest was recorded with the treatment T_1 having 1.814 g. Higher significant and statistically identical fresh weight of root with nodules 2.726, 2.910 and 3.026 g were recorded with treatment T_2, T_3 and T_4, respectively. Significant variation was observed among the treatments in respect of number of pods per plant. The highest significant and statistically identical number of pods per plant 11.20 and 10.00 were recorded with the control treatment T_0 and T_4 treatment (with the lowest inocula levels), respectively. Significantly lower and statistically identical number of pods per plant 5.20, 7.40 and 7.20 were observed with treatments T_1, T_2 and T_3, respectively. Significant effect was found among the treatments with respect to number of nodules per plant. Significantly the highest number 27.28 of nodules per plant was recorded with the control treatment T_0 followed by 24.64, 20.27, 15.27 and 9.86 with treatments T_4, T_3, T_2 and T_1, plants,

Table 1: Effect of different inocula levels of *Meloidogyne javanica* and *Sclerotium rolfsii* on the plant growth, yield and galling incidence of groundnut

Treatments	Length of shoot (cm)	Length of root (cm)	Fresh weight of shoot (g)	Fresh weight of root with nodules (g)	Number of pods per plant	Number of nodules per plant	Number of galls per g of root	Yield per plant (g)
T_0	44.50a	31.19a	37.46a	5.16a	11.20a	27.28a	0.00e	8.78a
T_1	15.02d	14.29d	17.32c	1.81c	5.20b	9.86e	27.70a	4.62c
T_2	31.90c	23.73c	30.46b	2.72b	7.40b	15.27d	21.46b	5.56c
T_3	35.70b	25.99b	32.08b	2.91b	7.20b	20.27c	18.96c	5.70c
T_4	31.60c	24.79bc	30.26b	3.03b	10.00a	24.64b	15.49d	7.40b
LSD	1.236	1.900	3.450	0.348	2.183	1.90	1.96	1.359

Table 2: Effect of different inocula levels on the growth of *Meloidogyne javanica* in groundnut inoculated with egg masses of *Meloidogyne javanica* and culture of *Sclerotium rolfsii*

Treatments	No. of adult females/10 galls	No. of J_2/10 galls	No. of J_3/10 galls	No. of J_4/10 galls
T_0	0.00d	0.00d	0.00d	0.00d
T_1	30.64a	28.07a	20.90a	18.79a
T_2	22.10b	19.36b	14.60b	13.43b
T_3	15.51c	15.72c	14.12b	12.31bc
T_4	15.27c	14.26c	11.75c	11.19c
LSD	2.102	2.534	2.236	1.427

Values are the means of five replications. In a column, values having same letter(s) do not differ significantly at p = 0.01.

T_0 = Control (without *Meloidogyne javanica* and *Sclerotium rolfsii*)
T_1 = *M. javanica* (10 egg masses) + *S. rolfsii* 0.2% (w/w)
T_2 = *M. javanica* (8 egg masses) + *S. rolfsii* 0.1% (w/w)
T_3 = *M. javanica* (6 egg masses) + *S. rolfsii* 0.05% (w/w)
T_4 = *M. javanica* (4 egg masses) + *S. rolfsii* 0.025% (w/w)
J = Juvenile

respectively. Significant differences were found among treatments in respect of galling.

Significantly the highest number of 27.70 per gram of root galls was recorded with the treatment T_1 followed by 21.46, 18.96 and 15.49 with the highest levels of inocula of *M. javanica* and *S. rolfsii* galls per gram of root in the treatments T_2, T_3 and T_4, respectively. Non-treated control treatment had no galling incidence. The effect of different treatment in respect of yield per plant was found to be significant. The highest significant grain yield/plant 8.78 g was observed with the non-inoculated treatment T_0. Higher significant effect on yield was recorded with the treatment T_4 having 7.40 g where the lowest levels of inocula of both the pathogens were used. The lowest significant and statistically identical response in grain yield per plant was recorded with the treatments T_2, T_3 and T_4 having 4.62, 5.56 and 5.70 g, respectively.

Results of different inocula levels on the growth of *Meloidogyne javanica* in groundnut inoculated with egg masses of *Meloidogyne javanica* and culture of *Sclerotium rolfsii* are presented in Table 2. The highest significant number 30.64 of adult females of *M. javanica* was found with the treatment T_1 where maximum levels of inocula of both the pathogens were used. Higher significant number 22.10 of adult females of *M. javanica* was found in treatment T_2 with higher inocula levels. Comparatively lower significant and statistically identical numbers of adult females were recorded in the treatments T_3 and T_4 having 15.51 and 15.27, respectively. Non-inoculated control treatment T_0 had no adult females. In case of J_2 juvenile stage, the effect of treatments was found to be statistically significant. Treatment T_1 with the highest levels of inocula gave the highest significant number 28.07 of J_2 juveniles followed by the treatment T_2 having 19.36 numbers of J_2 juveniles. Lower significant and statistically identical numbers 15.72 and 14.26 of J_2 were noted with T_3 and T_4, respectively, while no such nematode was found in T_0 (control) treatment. Different treatments were found to influence significantly the development of J_3 juveniles. The highest significant number 20.90 of J_3 juvenile were observed with treatment T_1 follower by higher significant and statistically identical numbers 14.60 and 14.12 of J_3 with the treatments T_2 and T_3, respectively. Lower significant number 11.75 of J_3 juveniles was noted with the treatment T_4, while no J_3 was found in T_0. Like that of the previous instances, the treatment T_1 having highest level of inocula of *M. javanica and S. rolfsii* gave maximum number 18.79 of J_4 juveniles (Table 2). This was followed by the treatments T_3 and T_4 having 13.43, 12.31 and 11.19 J_4 juveniles, respectively, which were found to be statistically identical. No J_4 juveniles were recorded with T_0.

Significantly higher effect in respect of shoot and root length, fresh weight of shoot and root with nodules, number of pods and nodules per plant and yield were found with the control treatment where no inocula of *M. javanica* and *S. rolfsii* were applied. In case of shoot length, more or less, similar trend of suppressing growth was noted with higher levels of inocula of the pathogens. In case of length of root, fresh weight of shoot and fresh weight of root with nodules there appeared less decreasing trend of growth with lower levels of inocula compared to the treatment with maximum levels of inocula. Bhagawati and Phukan (1993) similarly reported a progressive decreases in all plant growth characters with increasing inoculum levels of *M. incognita* alone with the leguminous crop like pea. Khan (1990) suggested that lethal products secreted by the fungus *Sclerotium rolfsii* though disturbed the development of *Meloidogyne javanica* in the first month, but ultimately their association suppressed the growth of shoot of brinjal in the subsequent months. Hazarika and Roy (1974) reported that combined effect of *Rhizoctonia solani* and *Meloidogyne incognita* decreased plant height, weight of shoot and root of brinjal plant to a higher significant level than their individual effect. The results obtained by Tripathy and Pandhi (1992) and Sarmah and Sinha (1995) also revealed a progressive decrease in plant growth characters with increasing inoculum levels of *Meloidogyne incognita* in rice, bean and cowpea, respectively. Identical response in respect of length of root, fresh weight of shoot and fresh weight of root with nodules in lower three. The development of nodules was influenced by the inoculum levels. Progressively lower number of nodules were produced with higher levels of inocula. Meena and Mishra (1993) and Ahmed and Srivastav (1996) observed reduction in the development of nodules in soybean with *M. incognita* alone. Anver *et al.* (1997) and Nejab and Khan (1997) similarly observed decreasing nodulation with increasing levels of inoculum of *M. incognita* in pigeon pea and chickpea, respectively. All these findings are in corroboration with the present findings. In the present study, the combined effect of *M. incognita* along with *S. rolfsii* rather made the situation more vulnerable for suppressing nodulation in both the legume crops. In respect of galling, a progressive increase in galling incidence was recorded with increasing levels of inocula. Similarly, Amarantha and Krishnappa (1989) and Hussain and Bora (1998) reported higher galling incidence with higher inoculum levels of *M. incognita* in sunflower and french bean, respectively. In the present study, the combined effect of *M. javanica* and *S. rolfsii* increased the galling incidence with the increase of their inocula levels. Hazarika and Roy (1974) working with mixed inocula of *M. incognita* and *R. solani* on brinjal and Ram Nath *et al.* (1984) working with *M. javanica* and *R. solani* on tomato found higher galling incidence. All these results are in agreement with the present findings. Higher inoculum levels of a single nematode like *Meloidogyne javanica* inoculated with peanut and *M. incognita* with rice bean and pigeon pea decreased the number of pods per plant as well as yield as reported by Bhat and Krishnappa (1989), Tripathy and Pandhi (1992) and Anver *et al.* (1997). In this study, higher levels of inocula of *M. javanica* and *S. rolfsii* reduced the yield significantly compared to the lower level and uninoculated control treatment. The interaction between *M. javanica* and *S. rolfsii* at higher levels of inocula might have created a complex situation in the environment which resulted in reduction of growth as well as yield. Similar report was given by Starr *et al.* (1996) working with *M. arenaria* and *C. rolfsii* on peanut. Anwar *et al.* (1996) observed decrease in yield of soybean cv. Clark-6 as well as growth parameters in simultaneous inoculation with *R. solani* and *M. incognita*. Their physiological studies showed significant alternation in chlorophyll-a and b, protein, oil and nitrate reductase enzyme of soybean. Such alterations in the plants of groundnut infected with *M. javanica* and *S. rolfsii* might have been responsible for reduction in yields of treatments with higher inocula levels in the present study.

The highest significant numbers of adult females, J_2, J_3 and J_4 populations of *M. javanica* were recorded with the highest inocula levels of the pathogens. In case of adult females, progressively higher numbers of females were recorded from lower to higher levels of inocula. More or less, similar trend of J_2 and J_4 populations were recorded with higher to lower levels of inocula of the pathogens. In respect of J_3 population, there was an identical response among the treatments. Amarantha and Krishnappa (1989) similarly observed that with the increase of inoculum density of *M. incognita* in fifteen days old seedlings of sunflower there appeared corresponding increases in the number of galls, egg masses and larval population. Hussain and Bora (1998) also reported that *M. incognita* population in french bean was found to be maximum with the maximum nematode inoculum level. Even the mixed inocula of *M. javanica* and *S. rolfsii* increased the nematode population in peanut *M. incognita* and *R. solani* in soybean (Anwar *et al.*, 1996) and *M. javanica* and *R. solani* in tomato (Ram Nath *et al.*, 1984). All these findings are in consonance with the present findings.

It is concluded that length of shoot, length of root, fresh weight of shoot, fresh weight of root with nodule, number of pods per plant, number of nodules per plant and yield per plant were highest and number of galls g^{-1} root, number of adult females of nematodes, J_2, J_3 and J_4 stages were lowest in control T_0 (without *Meloidogyne javanica* and *Sclerotium rolfsii*). But opposite trend of these parameters were found in T_1 *M. javanica* (10 egg masses) + *S. rolfsii* 0.2% (w/w).

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