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Bioefficacy of *azolla pinnata* as a function of soil amendment in rooy-knot nematode management

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ABSTRACT

Studies on pathogenecity of *Meloidogyne incognita* on Green gram, *Phaseolus aureus* were carried out in relation to different concentration of *Azolla pinnata*, a biofertilizer with reference to growth parameters of host plant and reproductive parameters of nematode pathogen. Amendments of Azolla resulted reduction in nematode infection and increment in growth characteristics of host plant. Azolla treated plants showed excessive shoot and root growth over infected untreated control plants. The growth increment was directly proportional to the dosage of Azolla amendments Reproductive parameters studied were also significantly affected by Azolla treatment. A progressive reduction in root – knot index, number of egg masses, eggs/egg mass, soil population and reproductive factor was recorded in different concentration of Azolla treatment. Alteration in protein content of plant tissue in response to Azolla treatment reveals that plants were put up resistance against nematode infection.

Introduction

One of the major obstacles to the production of adequate supplies of food in many developing countries is the damage caused by plant parasitic nematodes, especially the root-knot group, *Meloidogyne sp*. Their worldwide distribution, extensive host ranges and involvement with fungi, bacteria and viruses in disease complexes rank them among the top most plant pathogens affecting the world's food supply. Collectively, the various sp of *Meloidogyne* attack every crop grown. They affect not only the quantity of production but also the quality of production.

Several methods have been adopted to mitigate the nematode population among all methods; chemical method is an effective one. Amino acid such as Phenyl alanine, alanine, serine, threonine (Krishna prasad et.al., 1976, Sethy et al., 1977) and methionine (Reddy et al., 1975) have helped in reducing infection. Such chemotherapeutic studies especially impairing larval activity, growth etc. and influencing host metabolism towards reducing gall formation. However, the positive prohibitive cost hazards associated with their use makes it necessary to develop methods of effective nematode control with minimum use of chemicals or by use of soil amendments.

It is an established fact that the organic soil amendments can provide safe and pollution free control of nematode pests. A variety of organic additives of plant origin have been tested as nematode controlling agents (Muller and Gooch, 1982). Mishra *et al*., (1987) reported that chicken manure decrease nematode population and gall formation to maximum degree. Incorporation of plant parts with soil has been used by many workers (Singh and Sitaramaiah, 1966; Hameed, 1970) for the control of parasitic nematodes. Khan *et al*., (1974) suggested that the inhibitory effect of organic amendments resulted due to certain nematicidal compound liberated during decomposition of organic additives. Plants have been reported to have been reported to have nematicidal properties (Egunjobi and Afolmi, 1976; Mohamed et al., 1978)

Thakar *et al*., (1987) reported that root knot index was significantly reduced with increments in height of the host plant when wet and dry azolla amended with soil. Although information increased efficiency with degradation of phytotherapeutic substances is available (Singh and Sitaramiah, 1967) information regarding the effect of different levels of Azolla, a biofertilizer on the incidence of Meloidogyne incognita is lacking. The present study it was therefore attempted, to evaluate the efficiency of different levels of Azolla against *M. incognita* infecting green gram *P.aureus*.

Materials and Method

Experiments were carried out in 15cm earthen pots filled with sterilized sand soil mixture. Fresh *Azolla pinnata* were mixed at the rate of 5, 10, 15, 20 and 25 gm/kg of soil separately (T1, T2, T3, T4 & T5). The pots were left for 15 days for proper decomposition of Azolla. Seeds of green gram were surface sterilized with 0.1% mercuric chloride and were shown in the pots. The pots were watered at regular intervals. After germination the seedlings were thinned to one per pot. Two control pots, one without treatment and without infection and the other with treatment without infection were maintained for comparison. Ten days old seedlings of all treatments were inoculated with 1000 J2 larvae. Treatments were replicated thrice and maintained for 35 days under green house conditions.

At the end of 35 days infection plants were gently uprooted to study the impact of Azolla for growth characteristics of host plant, number of egg masses, eggs/egg mass, gall index, soil population, reproductive factor of pathogen and total protein content in root and shoot tissues. Gall index was calculated by adopting the method of Smith and Taylor (1947) and protein was by Lowry *et al*., (1951).

Result:

The effect of different concentration of Azolla on mean fresh and dry weight of the root system and shoot system of *P. aureus* infected with *M.incognita* are given in the table 1 and 2

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The growth characteristics of the host plant were significantly affected by the nematode. This influence consisted of reduction in length and weight of the host plant. The reduction in the total growth of the host plant was found to be 18.49% and 16.50% in wet weight of the whole plant over that of uninfected control plant. The percentage reduction due to nematode infection was 12.90% and 21.50% in the shoot and root systems respectively.

Statistical analysis of critical difference at 5% and 1% revealed that the different concentrations of Azolla significantly influence the growth of the host plant. The treatment T^4 and T^5 of Azolla exhibited better growth than other treatment. Similar trend has also observed in the weight of the host plant. The growth increment was increased with increase of concentrations of Azolla. Maximum % of total growth during infection was discernible at 25gm/kg of Azolla (T^5) the total growth was increased by 64.49% by shoot weight and 46.11% by root weight over that of infected untreated plants.

Perusal of table 2 reveals the influences of different concentration of azolla on reproduction and population of buildup of *M.incognita* on *P.aureus*. Statistical analysis of critical difference at 5% and 1% revealed that the parameters studied are significantly influenced by azolla treatments. A progressive reduction in root-knot index, number of egg masses, eggs/egg masses, soil population and reproduction factor increase was discernible in various concentration of treated infected plants. A maximum % of reduction in parameter studied was observed at T5 of azolla treated plant (egg mass 63.33 %; eggs/egg mass 51.93 %. Gall index 76.47% and soil population 64.54%).

The protein content of root tissues and shoot tissues of uninfected plant, infected plant and infected treated plants subjected to different concentration of Azolla is represented in the table 3. Generally the check for protein content is higher in root tissues than in shoot tissues. The statistical analysis of C.D at 5 % and 1 % revealed that protein content increment during pathogenesis was significantly checked by the azolla treatment. A significant relationship was observed between the protein levels and root gall numbers.

Discussion:

In the present investigation a significant reduction in growth characters observed. Irrespective of the weight of the root tissues as compared to healthy plants the absorption by galled roots is appreciably reduced (Bergeson, 1968). There by resulting in reduced top growth as observed in untreated infected plants. With the increase of azolla there was corresponding decrease in number of galls there by increments in weight of host plant noticed in treated host plants.

Statistical analysis revealed that all the treatment azolla was significantly influence both the root and shoot length. However the treatment T_3 and T_4 and T_5 of Azolla have significant influence in both root weight and shoot weight over that of infected untreated host plant. Extremes of environment such as oxygen concentration, temperature, alkalinity impeding the plant growth also impede pathogens fecundity (Vagundy *et al*., 1964). On the other hand a well nourished plant rallies around the infection by virtue of its nutritional status. Presumably under field conditions, judicious water management, fertilizers practices etc, play in this direction. Azolla used in the present studies seemed to alleviate the nutritional deficiency caused by the nematode and influenced the plant growth vigour. Increased availability of nutrients to the root system may compensate the damage caused in the root system. In the present study the azolla treated plants have shown excessive root and shoot growth with lesser root knot index.

The nematode development and reproduction was also observed to be reduced by azolla and there was corresponding increase in the growth of host plant was also observed. With the decrease of reproduction factor there was a corresponding increase in the root and shoot growth. There was a gradual reduction in nematode population, (Soil) total number of eggs, eggs/egg mass in all the treatments. Similar results have also seen reported by Mishra *et al*., (1987) using chicken manure for root-knot management in jute. The result suggest that there is almost a linear relationship between total number of eggs, eggs/egg mass, number of galls and nematode soil population and concentration of both the test materials.

The plant growth was more in T_5 (Azolla) treated plants with reduction in the root knot nematode development such reduction in root-knot nematode development might be due to liberation of some nematicidal compounds during decomposition of Azolla in the soil. Such liberated compounds might have suppressed the nematode population and its reproduction potentiality.

During decomposition of organic amendments in the soil ammonia (Miller *et al*., 1968; Kurk, 1971) and phenolic compounds (Singh and Sitaramaiah, 1978) are liberated. These two factors acted as nemostatic agents and the suppression of nematode population in the present investigation might be due to nemostatic agents of some liberated compounds during the decomposition of plant products. It was also pointed out that higher concentration of ammonia liberated during the decomposition of organic amendments inhibited the formation of syncitium which is essential for the development of nematode population. The amount of ammonia liberated during decomposition of organic additives seems to be dosage dependent. It was also observed that (Singh and Sitaramaiah, 1978) phenolic compounds liberated during the decomposition of organic amendments produced some changes in host plant which may be antagonistic to the larvae of *M.incognita*. These results are also in accordance with the finding of earlier workers. (Singh and Sitaramaiah, 1975; Miller *et al*., 1973)

Without organic treatment the plants suffer due to impact of infection, while organic amendments seem to overcome the infection stress. Changes in metabolism in plant tissue in response to treatment revealed that the treated plants were metabolically capable of combating infection and promoting growth of host plant. The effect of pathogenesis is reflected at tissue level with altered (elevated) protein. Similar results also obtained by many workers (Nattuthurai and Kannan, 1984, Salahi *et al*., 2009). The increased concentration of protein observed in the infected plants also thus presumed to be due to, increased rate of synthesis, alteration of translocation due to infection, decreased rate of degradation and deposition of nematodes.

Roy (1981) reported amino acid synthesis in the galls to be the chief contributory sources for the protein in the region. Increased level of protein and phenol have led to the concept that their role in defence reaction during pathogenesis (Dasgupta *et al*., 1981). In the present investigation the protein content decreased with that of increasing treatment corresponding decrease with gall numbers. A significant relationship was observed between the root protein level and root gall number from this it is evident that amount of protein could serve as a

reliable measure for evaluating nematode infestation at any stage of growth of the plant. This is suggestive of the observation that total protein is a good indicator of root-knot nematode infestation (Chatterjee and Sukul, 1981). The galled root protein was directly proportional to the root gall number as well as the population size of nematodes around the roots of the infected plant.

Results of the present investigation support that the presence of nemostatic compounds in the plant tissues grown in treated soil could be a reason for the suppression of host root knot nematode (Goswami and Swarup, 1971). Resistance of plants to nematode infection and development has been attributed to retarded giant cell formation. Tolerance level of susceptible host can be increased by controlling nematode population through growth mechanic of host plant. The various levels of metabolites associated with high energy content in the infected plant tissue, help the susceptible plant to put up resistance. Such resistance can be increased by treating plant product to the infected plant.

In conclusion the studies demonstrated that fortification of Azolla against nematode *M. incognita* due to presence of ammonia and phenol, the utilization of Azolla as a potential nematicide and as growth promoter seems promising.

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Table: 1 Influence of different concentration of Azolla on the growth characteristics of *Phaseolus aureus* infected with *Meloidogyne incognita*. Each value (mean ±S.D) represents an average performance of three observations

Treatment	Length (cm)			Wet weight (gm)		
	shoot	root	total	shoot	root	total
C_0	15.4±0.10	19.2±0.05	34.6±2.68	2.845±0.270	2.506±0.212	5.351±0.239
T_0	14.2±0.152 (-7.79)	14.0±0.152 (-27.08)	28.2±0.141 (-18.49)	2.501±0.303 (-12.09)	1.967±0.279 (-21.50)	4.468±0.377 (-16.50)
T_1	15.1±0.251 (+6.33)	15.0±0.35 (+7.14)	30.1±0.707 (+6.73)	2.692±0.234 (+7.63)	2.106±0.20 (+7.06)	4.798±0.414 (+7.18)
T_2	16.0±0.152 (+12.67)	16.2±0.40 (+15.71)	32.2±0.141 (+14.18)	2.941±0.455 (+17.59)	2.334±0.302 (+18.65)	5.275±0.429 (+18.06)
T_3	16.9±0.152 (+19.01)	17.4±0.40 (+24.28)	34.3±0.353 (+21.63)	3.314±0.360 (+32.50)	2.404±0.226 (+22.21)	5.718±0.643 (+27.97)
T_4	17.9±0.200 (+26.05)	18.6±0.55 (+32.85)	36.5±0.494 (+29.43)	3.857±0.286 (+54.21)	2.670±0.270 (+35.73)	6.527±0.839 (++46.08)
T_5	18.6±0.208 (+30.98)	21.8±0.30 (+55.71)	40.4±2.262 (+43.26)	4.114±0.199 (+64.49)	2.874±0.340 (+46.11)	6.988±0.876 (+56.40)
C.D at 5 %	0.348	0.230	-	0.5809	0.307	-
C.Dat1 %	0.495	0.328	-	0.8263	0.436	-

Values in paranthesis indicate percent decrease (-) over control; percent increase (+) over untreated control

Table 2. Influence of different Concentration of Azolla on Reproduction and population build-up of *M. incognita* on *P. aureus*. Each value mean (± S.D) represent on average performance of three replicates.

Treatment	No. of egg masses per plant	Eggs/egg mass	Gall index	Soil population	Reproduction factor
T_0	30±2.51	310±4.0	3.4±0.152	2200±114.564	2.2
T_1	25±1.52 (-16.66)	298±2.08 (-3.87)	3.2±0.11 (-5.88)	2010±137.470 (-8.63)	2.01
T_2	20±2.0 (-33.33)	245±2.0 (-20.96)	2.1±0.1 (-38.23)	1811±103.630 (-17.68)	1.8
T_3	16±1.52 (-4 6.66)	230±2.51 (-25.80)	21.7±0.1 (-50.00)	1657±110.340 (-23.86)	1.67
T_4	13±1.15 (-56.66)	195±3.51 (- 37.09)	1.7±0.15 (-67.64)	1102±91.016 (-49.90)	1.1
T_5	11±1.0 (-63.33)	149±1.52 (-51.93)	0.8±0.05 (-76.47)	780±77.674 (-64.54)	0.78
C.D value at 5 %	3.107	5.001	0.0274	209.32	--
C.D value at 1%	4.419	7.113	0.390	297.7	--

Values in paranthesis indicate percent decrease (-) over control; percent increase (+) over untreated control

Table 3. Influence of Azolla on Protein content (mg/gm) the host plant *P.aureus* infected with *M. incognita*. Each value represents on average performance of three observations

Treatment	Shoot (mg/gm)	Root (mg/gm)
C_0	25.0±1.006	16.7±1.20
T_0	66.5±5.008 (-166.0)	49.5±1.75 (-196.4)
T_1	59±1.51 (+11.12)	40.2±1.75 (+18.78)
T_2	52.7±0.461 (+20.75)	35.2±0.69 (+26.86)
T_3	51.7±0.986 (+22.25)	27.7±0.66 (+44.04)
T_4	42.3±0.59 (+36.39)	24.7±0.68 (+50.10)
T_5	40.2±1.80 (+39.54)	20.7±1.59 (+58.18)
C.D at 5 %	4.223	2.378
C.Dat1 %	6.006	3.383

Values in paranthesis indicate percent decrease (-) over control; percent increase (+) over untreated control