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Effect of Inocula Levels of *Meloidogyne javanica* and *Sclerotium rolfsii* on the Growth, Yield and Galling Incidence of Soybean

K.M. Khalequzzaman
Agricultural Research Station, Bangladesh Agriculture Research Institute,
Seujgari, Bogra-5800, Bangladesh

Abstract: The experiment was conducted both in the laboratory and glasshouse of the Department of Plant Pathology, BAU, Mymensingh during the period of March to July, 2001. Mixed inocula of ***Meloidogyne javanica*** and ***Sclerotium rolfsii*** in five different treatments including control were tested on the growth, yield, galling incidence and development of the nematode in soybean. Maximum length of shoot and root, fresh weight of shoot and root with nodules, number of pods, number of nodules and yield per plant were observed with the control treatment. Progressively higher galling incidence and higher number of adult females and juvenile populations of ***M. javanica*** correspondinfly with lower plant growth, nodulation and yield per plant were recorded from lower to higher levels of inocula ranging from 4-10 eggmasses of ***M. javanica*** with 0.025-0.1% w/w of ***S. rolfsii***. Galling incidence was negatively correlated with plant growth, nodulated and yield of soybean.

Key words: ***Meloidogyne javanica***, ***Sclerotium rolfsii***, growth, yield, galling incidence, soybean

Introduction

Soybean (***Glycine max*** L. Merril) is the important and well-recognized grain legume, vagetable oil and protein crop in many countries of the world. Soybean has occupied the top position in terms of oil source in the world and has been placed in the second position in Bangladesh. Soybean contains higher amount of both protein and oil than any other legume crops. The protein content in soybean is 40-45%, the oil content is 18-20% and for carbohydrates is 24-26%. The traditional soybean producing and concuming countries make various soybean products such as soymilk, soysauce, curd and high protein biscuits, bread etc. soybean and groundnut plants like many others legumes are capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with ***Rhizobium*** bacteria at the root of the crops. The crop thus improve soil fertility and economize crop production not only for themselves but also for the next cereals and non-legume cropes grown in rotation and there by minimizing the regular rate of nitrogen fertilizer.

Soybean is subjected to attack by many disease caused by fungi, bacteria, viruses, mycoplasma and nematodes (Ahmed and Hossain, 1985; Mian, 1986) reported that 17 genera of nematodes attack different crops along with soybean in Bangladesh were ***Meloidogyne*** spp. are predominant. The common species of root-knot nematode attack wide varities of fruits, vegetables and field crops including soybean. Powell ***et al.*** (1971) reported that disease complexes are formed by ***Meloidogyne javanica*** in association with ***Fusarium axysporum*** in cowpea, tobacco and tomato; with ***Rhizoctonia solani*** in soybean. ***Sclerotium rolfsii*** is a soil brone pathogen and it causes wilt disease in Bangladesh (Ashrafuzzaman, 1986). It is often found that ***Sclerotium rolfsii*** and root-knot nematodes ***Meloidogyne*** spp. 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 throughly studies in Bangladesh, although this type of study has already been done in other countries (Khan and Saxena, 1969 and Lanjewar and Shukla, 1985). So, the present research work was undertaken i) to determine the effect of different inocula levels of ***Meloidogyne javanica*** and ***Sclerotium rolfsii*** separately on the plant growth, nodulation, galling incidence and yield of groundnut and soybean, and ii) to determine the combined effect of ***Meloidogyne javanica*** and ***Sclerotium rolfsii*** on the development of nematode populations in soybean.

Meterials and Methods

The experiment was conducted both in the laboratory and glasshouse of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), 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 orser to remove excess vapour of formalin. Earthen pots (30 cm diameter) were filles 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 soybean (var. sohag) were collected from the Seed Foundation of Trasal 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 soybean were sown per pot. Only one healthy seedling per pot was allowed to grown. Sowing of seed 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 pot 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 eggmasses)+***S. rolfsii*** 0.2% (w/w), T_2= ***M. javanica*** (8 eddmasses)+***S. rolfsii*** 0.1% (w/w), T_3= ***M. javanica*** (6 eggmasses)+***S. rolfsii*** 0.05% (w/w) and T_4= ***M. javanica*** (4 eggmasses)+***S. rolfsii*** 0.025% (w/w).

The fungus ***Sclerotium rolfsii*** was sub-cultured in PDA medium in petri dishes. Oat seeds was washed and soaked in water for 48 h. About 50 gm of soaked seed were taken in 250 ml. Erlenmeyer flask plugged tightly with cotton and than autoclaved for 20 min. under 15 lbs pressure at 120°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°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*** grown on oat seeds. Four different levels of inoculum of the pathogen were used. The levels were 0.2, 0.1, 0.05 and 0.0025% 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 uniformaly in the soil. Eggmasses of ***Meloidogyne javanica*** were collected from the roots of brinjall 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 eggmasses were done with 0.001% solution of Mercuric chloride for about one minute following by subsequent washing with water. Groundnut seedlings of 25 days age grown in pot soil were inoculated with sterilized eggmasses of ***Meloidogyne javanica***.

The data were collected from uprooted groundnut plants from pots after 90 days of inoculation on 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 per g root and yield per plant (g) and number of adult females of nematodes, J_2, J_3 and J_4 stages. The collected data were analyzed statistically to find out the levels 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

Effect of different inocula levels of ***Meloidogyne javanica*** and ***Sclerotium rolfsii*** on the plant growth, yield and galling incidence of soybean are presented in Table 1. The highest shoot length 47.38 cm was observed with treatment T_0, where no inocula of ***M. Javanica*** and ***S. rolfsii*** were used. Higher significant and statistically indentical lengths of shoots were found with the treatments T_3 and T_4 having 41.39 and 41.89 cm, respectively. Lower length of shoot 38.90 cm was noted with the treatment T_2 where higher levels of inocula of both the pathogens were applied. The effect of treatments in respect of length of root was found significant. The highest

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

	Treatments					
	T_0	T_1	T_2	T_3	T_4	LSD
Length of shoot (cm)	47.38a	28.06d	38.90c	41.39b	41.89b	1.084
Length of root (cm)	34.26a	17.30e	26.26d	27.78c	31.82b	0.4890
Fresh weight of shoot (g)	29.58a	13.57e	20.85d	23.51c	27.23b	0.4606
Fresh weight of root with nodules (g)	5.06a	1.45d	2.64c	2.76c	3.15b	0.1799
Number of pods per plant	32.97a	18.57e	24.05d	25.84c	30.91b	1.659
Number of nodules per plant	20.20a	4.80d	5.80d	8.00c	14.00b	1.867
Number of galls per g of root	0.00ae	30.54a	16.63b	14.17c	12.63d	1.10
Yield per plant (g)	10.30a	5.30d	7.68c	8.04bc	9.26ab	1.289

Table 2: Effect of different inocula levels on the growth of ***Meloidogyne javanica*** in soybean inculated with eggmasses 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	0.00c	0.00c	0.00d	0.00d
T_1	35.03a	30.51a	23.09a	21.64a
T_2	31.43b	15.54b	18.34b	14.13b
T_3	20.18c	20.78b	15.56b	13.86b
T_4	15.27d	15.27b	12.84c	11.47c
LSD	3.064	7.182	2.038	1.828

Values are the means of five replications. In a column, values having same letter(s) do not differ significanty at p=0.01, T_0= Control (without ***Meloidogyne javanica*** and ***Scleroium rolfsii***)
T_1= ***M. javanica*** (10 eggmasses) + ***S. rolfsii*** 0.2% (w/w), T_2= ***M. javanica*** (8 eggmasses) + ***S. rolfsii*** 0.1% (w/w), T_3= ***M. javanica*** (6 eggmasses) + ***S. rolfsii*** 0.05% (w/w), T_4= ***M. javanica*** (4 eggmasses) + ***S. rolfsii*** 0.025% (w/w), J= Juvenile

significant root length 34.26 cm was recorded with T_0 where no inocula of both the nemic and fungal pathogens were used. Higher significant effect on root length was found with the treatmenst T_4 having 37.82 cm where minimum levels of inocula of the pathogens were applied. Lower significant root length 27.78 cm was observed with the treatment T_3 with lower inocula levels of the pathogens. Treatment T_2 having the highest levels of inocula of both the pathogens was found have the ower length root 26.26 cm followed by T_1 having 17.30 cm with the maximum levels of the pathogens inocula. A significant variation was observed in respect of fresh weight of shoot among the treatments. Control treatment T_0 (without any pathogenic inocula) gave the highest significant fresh weight 29.58 g of shoot followed by the treatment T_4 having 27.23 g with the lowest levels of the pathogenic inocula. Lower significant fresh weight 23.51 g of shoot was

observed with treatment T_3 with lower inocula levels followed by 20.85 g fresh shoot weight in T_2 with comparatively higher inocula levels. Minimum fresh weight of shoot 13.57 g was recorded with T_1 having the highest inocula levels. Significantly the highest root weight with nodules 5.06 g was found with the non inoculated control treatment T_0, while the lowest significant effects was recorded with treatment T_1 having 1.45 g, weight with maximum levels of inocula of ***M. Javanica*** and ***S. rolfsii***. Higher significant effect on fresh weight of root with nodules 3.15 g was recorded with the treatment T_4, having minimum levels of pathogenic inocula. Lower significant and statistically identical fresh weights of root with nodules 2.76 and 2.64 g were recorded in the treatment T_3 and T_2 respectively. The lowest effect was found in T_1 having 1.45 g weights where maximum, levels of inocula of the pathogens were used. With respect to number of pods per plant, effect of the treatments were found to be statistically significant. The highest significant number of pods per plant 32.97 was found in the non-inoculated control treatment T_0, while higher significant effect was recorded with treatment T_4 having 30.91 where minimum levels of the pathogenic inocula were applied. It was followed by the treatments T_3 and T_2 with 25.84 and 24.05, respectively where comparatively lower levels of inocula were used. The lowest response was found with treatment T_1 with 18.57 pods where maximum levels of inocula of both the pathogens were incorporated. Significantly the highest number 20.20 of nodules per plant was recorded with the treatment T_0 where no inocula of the two pathogens were applied. Higher significant effect was found with the treatment T_4 having 14.00 where minimum levels of the inocula of the pathogens were used. Lower significant number 8.00 nodules per plant was noted with the treatment T_3 followed by the lowest significant and statistically identical number 5.80 and 4.80 of nodules per plant were found in the treatments T_2 and T_1, respectively. The effect of treatments in respect of galling was found to be significant. The highest number 30.54 of the galls per g root was observed with the treatment T_1 where the maximum levels of inocula of ***M. Javanica S. rolfsii*** were applied. Higher number 16.63 of galls was recorded with T_2 followed by 14.17 and 12.63 galls in the treatments T_3 and T_4, respectively. Incase of control treatment T_0 no galling incidence was observed. With respect to pod yield per plant, the effect of the treatments was found significant. The highest significant pod yield 10.30 g per plant was observed with T_0, where no inocula of the two pathogens were applied. Higher significant and statistically identical response on pod yields per plant were noted with T_4, T_3 and T_2 having 9.26, 8.04 and 7.68, respectively. The treatment T_1 was found to give lowest pod yield 5.30 g per plant having the maximum levels of inocula of ***M. javanica*** and ***S. rolfsii***.

Effect of different inocula levels on the growth of ***Meloidogyne javanica*** in soybean inoculated with eggmeasses of ***Meloidogyne javanica*** and culture of ***Sclerotium rolfsii*** are shown in Table 2. The effect of different treatments on the development of adult female was found to be significant. The highest significant number 35.03 of adult females was observed with the treatment T_1 where maximum levels of inocula of ***M. javanica*** and ***S. rolfsii*** were maintained.

Higher significant number 31.43 of adult females was recorded with the treatment T_2 followed by 20.18 and 15.27 adult females noted with T_3 and T_4, respectively. No stage of development of the nematode including adult was noted in the control treatment T_0. In case of J_2 juvenile stage, the effect of treatments was found to be statistically significant. The highest significant number 30.51 of J_2 juveniles was observed with treatment T_1 having the highest levels of inocula of ***M. Javanica*** and ***S. rolfsii***. Lower significant and statistically identical numbers of J_2 juveniles 20.78, 15.54 and 15.27 were noted with the treatments T_3, T_2 and T_4, respectively. Non inoculated control treatment T_0 had no J_2 juveniles. Different treatments were found to influence significantly the development of J_3 juvenile stage. The highest number 23.09 of J_3 juvenile was found with the treatment T_1 having maximum inocula of both the pathogens. Higher significant and statistically identical numbers 18.34, and 16.56 of J_3 juveniles were recorded with the treatments T_2 and T_3, respectively. Lower significant number 12.84 of J_3 juveniles was found having minimum levels of inocula of both the pathogens while no J_3 was present in the control treatment T. Significant differences were found among the treatments with respect to the number of J_4 juvenile (Table 2). The highest significant number 21.64 of J_4 juveniles was found with the treatment T_1 with the highest levels of inocula of ***M. javanica*** and ***S. rolfsii***. Comparative higher significant and statistically identical numbers of J_4 juvenile were recorded with the treatments T_2 and T_3 having 14.13 and 13.86, respectively. Lower significant number 11.47 of J_4 juveniles was observed with the treatment T_4 having minimum inocula of both pathogens. Control treatment T_0 appeared without any stage of the nematode.

The study revealed that maximum lenght of shoot and root, fresh weight of shoot and root with nodules, number of pods, number of nodules per plant and yield were encountered with the nontreated control treatement in soyabean. On the other hand, the lowest significant responses in respect of length of shoot and root, fresh weight of shoot and root with nodules, number of pods and nodules per plant and yield were recorded in soyabean with the maximum levels of inocula of ***M. javanica*** and ***S. rolfsii***. Simultaneously, the highest galling incidence was obtained under this treatment with maximum levels of inocula in this crop.

In case of shoot length, more or less, similar trend of supressing growth was noted with higher levels of inocula of the pathogens. Comapartively, lower and identical response in shoot length with lower levels of inocula was observed. In soyabean, the effect of the treatments were, more or less, found to be with reducing growth characteristics from higher to lower levels of inocula in all these plant growth characters. Bhagawati and Phukan (1993) similarly reported a progressive decrease in all plant growth characters with increasing inoculum levels of ***M. incognita*** alone with the leguminous crop like pea. Working with ***Rhizoctonia solani*** and ***Meloidogyne incognita*** as mixed inocula in soyabean, Anwar ***et al.*** (1996) also observed suppressed growth parameters of the plant. Khan (1990) suggested that lethal products secreted by the fungus ***Sclerotium rolfsii*** through 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) repoted 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. The development of nodules were 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 soyabean with ***M. incognita*** alone. Anver ***et al.*** (1997), Nejab and Khan (1997) similarly obsereved 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 codulation in both the legume crops. In respect of galling, aprogressive increase in galling incidence was recorded with increasing levels of inocula. Similarly, Amarantha and Krishnappa (1989), 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 oncrease 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 the present study, higher levels of inocula of ***M.javanica*** and ***S.rolfsii*** more or less 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 mught have created a complex situation in the environment that 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 alteration in chlorophyll-a and chlorophyll-b, protein, oil and nitrate reductase enzyme of soybean. Such alterations in the plants of groundnut and soybean 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 number 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. Amaranatha 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, eggmasses and larval population. Hussain and Bora (1989) 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. 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.

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