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Influence of *Azolla pinnata* in Combination with Mustard Oil Cake on the Galling of *Meloidogyne javanica* and the Growth of Eggplant

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Abstract: Effect of Azolla (*Azolla pinnata*) in combination with mustard oil cake (MOC) was studied on the galling in roots and growth of eggplants (*Solanum melongena*) inoculated with *Meloidogyne javanica*. Fresh weight and length of both shoot and root were vigorous when dry Azolla (5g) or fresh Azolla extract (10ml) in mixing with the half standard dose (1.25g) of MOC was applied. Similarly, the galling incidence was lower in the same treatment that was identified for the vigorous growth of the plant. *A. pinnata* was found as a very effective agent for the normal growth of root and shoot with half-standard dose of MOC. Both of these agents in together created resistance to the plants and its toxic materials might suppress the nematode activity, which ultimately confirm the lower presence of galling by *M. javanica*

Key words: Azolla, eggplant, gall, *Meloidogyne javanica*, mustard oil cake

Introduction

Soils of Bangladesh are the harbour of plant parasitic nematodes due to its hot and humid climate. Population of nematode has been increasing in the country (Chowdhury, 1976). Root knot nematodes are very harmful as they cause severe loss of both field and horticultural crops not only in Bangladesh but other tropical countries also. Page (1979) reported that 36 different crops grown in Bangladesh are susceptible to root knot nematode, *Meloidogyne javanica*. No specific and economically viable control measure has yet been suggested in Bangladesh to save the eggplant (*Solanum melongena*) and other common vegetable crops from this nematode. Besides the chemicals are very expensive and not available in the market especially in the developing countries. Moreover, it is a difficult task for the common farmers to determine the precise dose of the chemical for its application. In addition, their harmful effects are responsible for air, soil and water pollution.

Various plant parts have recently been reported to have nematicidal effects (Mahmood *et al*., 1982; Sartaj *et al*., 1985; Pathak *et al*., 1989; Ahmad *et al*., 1990). Organic amendments in the form of oil cakes have also been found effective in controlling root knots and other plant parasitic nematodes (Mammen, 1973; Khan *et al*., 1973 and 1974; Goswami and Vijaylakshmi, 1986; Darenkar *et al*., 1990). But supply of mustard oil cake (MOC) is limited and relatively expensive. Moreover, soil acidity might increase owing to continuous and higher application of this oil cake. *Azolla pinnata*, a bryophyte naturally grown in the rice fields and other low lands has been found to reduce egg hatching of *M. javanica* and *M. incognita* (Thakar *et al*., 1988). In the previous study we reported the single effect of Azolla in different forms on the control of *M. javanica* (Malek *et al*., 1996). Here we investigated the combined effect of *Azolla pinnata* and MOC to reproduce economically viable method of control with increasing the soil organic matter level for good plant growth. Proper combination of Azolla and MOC is also important in this context. Therefore, an attempt was made in this study to see the effect of *A. pinnata* in combination with MOC on the growth and galling of eggplant infected with *M. javanica*.

Materials and Methods

Study area: The experiment was conducted both in the laboratory and glass house during 1992-93 at Bangladesh Agricultural University, Bangladesh. This area is situated in Mymensingh district under Dhaka division, Bangladesh (Fig. 1) at 20°34′-26°38′ N and 88°01′-92°41′ E. During the study period, the temperature ranged from 34–36°C and relative humidity 85-92% in the glass house.

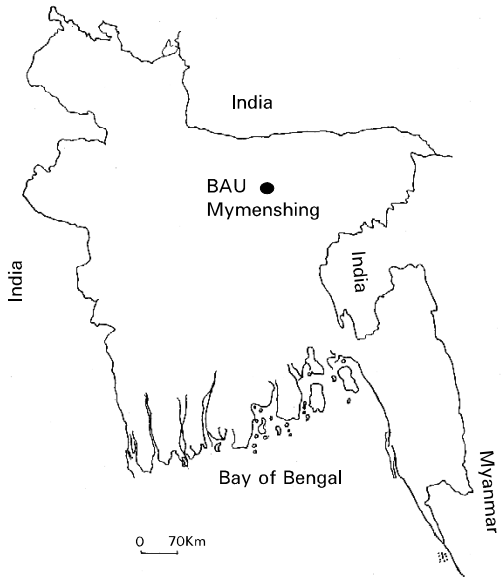

Fig. 1: Map of Bangladesh displaying the experimental site.

Preparation of pot soil: Sandy loam soil was mixed thoroughly with cowdung at 5:1 ratio and dried for 3 days. This soil was sterilized with 3% formalin covering with polyethylene sheet. It was then exposed to air under shed for three consecutive days and poured into earthen pot (30 cm dia.) at 6 kg/pot.

Plant material and management: Seedlings of root knot susceptible eggplant cultivar, Singnath (Ahmad *et al*., 1990) were raised in a nematode free soil using 0.001% mercuric chloride treated seeds. Each pot was planted with a thirty days old uniform healthy seedling. Water was given in every morning and afternoon for the first seven days after planting. When the plants were established, the soil around the base of the plant was loosened with a khurpi (hand weeding sickle). Soil moisture content (18-20%) was maintained by sprinkling water in the rhizosphere soil every day.

Soil amendment/treatment: The pot soil were treated or amended separately with dry Azolla and Azolla extract in combination with powdered MOC. Azolla was dried in the sunny days and powdered. Fresh viable Azolla was crushed in the blender and the extract was used. MOC was mixed with dry Azolla and Azolla

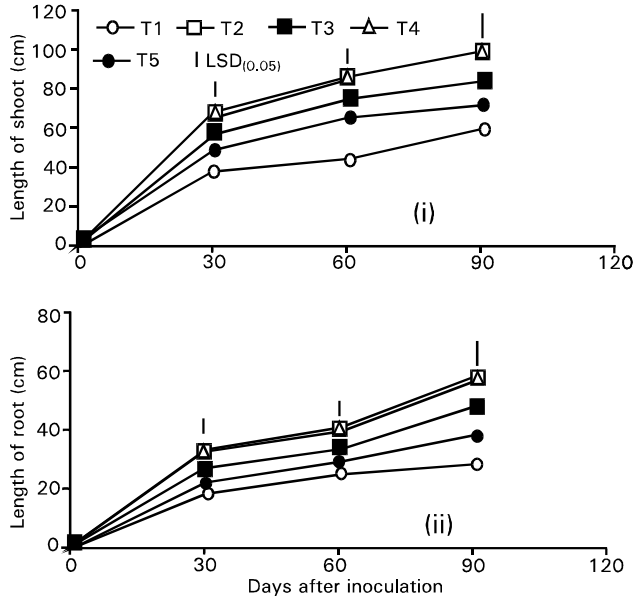

Fig. 2: Length of shoot (I) and root (ii) of eggplant as influenced by *A. pinnata* in combination with mustard oil cake after inoculation with *M. javanica*.

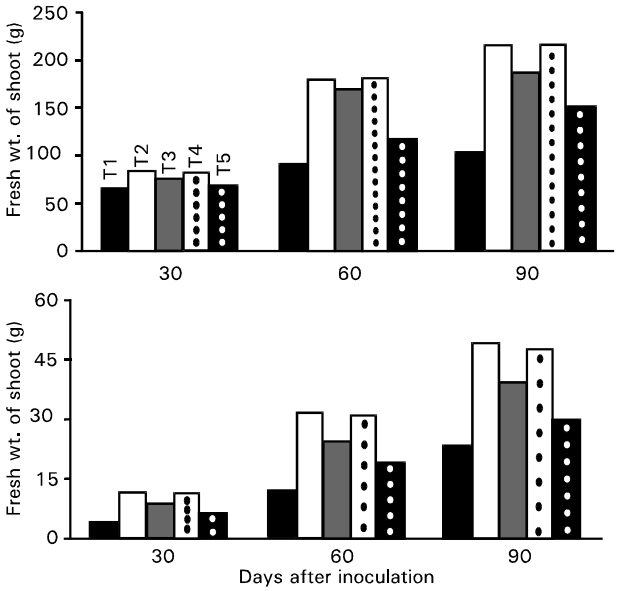

Fig. 3: Fresh weight of shoot (I) and root (ii) of eggplant planted to soils amended with *A. pinnata* in combination with mustard oil cake and inoculated with *M. javanica*.

extract at two different rates. Two rates of MOC were one-half (1.25g) and one-forth (0.625g) of the standard rate of application per plant. Therefore five treatments including a control were laid out in a completely randomized design with five replications. More clearly the treatments per plant and their rate of applications were- T1: Control (without Azolla and MOC), T2: Dry Azolla (5g) + MOC (1.25g), T3: Dry Azolla (5g) + MOC (0.625g), T4: Azolla extract (10ml) + MOC (1.25g) and T5: Azolla extract (10ml) + MOC (0.625g). First amendment was done after seven days of transplanting and subsequent applications of azolla in different treatments were done at every 10 days interval. Three sets of this experiment were conducted considering the three samples.

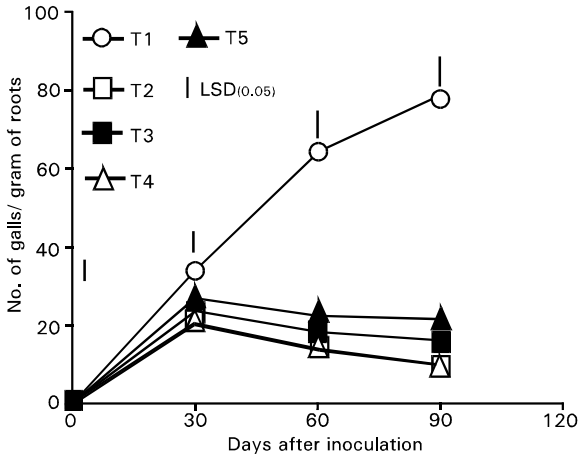

Fig. 4: Number of galls by *M. javanica* in eggplant roots after the rhizosphere soil amended *with A. pinnata* in combination with mustard oil cake at different duration.

Preparation of inoculum and inoculation: Egg masses collected from roots of eggplant (cv. Singnath) which were previously inoculated with a single egg mass of *M. javanica*. Surface sterilization of the egg masses was done with 0.1% mercuric chloride solution for about 1min and then placed in watch glasses containing distilled water with the water level just touching the mesh. The second stage juveniles were collected from the total quantity of the suspension of the larvae and diluted in such a way that each ml suspension contained approximately 500 larvae as counted with the help of sterio-binocular microscope. After first application of azolla, eggplant seedlings were inoculated with freshly hatched second stage larvae of *M. javanica* suspended in water. One ml of larvae suspension was applied in each pot in equal proportion in 2 holes (2.5cm deep) one on each side of the plant.

Parameters studied: Plants were uprooted from pots after 30, 60 and 90 days of inoculation and roots were washed out and cleaned under gentle running tap water. Length of shoot and root, fresh weight of shoot and root and number of galls per gram of root were recorded. Length of root was measured from the starting point of root to the largest viable lateral root apex. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. The shoot and root portions were weighed after blotted with fine tissue paper and before the materials desiccated. Number of gall per gram of roots was counted after washing and staining with phloxine-B (Daykin and Hussey, 1985).

Results

Length of shoot and root: Length of shoot increased at the highest significance level with T2 and T4 having 68.5 and 66.8 cm at 30 days, 86.0 and 85.8 cm at 60, 101 and 100 cm at 90 days after inoculation, respectively (Fig. 2i). A lower shoot length was observed with treatments T3 and T5 in all the observations. The lowest shoot growth was recorded with T1.

Treatment T2 and T4 appeared to give the highest root length, while comparatively lower significant root length was recorded with T3 on successive investigation at every 30 days after inoculation (Fig. 2ii). The lowest and identical root growth was recorded with T1 at 30 and 90 days after inoculation. But in the second observation, treatments T1 and T5 were found to give identical lower root growth. Similarly, there was no significant difference in root length between T3 and T5.

Fresh weight of shoot and root: Significant common trend in fresh weight of shoot was recorded among the treatments (Fig. 3i). The highest response in fresh weight of shoot was recorded with T2 and T4 in all the three continued observations at every 30 days after inoculation. Lower response was noted with T3 having 73.8, 168.5 and 187.0g fresh weight of shoot in the first, second and third observations differing each at 30 days after the incorporation of second stage larvae of *M. javanica* respectively. At the same time comparatively lower response in fresh weight of shoot was recorded with T5. T1 appeared to give the lowest fresh weight of shoot in comparison with other treatments.

Similar trend was found in the fresh weight of root. Treatments T2 and T4 gave the highest response followed by a lower response in fresh weight of root recorded with T3 (Fig. 3ii). T2 produced the longest 48.8cm root followed by T4 (47.5cm), T3 (38.8cm), T5 (30.1cm) and T1 (22.9cm) at 90 days after inoculation. Comparatively, lower and the lowest fresh weight of root was observed with treatments T5 and T1 respectively in all the successive observations.

Incidence of galling: All treatments had positive controlling effect on the galling by *M. javanica* except T1 (Fig. 4). No significant difference in galling observed among T2, T3 and T4 in the first and second counting at 30 and 60 days after inoculation. Untreated plant roots (T1) were severely infected with significantly highest number of galls in comparison with the others. The plants having no amendment in rhizosphere soil produced 34.8, 64.4 and 78.6 galls. Whereas T2 amended plants produced 20.6, 14, 9, and T4 amended plants produced 20.8, 14.2 and 9.6 galls at 30, 60 and 90 days respectively. Treatment T2 and T4 gave the best gall controlling response followed by T3, T5 and T1 at 90 days after inoculation which can show as T2= T4> T3> T5> T1 for gall control.

Discussion

Treatment with dry azolla and azolla extract each with 1.25g MOC/plant gave significantly the highest plant growth in respect to length of shoot and root, fresh weight of shoot and root corresponding with the lowest significant galling incidence. On the other hand, dry azolla with 0.625g MOC/plant gave significantly higher plant growth correspondingly with lower galling incidence. The nematode activity is interrupted by less extent with dry azolla due to

requirement of longer time for decomposition and release of nematicidal toxic substances and nutrient elements into the soil (Malek *et al.*, 1996). They also reported that due to diluted condition of the toxic substances in azolla extract as well as leaching of it into the soil, their efficacy against the nematode could not be well marked. But, combination of ½ standard dose of MOC improved the situation in this experiment. In T2 and T4, dry azolla and azolla extract compensated their shortcomings with the addition of 1.25g MOC/per plant as powder form. Probably, acidic properties of MOC increased the microbial activity particularly the fungal activity for which the dry azolla decomposed rapidly and powder form of MOC might have more adhesion capacity to hold the liquid extract of azolla in the rhizosphere soil. Further, released decomposition materials from MOC and azolla, like protein, amino acid, low molecular weight compound, enzyme might be absorbed more by the plant that ultimately created resistance to nematode. As a result, the longest plant growth corresponding with the lowest galling incidence was recorded with these treatments. Dry azolla and azolla extract each with 0.625g MOC/plant as evident with lower significant plant growth with corresponding higher galling incidence. This might be owing to a compatible combination ratio of both form of azolla with MOC. The growth of control plant was in similar pattern but lower to other treated plants and it had the highest number of galls. Probably previously decomposed cowdung worked well on the growth was more than MOC and Azolla. Therefore, MOC and Azolla was effective as an additive with cowdung for plant growth. Absence of these might have the effect on lower growth but the presence of larger number of galls in roots strongly influenced the lower growth. On the other hand, treated plants had higher growth due to both lower number of galls and positive effects on growth by azolla and MOC. Results indicate the influence of both azolla and MOC in a proper combination made the plant resistant to nematode with healthy growth.

Many oil cakes have been found to be nematicidal against plant parasitic nematodes (Singh and Sitaramaiah, 1966; Sharma *et al.*, 1971; Khan *et al.*, 1973 and 1974; Mishra and Prasad, 1975; Mian and Rodriguez-Kabana, 1982; Ahmad, 1989). Hameed (1968) observed greater reduction of the population of *Meloidogyne* on tomato with pre-plant application of mustard oil cake. Similar report on effective control of *Hoplolaimus indicus* associated with brinjal and tomato with extracts of whole and de-oiled cakes of ground-nut, safflower, sesamum, castor, and mustard have been made by Deshmukh and Prashad (1969). The mechanism of control due to application of organic amendment with oil cakes and chicken litter to soil reviewed by Mian and Rodriguez-Kabana (1982) who indicated that decomposition products like formic acid, acetic acid, phenolic compounds are toxic to the plant parasitic nematode, *Meloidogyne alternaria*. Similar toxic products especially phenolic butyl acid, ammonia and phenolic compounds liberated from amended MOC along with the toxic substance of *A. pinnata* might suppress the nematode activity to greater extent in T2 and T4 where ½ standard dose of MOC was used. It is more important to know whether azolla released amino acids, protein, growth hormone like auxin and other low molecular organic acids that might have effect on the increase of beneficial microbial community and plant growth. Such microbial population might enhance the natural control of *M. javanica*.

This study extends the greater scope of utilizing *A. pinnata*, a commonly available indigenous bryophyte plant in Bangladesh to control the plant parasitic nematode like *M. javanica*. Use of *A. pinnata* has got both way benefits of controlling nematode like *M. javanica* and adding nitrogen and potassium to soil to increase the fertility level (Ramswamy *et al.*, 1988; Ventura *et al.*, 1989; Srinivasan and Pothiraj, 1989; Kolhe and Mittara, 1990; Malek *et al.*, 1996). Further investigation on azolla and / or MOC will be needed to understand more of its chemical composition, influence of the released chemicals on microbial community and their interaction with *M. javanica*, direct effect of chemicals on the *M. javanica* and finally the biochemical status of soil before and after application. All these investigations might clear the mechanism to control the galling. So, *A. pinnata* has been proved primarily as a very good agent both for plant growth and control of *M. javanica* when applied in proper combination with MOC.

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