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Role of Different Sawdusts and Bioinoculant in the Management of Root-Knot Nematode Infesting Chickpea

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ABSTRACT

The impact of amending soil with composted sawdusts derived from different woods [Neem (*Azadirachta indica*), sheesham (*Dalbergia sissoo*), teak (*Tectona grandis*) and chir (*Pinus roxburghii*)] at different concentrations (12.5, 25 and 50 g per pot) and a bio-inoculant, *Pseudomonas fluorescens,* singly and in combination, was investigated in terms of plant growth parameters of chickpea, both in the presence and absence of root-knot nematode, *Meloidogyne incognita.* Effect of these amendments on nematode reproduction was also assessed. All the sawdusts at all the concentrations and the bioinoculants either singly or in combination, improved plant growth parameters in terms of plant length, fresh weight, dry weight and number of nodules per plant and suppressed root-knot nematode infection in terms of number of galls/plant and nematode population. Among the four sawdusts, neem was found to be most effective followed by sheesham, teak and chir. The effectiveness of all the four sawdusts was proportional to their doses. However, addition of *Pseudomonas fluorescens* along with the different sawdusts was more efficient than either of them applied alone, the maximum improvement was recorded in all growth variables in the plants those received the combined application of neem sawdust at 50 g/pot+*Pseudomonas fluorescens*.

Key words: *Pseudomonas fluorescens, Meloidogyne incognita,* plant growth parameters

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an essential pulse crop grown and utilized worldwide, especially in the Afro-Asian countries. It is a rich source of protein and carbohydrates and the protein quality is regarded to be better than other pulses (Jukanti *et al*., 2012). In addition to satisfying the dietary needs of people, chickpea also helps to fix large amounts of environmental nitrogen in soil and thus improves soil fertility, due to its association with nitrogen fixing rhizobia. It is an important pulse crop, ranking second in growing area (15.3% of total pulse area) and third in production (14.6% of total pulse production) around the world (Knights *et al*., 2007). During 2013, chickpea was grown on approximately 13.5 mha, of which, about 89.2% area was contributed by Asia, 4.2% in Oceanica, 3.6% in Africa, 2.4% in Americas and 0.5% in Europe (FAO., 2014). India is the chief producer of chickpea in the world with a production of 8,832,500 MT (about 67.4% of the total production) in 2013 (FAO., 2014). The production of chickpea remains short of expectations due to some constraints which come in a way in production of chickpea, such as

ecological, economical and agricultural constraints. Among biological threats, root-knot nematodes (*Meloidogyne* spp.) are one of the worst enemies of this crop in both tropical and subtropical crop production regions and leads to extensive economic damage worldwide (Sikora and Fernandez, 2005). Root-knot in chickpea has been reported from various states of India (Jamal, 1976; Khan and Siddiqui, 2005). Nematode harm the plant not only by suppressing plant growth but also interferes in the nodulation, nitrogen fixation and negatively affects the overall yield.

Current methods of nematode control are absolutely based on the chemical nematicides which are not only poisonous to various organisms but also leads to environmental perturbation, bio magnification and even the depletion of stratospheric zone (Wheeler *et al*., 1979). Thus, an immediate option is needed to combat this problem, that should be eco-friendly. Plant parts/products have proved to be the dependable substitute and showed lethal effects to pest up to a certain extent and their utilization offers complete economic advantage. A wide range of botanicals are known to be favorable to plant growth and deleterious to nematodes (Muller and Gooch, 1982). When these materials are amended in soil, their harmful impact on nematodes may be due to the release of toxic compounds during decomposition, through instigation of natural antagonists of nematodes or through alteration in the level of plant resistance. On the other hand, these amendments may ameliorate the soil structure and fertility so that plant health improves and plants are better able to tolerate the effects of nematodes (Stirling, 1991).

Sawdust is a voluminous by-product generated by the timber industry and due to its poor degradability and low bulk density, dumping of this solid waste has become an increasing economic and environmental trouble. Although, huge amounts of sawdust are incinerated in India and other countries for the production of energy (Kandpal and Maheshwari, 1993) but its low bulk density and high surface area make energy retrieval an incomplete combustion, at the same time it also generates volatile pollutants. However, some reports are available suggesting the role of sawdust in improving soil nutritional pool (Obasi *et al*., 2013), refining the soil physico-chemical properties such as porosity (Vano *et al*., 2011) and also its potential for nematode control (Miller and Edgington, 1962; Singh *et al*., 1967; Singh and Sitaramaiah, 1971; Siddiqui and Alam, 1990; Vawdrey and Stirling, 1997). Thus, there are the possibility of transforming sawdust into a plant growth promoting substance and also useful for the management of plant parasitic nematodes.

Rhizosphere organisms offer the primary level of resistance against pathogens attacking the roots (Weller, 1988) thus, playing important roles in natural and induced suppressiveness of soil-borne diseases. A number of cultivable bacteria have been examined for their bio-control potential against plant parasitic nematodes (Becker *et al*., 1988; Kloepper *et al*., 1992; Oka *et al*., 1993; Hallmann *et al*., 1997; Siddiqui and Shauqat, 2002, 2003; Khan *et al*., 2008; Son *et al*., 2008, 2009; Youssef and Eissa, 2014). Out of these microorganisms, *Pseudomonas* is a free living bacterium which colonize roots and intensify the overall plant growth. In addition, it also improves seed germination, root development, mineral nutrition and water utilization and can also check plant diseases. They are reported to release some metabolites, toxic to nematodes and induce systemic resistance against root-knot nematodes (Siddiqui and Shauqat, 2002, 2003). Various reports regarding the exploitation of *Pseudomonas fluorescens* for bio-control of plant parasitic nematodes are available (Siddiqui and Mahmood, 1999; Nelson, 2004; Siddiqui, 2005).

The general objective of our study was to evaluate the bio protective potential of sawdusts from different woods (Neem (*Azadirachta indica*), sheesham (*Dalbergia sissoo*), teak (*Tectona grandis*) and chir (*Pinus roxburghii*)) and the bio-control agent, *Pseudomonas fluorescens,* singly and combinedly, in the management of root-knot nematode, *Meloidogyne incognita* infesting chickpea.

MATERIALS AND METHODS

Present experiment was conducted during 2013-14 in the net house of Department of Botany, Aligarh Muslim University, Aligarh. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White), Chitwood (2002) was selected as the test pathogen and chickpea (*Cicer arietinum* L.) var. Avrodhi as a test plant. *Pseudomonas fluorescens* and sawdusts of teak, sheesham, neem and chir were used alone and different combination for the management of root-knot nematode on test plant.

Preparation and sterilization of soil mixture: Sandy loam soil collected from a field of Department of Botany, AMU, Aligarh was passed through 10 mesh sieve. The soil, river sand and organic manure mixed in the ratio of 3:1:1. Clay pots of 15 cm diameter were filled with the soil mixture at 1 kg/pot. A little water was poured in each pot to wet the soil before transferring it to an autoclave for sterilization at 20 lb pressure. These sterilized pots were allowed to cool down at temperature before use for the experiments.

Soil amendment with organic fertilizers: Saw-dusts of different woods i.e., neem (*Azadirachta indica*), sheesham (*Dalbergia sissoo*), teak (*Tectona grandis*) and chir (*Pinus roxburghii*) were mixed in the soil of the pot in different concentrations, i.e., 12.5, 25 and 50 g and were allowed to decompose for 20 days by giving water daily twice. Plants without amendments served as control (C). All pots were arranged in a completely randomized block design on a bench in a greenhouse. Each treatment was replicated five times.

Raising and maintenance of test plant: Seeds of test plant were surface sterilized with 0.01% $HgCl_2$ for 2 min and washed twice with distilled water. Five seeds/pot were sown and thinning was done to maintain one plant/pot. Watering was done whenever necessary. One week old, well established and healthy seedlings were used for experimental purpose.

Preparation of nematode inoculum: Large number of egg masses was handpicked with the help of sterilized forceps from heavily infected roots of eggplant on which pure culture of *M. incognita* was maintained. These eggmasses, after being washed with distilled water were placed in a 10 cm diameter coarse sieve mounted with crossed double layered tissue papers. The sieves were placed in petridishes containing water. The water level was kept in such a way that it just touched the bottom of the sieve having egg masses. The petri dishes were incubated at 25°C in the dark. Three days later the majority of eggs had hatched and j_2 were collected by rinsing the petridishes with distilled water.

The hatched out second stage juveniles (j_2) of *M. incognita* were thoroughly stirred for making homogenous distribution of nematodes. One milliliter suspension of nematodes was taken for counting the number of j_2 in each sample under the stereoscope. An average of five counts was made to determine the density of nematodes in the suspension. Volume of water in nematode suspension was so adjusted that each may contain 200+5 nematodes per milliliter. It was done by adding more water or decanting the excess amount, depending upon his situation. Ten milliliter of this suspension containing 2000 freshly hatched j_2 per plant were inoculated.

Soil amendment with biocontrol agent: Pure culture of *Pseudomonas fluorescens* was acquired from the Institute of Microbial Technology, Chandigarh, India. The bacterium was cultured by streaking on solidified nutrient agar medium in Petri plates. Plates were incubated at 37.8±2°C for

24 h. Mass culture of *P. fluorescens* was prepared in conical flasks containing King's B medium (King *et al.*, 1954). Each flask was inoculated with a similar colony of the bacteria from Petri plate whose gram-negative response had been tested. Bacteria were applied to soil around roots of chickpea plants at 2 mL pure culture/pot containing load of 1.0×10^{12} CFU mL^{-1}.

Inoculation technique of nematode: For inoculation of *M. incognita,* soil around the roots was carefully removed so that the roots were not damaged. The inoculum suspension of nematode of 2000 j_2 was poured around the roots of chickpea and the soil was replaced on the roots. In control, water was poured equal to the inoculum suspension in the same way.

Observations

Plant growth parameters: The plants of each treatment were taken out from the pots after 90 days of nematode inoculation and soil particles adhering to roots were removed by washing gently under tap water and properly labeled. Length of the plants was measured by measuring tape while fresh weight of the plants was determined with the help of a physical balance. Excess water was removed by blotting paper before weighing the plants. For dry weight determination, plants were kept in labeled envelopes and dried in a hot air oven running at 60°C for 24-48 h before weighing.

Galls/root system and extraction of nematode population: On termination of the experiment, roots of harvested plants were washed under the tap water and examined for the presence of galls. Number of galls per root system were counted. A 250 g sub-sample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method followed by Baermann's funnel technique to determine the final nematode population in soil (Southey, 1986). Nematode suspensions were collected after 24 h and the number of nematodes were counted in five aliquots of 1 mL of suspension from each sample. The mean of the five counts were used to calculate the population of nematodes per kilogram soil. To estimate the number of juveniles, eggs and females inside the roots, 1 g sub-sample of root was macerated for 30-40 sec in a waring blender and counts were made from the suspension thus obtained. The total number of nematodes present in the roots was calculated by multiplying the number of nematodes present in 1 g of root by the total weight of root.

Statistical analysis: All the data collected was analyzed statistically and Least Significant Differences (LSD) were calculated at $p = 0.05$.

RESULTS

Symptoms of root-knot disease on chickpea: Roots of chickpea plants exhibited extensive galling upon harvest. Nematode inoculated plants showed extensive galling and egg masses than the other treatments as compare with nematode uninoculated control. Number of galls were decreased when treated with all the sawdusts and bioinoculant, either alone or in combination , however, galling were recorded more in single treatment than combined one.

Plant growth parameters: A significant increase in plant growth parameters was recorded when chickpea plants were grown in soil amended with all the four sawdusts (i.e., neem, sheesham, teak and pinus) and a bioagent (i.e., *Pseudomonas fluorescens*) in the absence of pathogen (i.e., *Meloidogyne incognita*), over uninoculated control plants. Improvement in plant-growth parameters was further enhanced with an increase in the doses of these sawdusts along with bioagent.

Among all the four sawdusts, neem was found to be best in enhancing plant growth, followed by sheesham teak and pinus (Table 1). Out of three doses of all the four sawdusts (12.5, 25 and 50 g), the most efficient dose was found to be 50 g. The highest improvement in plant length, fresh weight, dry weight and number of nodules per plant (26.70, 29.74, 33.90 and 33.89%) in the absence of both, the pathogen and the bio-inoculant was recorded in case of plants treated with neem sawdust at 50 g/pot.

Inoculation of bio-agent, *Pseudomonas fluorescens* alone caused significant improvement in plant growth parameters, however, this increase (12.87, 15.26, 17.39 and 20.24%) was lower than those caused by all the four sawdusts in their highest doses (Table 1). Combined application of sawdusts and bio-agent caused higher and significant increase in plant growth parameters as compared to control as well as those amended singly with sawdusts at different doses (Table 1).

Inoculation of *M. incognita* (2000 j_2/plant), caused a significant reduction in the growth parameters of chickpea plants over uninoculated control (Table 2). Application of all the sawdusts and bio-inoculant resulted in enhanced plant growth even in the presence of pathogen. However, the enhancement was lesser as compared to the plants uninoculated with *M. incognita* (Table 2). The most efficient combination was found to be that of neem sawdust at 50 g/pot+*P. fluorescens* while the least effective was pinus sawdust+*P. fluorescens,* in enhancing plant growth parameters, over both pathogen inoculated and uninoculated control (Table 2).

Nematode related parameters: Data given in Table 2 revealed that the different treatments of all the four sawdusts and bioagent, alone and in combination significantly reduced the nematode density in terms of nematode population both in soil and roots and number of galls/root system as compared with the untreated nematode inoculated plants. The number of nematodes as well as galls/root system in plants treated with sawdusts along with bio-agent, were fewer than in plants

Table 1: Effect of sawdusts and/or PGPR, *Pseudomonas fluorescens* on growth parameters of chickpea

Treatments	Doses (g)	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	No. of nodules/root system
Control	-	48.20	77.10	25.57	16.32
Neem	12.5	57.61	95.02	31.99	25.19
	25.0	58.93	97.13	32.77	30.24
	50.0	61.07	100.03	34.24	33.89
Pinus	12.5	53.09	87.50	29.46	18.90
	25.0	54.76	89.87	30.29	21.28
	50.0	56.66	92.66	31.35	24.82
Sheesham	12.5	56.56	93.85	31.49	24.20
	25.0	57.78	95.40	32.21	27.63
	50.0	59.53	97.92	33.35	31.60
Teak	12.5	55.50	91.60	30.66	22.17
	25.0	57.00	93.57	31.57	25.02
	50.0	58.95	96.70	32.46	28.73
Pf	1.0×10^{12} CFU	54.40	88.87	30.02	20.24
Neem+Pf	12.5	59.42	98.29	33.34	30.50
	25.0	60.64	99.84	34.39	32.20
	50.0	62.61	102.66	35.49	35.50
Pinus+Pf	12.5	55.30	90.67	30.49	22.29
	25.0	56.44	92.68	31.32	23.10
	50.0	57.74	95.45	32.28	26.25
Sheesham+Pf	12.5	58.55	96.22	32.50	28.30
	25.0	59.40	98.65	33.22	30.30
	50.0	60.78	101.42	34.03	34.10
Teak+Pf	12.5	57.03	94.63	32.01	26.80
	25.0	58.57	96.57	32.76	28.50
	50.0	59.74	100.01	33.78	31.75
LSD $p\leq0.05$		03.54	004.51	02.13	01.59

Pf: *Pseudomonas fluorescens*

Table 2: Effect of sawdusts and/or PGPR, *Pseudomonas fluorescens* on plant growth, galling and nematode reproduction on chickpea

Treatments	Doses (g)	Plant length (cm)	Plant weight (g)	Plant dry weight (g)	No. of Nodules/root system	No. of Galls/root system	Nematode population
Control	-	48.20	77.10	25.57	16.32	-	-
MI	2000J_2	29.11	44..94	13.15	11.40	245.00	31810
Neem+MI	12.5	53.29	87.08	29.19	19.50	157.00	18515
	25.0	55.73	90.29	30.24	22.21	139.00	17716
	50.0	58.71	95.10	31.76	24.95	112.00	15515
Pinus+MI	12.5	50.78	81.84	27.56	14.61	218.00	25817
	25.0	52.72	84.96	28.15	16.85	196.00	24610
	50.0	54.78	88.90	29.69	19.10	171.00	23117
Sheesham+MI	12.5	52.19	84.24	28.67	18.15	168.00	22420
	25.0	54.57	88.43	29.97	20.35	145.00	20515
	50.0	57.18	92.44	30.94	22.13	121.00	18610
Teak+MI	12.5	51.73	84.42	28.72	16.14	187.00	23990
	25.0	53.62	87.78	29.71	18.22	162.00	21815
	50.0	57.18	91.83	30.71	21.53	129.00	20710
Pf+MI		51.68	84.20	28.34	18.55	182.00	22354
Neem+MI+Pf	12.5	56.49	89.94	30.75	28.10	130.00	14510
	25.0	57.96	91.13	31.48	30.44	112.00	13517
	50.0	59.46	92.37	32.09	32.50	84.00	12816
Pinus+MI+Pf	12.5	52.30	85.24	28.95	19.80	189.00	20616
	25.0	53.07	86.58	29.48	22.55	165.00	19530
	50.0	54.15	87.81	29.76	24.85	143.00	18215
Sheesham+MI+Pf	12.5	55.48	89.09	30.35	24.66	151.00	17815
	25.0	56.64	90.52	30.84	26.57	140.00	16100
	50.0	58.13	92.13	31.19	28.75	119.00	14510
Teak+MI+Pf	12.5	55.38	87.66	29.69	21.20	172.00	18514
	25.0	56.03	88.74	30.23	24.58	156.00	16616
	50.0	57.41	90.59	30.97	26.20	131.00	15617
LSD p≤0.05		03.47	04.36	02.02	01.46	008.42	132.64

MI: *Meloidogyne incognita*, Pf: *Pseudomonas fluorescens*

treated with either bio-agent or sawdust alone. However, their effectiveness increased with the increase in their doses. The higher doses of the sawdusts resulted in greater reduction in disease incidence caused by *M. incognita*. The most effective combination in the management of nematode was found to be neem sawdust at 50 g/pot+*P. fluorescens* while the least effective was pinus sawdust+*P. fluorescens*. Thus, combined treatment has proved to be more effective in increasing the plant growth and reducing the nematode density than the individual treatment.

DISCUSSION

Results from the pot experiments indicated that soil amendment with various sawdusts suppressed root galling and final population of *M. incognita* and promoted plant growth. These results confirm previous observations of management of plant parasitic nematodes by the application of different sawdusts (Brezeski and Szczech, 1999; Hassan *et al*., 2010; Ganaie *et al*., 2011; Prakash and Singh, 2014). Improvement in plant growth variables and suppression of nematode population may be due to adequate availability of nutrients which is released after decomposition of the organics. These chemicals are also nematostatic to phytoparasitic nematodes. Sawdust implement the soil with organic matter, which eventually get decomposed and forms humus. It has been considered that the depletion in nematode population might be due to accrued noxiousness of the decomposing products (Khan *et al*., 1974a; Alam *et al*., 1979), alteration in physical and chemical qualities of soil (Ahmad *et al*., 1972) or to enhanced host resistance (Van der Laan, 1956; Alam *et al*., 1977). According to Sitaramaiah and Singh (1978), in the soil amended with saw dust, phenols are released which hamper the root-knot nematode multiplication.

The present findings also indicate that the neem sawdust proved to be most efficacious for the management of *M. incognita*. Rodriguez-Kabana *et al.* (1987) speculated that the effectiveness of an organic amendment depends upon its chemical composition and the type of microbial population that flourish during the decomposition of the amendments. This view fully supports our results, as the neem sawdust was proved to be more effective than other sawdusts at the same concentration. Neem is reported to be nemato-toxic because it contains triterpenoids and flavonoids such as azadirachtin, nimbin, nimbidin, nimbidic acid, thionemone, kaempferol and quercetin which checks the nematode population (Khan *et al.*, 1974b; Siddiqui, 1986). However, the mode of action of sawdust in nematode control has not been extensively studied so far. Perhaps the activity of nematophagous fungi increased in response to sawdust application to soil (Hassan *et al.*, 2010). Barron (1992) mentioned that these fungi were cellulolytic and lignolytic and were commonly associated with rotting wood. He supposed that their nematophagous habit may be an evolutionary response which allow them to get nitrogen in a nitrogen limiting habitat.

Biofertilizers offer economically promising and ecologically harmless substitute for providing nutrients to the plants (Bhattacharjee and Dey, 2014), making them more resistant to the pathogens. *Pseudomonas fluorescens* was found to enhance plant growth variables significantly both in the presence and absence of *M. incognita. Pseudomonas fluorescens,* PGPR, ameliorate plant growth by restricting the parasitic and nonparasitic root pathogens (Oostendorp and Sikora, 1989) through the production of biologically active substances (Gamliel and Katan, 1993; Nielsen *et al.*, 1998) or the conversion of unavailable minerals and organic compounds into forms that are available to plants (Broadbent *et al.*, 1977; Siddiqui and Mahmood, 1999). In addition, induced systemic resistance by *Pseudomonas* spp. is also thought to be a biocontrol mechanism against plant pathogens (Wei *et al.,* 1996; Gajalakshmi and Abbasi, 2004). Pseudomonas can synthesize enzymes that can monitor the level of plant hormones and limit the available iron via siderophores (Glick, 1995) and can also kill the pathogen with antibiotics (Siddiqui, 2005).

The ability of sawdusts to manage the nematode disease increased, when it was integrated with the biofertilizer, *P. fluorescens*. It is assumed that the combined application of sawdusts with *P. fluorescens* resulted in build up of high bacterial population leading to improved plant growth, which provides the appreciable extent of resistance against *M. incognita.*

CONCLUSION

The inference can be drawn from the present findings that the combined application of sawdust(s) along with *P. fluorescens* could be exploited for the management practices of root-knot nematode, *M. incognita* infesting chickpea. However, more work is needed under field conditions to determine the actual contributions of the organics towards controlling these nematodes which contain toxic compounds released during the decomposition of organic matter. The present study also advocates and supports the organic farming in pulse cultivation especially chickpea. This type of study may provide an assistance to the farmers to control this hidden enemy, especially where this work has been conducted and maintain soil health which has been a hot talk in the present scenario.

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