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Potential role of organic matters and phosphate-solubilizing bacteria on growth of mungbean (*Vigna radiata*) and their response to management of phytonematodes

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ABSTRACT

An experiment was conducted during 2007–09 to eplore addition of organic matters and phosphate-salubilizing bacteria (PSB) to soil as an alternative means of organic management of nematodes under field condition at Aligarh, Uttar Pradesh. Organic matters in the form of oilseed cakes of neem (*Azadirachta indica* Adr. Juss.), castor (*Ricinus communis* L.), groundnut (*Arachis hypogaea* L.), linseed (*Linum usitatissimum*) and sunflower (*Helianthus annuus* L.) were found to be highly efficacious in reducing the multiplication of plant-parasitic nematodes *Meloidogyne incognita* (Kofold & White) Chitwood, *Rotylenchulus reniformis* Limnford & Oliveira, *Tylenchorhynchus brassicae*, etc. The plant growth parameters such as plant weight, pollen fertility (%), pods/plant, root-nodulation and chlorophyll content of mungbean [*Vigna radiata* (L.) R. Wilczek] increased significantly. The multiplication rate of phyto-nematodes and number of root-galls caused by *M. incognita* greatly affected in the presence of *Pseudomonas fluorescens* (Trevisan) Migula as compared to its absence. Damage caused by the nematodes was further reduced when *Pseudomonas fluorescens* was added along with the oilseed cakes. Neem-cake was found most effective in combination with *Pseudomonas fluorescens.*

Key words: Oilseed cakes, Plant-growth parameters, Plant-parasitic nematodes, *Pseudomonas fluorescens,* Mungbean, *Vigna radiata*

Mungbean (*Vigna radiata* (L.) R. Wilczek) is an important pulse crop grown in the northern part of India. Being a photo-sensitive and short-duration crop, it can be grown during summer season. Pest and pathogens are major biotic factors responsible for reduced biomass production and uncertainty in the yield of pulses. However its productivity is generally low ranging from 0.5 to 0.8 tonne/ha (Sharma and Prasad, 2001). Low and erratic rainfall, improper nutrient management and occurrence of many diseases are principal factors for its low productivity in semi-arid and arid regions of India. The crop suffers from various pathogens like fungi, bacteria, viruses and nematodes, etc. Plant-parasitic nematodes are one of the major biological constraints causing severe damage to the productivity of mungbean (Tiyagi *et al*. 2010). Sasser and Freckman (1987) have estimated 10.9% worldwide yield losses in mungbean due to various plant-parasitic nematodes. Therefore, it is a matter of great concern to manage pathogens in order to produce more plant biomass and increased quality of grains. This management could be achieved with the help of chemical fertilizers, broad-spectrum pesticides, etc. It has been accepted for decades that effective control of plant-parasitic nematodes is dependent on various chemicals. Pesticides, though efficient and working quickly, are now being reappraised with respect to the environmental hazards, poor soil health, fertility and productivity, shows direct toxicity to predators, pollinators, fish and man, pesticide resistance (Schmutterer, 1981), their high costs and limited availability in many developing countries. Deregistration of some of these hazardous pesticides has emphasized the need for new methods to control nematodes. There is now tremendous pressure on growers to use another methods of pest control which do not pollute or degrade the environment. Organic amendment offers an alternative or supplementing control tactic to chemical or cultural control of nematodes on different agricultural crops. During the last several years, considerable progress has been made in the utilization of organic materials of plant origin as soil amendment such as oilseed cakes, chopped plant parts and seed dressing with plant extracts for the control of plant-parasitic nematodes. Organic sources of nutrients like farmyard manures, compost, decomposed oil-cakes and botanical residues are extensively used in various crops. Some antipathogenic principles derived from plant origin have been recognised as important factors

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towards disease management.These organic additives can be used to promote the development of beneficial organisms in the soil. Mujahid and Gupta (2010) also used organic additives to enhance the growth, yield and quality of lettuce. The utility of diverge groups of microbes in maintenance and buid-up of soil fertility, thereby enhancing plant growth and yield is indispensable. They enrich the soil by addition of 25-40 kg N/ha, solubilize/mobilize 30-50 kg P_2O_5/ha, liberate growth-promoting substances and vitamins, improve soil tilth and fertility and suppress the incidence of soil-borne plant pathogens (Motsara *et al*. 1995, Chattoo *et al*. 2003).

The concept that organic amendments generally increased the population of biofertilizers in this manner that has been considered as promising for the biological control of plant-parasitic nematodes. Such biofertilizers are cheaper, eco-friendly and based on renewable energy sources has gained momentum in recent years to supplement the parts of chemical fertilizers. The rhizosphere is inhabited by actively growing microbial population that immensely affect the root and plant metabolic activities. Some of these microorganisms are plant pathogens like plant-parasitic nematodes whereas others are beneficial one. Among the beneficial organisms a potential bacteria *Pseudomonas fluorescens* is one such proven biological control agent has been found detrimental against the population of phyto-nematodes and specially the root-knot nematodes (Siddiqui *et al*. 2001). The plant growth promoting abilities of *P. fluorescens* are attributed to release of siderophores (Goel and Katiyar 2004), production of antibiotic (Anita and Rajendran 2006), production of lytic enzymes (Bhaskaran, et al. 2004), induction of systemic resistance, production of plant growth promoting factors and phosphorus solubilization. Similary, the significance of root-nodule forming bacteria collectively known as rhizobia, as potential microbial inoculants has been convincingly emphasized in recent years for its N-fixing ability. But information about the association of oilseed cakes, *Rhizobium* (N-fixing bacteria) and *P. fluorescens* are limited on this crop. The aim of present study was to test the efficacy of some easily available and biodegradable oilseed cakes in the presence and absence of *P. fluorescens* on the multiplication of nematodes and different growth parameters such as plant weight, pollen fertility (%), pod numbers, and chlorophyll content in relation to root-nodule forming bacteria under field condition.

MATERIALS AND METHODS

The nematode-infested experimental field was selected and enclosed with barbed wire in the Agricultural Research Farm of Aligarh Muslim University. The experimental field was thoroughly ploughed and small beds of 6 m^2 prepared leaving 0.5 m buffer zones between them. These beds were separately treated with oilseed cakes of neem (*Azadirachta indica* Adr. Juss), castor (*Ricinus communis* L.), groundnut (*Arachis hypogaea* L.), linseed (*Linum usitatissimum* L.) and sunflower (*Helianthus annuus* L.) at 110 kg N/ha. Untreated beds alone served as control. The treatments were randomized with five replications. The beds were watered immediately to assist the decomposition of oilseed cakes and after 10 days, *Rhizobium* inoculated seeds of K 851 mungbean were sown. Weeding and watering were done whenever required during the growing period of four months. This experiment was conducted during the summer seasons of 2007-09.

Second trial on above lines was also laid out in the presence of *P. fluorescens* in the vicinity of first one. For the bacterial inoculation, charcoal-soil-based commercial culture of fluorescent pseudomonads GRP3 was obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi. Culture (100 g) of this strain of *P. fluorescens* was suspended in 1 000 ml distilled water and 10 ml (equivalent to 1 g culture) were added around each plant. One gram culture of GRP3 strain had 26×10^6 viable bacterial cells.

The plants were uprooted four months after seed germination. Fresh weight of shoots and roots were separately taken and pods/plant counted. The number of nodules/plant were also counted and assessed their efficiency to increase soil fertility. At the flowering stage, the pollen fertility (%) was estimated by the method of Brown (1949), using stainability of pollen-grains in 1% acetocarmine solution. Chlorophyll content of leaves was estimated as per the method of Hiscox and Israelstam (1979). One hundred milligram of leaf pieces were placed in a vial containing 7 ml dimethyl sulfoxide (DMSO) and the chlorophyll extracted by incubating for 60 minutes. The extract was transferred to a graduated tube and brought to 10 ml with DMSO and assayed immediately. A sample of 3 ml chlorophyll extract was transferred to a cuvette and the optical density (O.D.) values at 645 and 663 nm were read in a Spectronic-1001 Spectrophotometer against a DMSO blank.

The population of plant-parasitic nematodes were determined from each bed before treatment as well as after terminating the experiment by processing representative soil sub-samples by Cobb's sieving and decanting method followed by Baermann funnel technique (Southey 1986). The nematodes were counted seperately and identified. An average of five counts was taken in each case to determine the population density of each nematode species. Number of root-galls caused by *M. incognita*/plant were counted and assessed the disease intensity.

The data of two years were pooled and the critical differences (CD) was calculated at P=0.05 to test for significant differences between treatment (T) (Pansey and Sukhatme 1978). A factorial analysis was also taken with regard to *P. fluorescens* (*Pf*) and treatment (T) × *P. fluorescens* (*Pf*).

RESULTS AND DISCUSSION

Plant growth parameters

The results (Table1), clearly revealed that the plant-growth parameters such as plant weight, pollen fertility (%), number of pods and nodules and chlorophyll content of mungbean improved significantly which seems to be due to the soil application of various oilseed cakes in comparison to untreated control. Maximum improvement was noticed in plant weight (84.47 g), pollen fertility (94.40%), number of pods (78.60), root-nodulation (83.5) and chlorophyll content (3.436 mg/g) in the beds treated with neem-cake as compared with other oilseed cakes and the untreated control. In the untreated control, plant weight (27.52 g), pollen fertility (50.64%), number of pods (33.59), root-nodulation (40.7) and chlorophyll content (1.850 mg/g) were reduced by the presence of plant-parasitic nematodes. However, significant increase in growth parameters were also noted in linseed cake, followed by sunflower, groundnut and castor cakes. The improvement was more pronounced in the presence of *P. fluorescens.*

Amongst inoculations, *Rhizobium* and *Pseudomonas fluorescens* along with the oilseed cakes significantly improved growth parameters over untreated as well as in those plants not treated with *P. fluorescens.*This marked improvement could be due to pivotal role of *Rhizobium* in fixation of atmospheric nitrogen and *P. fluorescens* in solubilization of insoluble P through production of organic acids. The adequate supply of P seems to be due to *P. fluorescens* have to promoted root-growth, resulting in higher production of root-nodules, thus better exploitation of N from soil as well as from atmosphere and its re-allocation in plant system. Elanchezhian *et al*. (2009) also reported the importance of fixed N by bacteria in nodules. Root-nodule bacteria fixed as much as 200-300 kg N/ha/year which plays an important role in nitrogen enrichment of the soil. Rhizobia have also been increasingly associated with secretion of plant growth promoting substances and antagonistic action against pathogens.

Addition of oilseed cakes become very important in organic agricultural system which are increasing due to demand of chemical free products and due to the harmful effect of fertilizer on nodule formation. Several reports revealed that the combined effect of organic matters and biofertilizers increases yield and influences the quality parameters in several crops. Our results are in conformity with those of Bahadur *et al*. (2006). Organic matter acts as nutrient reservoirs and upon decomposition produces organic acids and they might be absorbed for leading to higher yields (Kumar *et al*. 2009). The positive effect of *P. fluorescens* observed in the plants may be due to better mobilization and supply of available phosphorus for crop growth and other yield attributes. The chlorophyll content was also found increased in all the treatments receiving oilseed cakes, *Rhizobium* and *P. fluorescens* simultaneously. Since the oil-seed cakes are also rich in nutrients, the increased amount of N might have led to better root development, photosynthesis and transportation of water and minerals. Our results are in agreement with those of Jain *et al*. (2003).

Root-galling

The root-galling due to *M. incognita* was also reduced due to soil application of oilseed cakes. The greatest reduction occurred in the beds treated with neem-cake (Table 1). Although the reduction in root-galling was statistically significant for other oilseed cakes also. These results were in agreement with those of (Dwivedi and Pandey 1992). The population of plant-parasitic nematodes increased beyond the initial level in untreated and treated beds with oilseed cakes. *M. incognita*, *R. reniformis* and *T. brassicae* were the dominant species in all the beds (Table 2). The population of plant-parasitic nematodes increased from the initial level of 1 123/250 g soil to 2 827 in untreated beds, whereas treatments with oilseed cakes reduced the nematode population. The greatest reduction occurred in neem-cake-treated beds (376), followed by linseed cake (457), sunflower cake (590), groundnut cake (740) and castor cake (889). However, the reduction in the population of plant-parasitic nematodes was more pronounced in the presence of *Pseudomonas fluorescens.*

The materials used for soil amendment were allowed to decompose in the field itself, where the target pathogens as well as decomposers are supposed to be present. Another example of nematode suppression by abiotic factors is the use of olive mill waste compost which contains nematicidal compounds and has been suggested for use as a biopesticide (Cayuela *et al*. 2008). The decomposition of organic matter helps in changing the physical, chemical and biotic conditions of the soil**.** The altered conditions of soil render the soil atmosphere unfavourable for nematode activity. In addition, the improved soil structure promotes root growth of the hosts. Organic materials like oilseed cakes on decomposition released ammonia which is known to play a important role in the suppression of plant-parasitic nematodes and fungi (Lazarovits *et al*. 2000).

Plant-parasitic nematodes

The oilseed cakes reduced the multiplication of phytonematodes significantly but more effect was observed in the presence of *P. fluorescens* (Table 2). Nemato-toxic substances present in the amendments are released after dissolution in water. The addition of organic matter to soil stimulates the activity of bacteria, fungi and other microorganisms (Rodriguez-Kabana *et al*. 1987). Increased microbial activity in amended soil causes enhanced enzymatic activities and accumulation of decomposition end-products and microbial metabolites, which are deleterious to plant-parasitic nematodes. Jaffee *et al*. (1998) showed that the number of species of nematode trapping fungi was higher in

Table 1 Effect of oilseed cakes in absence and presence of *Pseudomonas fluorescens* on growth parameters of mungbean*

Treatment	Plant weight (g)			Pollen	No. of	No. of	No. of	Chlorophyll
	Shoot	Root	Total	fertility (%)	pods/plant	root-galls/ plant	root-nodules/ plant	content (mg/g)
In absence of Pseudomonas fluorescens								
Untreated	18.25	9.27	27.52	50.64	33.59	135.30	40.7	1.850
Neem cake	62.3	22.17	84.47	94.40	78.60	35.28	83.5	3.436
Castor cake	46.32	13.87	60.19	70.62	49.17	73.17	59.2	2.367
Groundnut cake	51.62	15.88	67.50	76.29	54.68	67.10	62.3	2.693
Linseed cake	57.86	20.00	77.86	84.00	66.50	50.44	76.0	3.325
Sunflower cake	55.97	18.25	74.22	80.56	63.86	60.12	74.1	3.248
CD (*P*=0.05) for treatment			5.97	6.48	4.49	6.80	4.15	0.245
In presence of Pseudomonas fluorescens								
Untreated	23.1	13.64	36.74	74.58	42.44	87.60	55.4	2.023
Neem cake	74.34	31.22	105.56	98.67	87.50	15.56	96.3	4.358
Castor cake	52.78	17.00	69.78	80.62	57.37	55.00	66.7	2.879
Groundnut cake	57.29	19.21	76.50	86.90	61.56	51.14	78.2	3.460
Linseed cake	70.13	27.52	97.65	93.50	77.07	32.14	92.0	3.992
Sunflower cake	65.98	24.44	90.42	90.33	70.69	40.26	86.5	3.867
CD (*P*=0.05) for treatment			6.11	6.63	4.73	4.22	4.69	0.361
P.fluorescens(*Pf*)			5.46	5.73	3.88	3.31	3.56	0.274
T × *Pf*			6.73	6.80	5.03	4.58	4.80	0.404

*Each value is an average of five replicates

Table 2 Effect of oilseed cakes in absence and presence of *Pseudomonas fluorescens* on the population of plant-parasitic nematodes associated with mungbean*

Treatment	Population of plant-parasitic nematodes per 250 g soil										
	Hop	Hel	Rot	Trh	Tyl	Mel	Hem	Pra	Dor	Others	Total
In absence of Pseudomonas fluorescens											
Untreated	120	82	525	183	63	1 580	70	57	105	42	2 827
Neem cake	17	17	75	44	11	17 111	7	5	25	4	376
Castor cake	46	42	166	99	33	374	30	27	52	20	889
Groundnut cake	40	40	118	88	30	315	24	20	45	20	740
Linseed cake	22	21	80	55	15	204	10	10	31	9	457
Sunflower cake	31	27	85	67	20	283	19	13	33	12	590
Initial population	60	51	235	130	37	455	40	33	60	23	1 124
CD (*P*=0.05) for treatment	3.76	3.57	8.14	4.72	2.72	10.19	2.87	2.13	3.07	2.23	
In presence of Pseudomonas fluorescens											
Untreated	103	69	405	144	45	1212	56	44	75	36	2 189
Neem cake	6	10	53	25	0	96	0	2	0	3	195
Castor cake	38	35	133	92	20	324	19	21	43	15	740
Groundnut cake	34	30	105	75	15	264	15	13	39	11	601
Linseed cake	14	17	62	33	3	120	5	1	6	2	263
Sunflower cake	22	18	81	45	10	146	10	08	20	4	364
Initial population	60	51	235	130	37	455	40	33	60	23	1 124
CD (P=0.05) for treatments	3.49	3.17	6.30	3.66	2.14	8.10	2.21	2.03	2.69	1.53	
P. fluorescens(*Pf*)	2.77	2.35	4.39	2.82	1.50	5.72	1.55	1.45	1.76	1.09	
T × *Pf*	3.98	3.67	6.79	4.13	2.49	9.50	2.64	2.37	2.99	1.68	

*Each value is an average of five replicates

Hop, *Hoplolaimus indicus*; Hel, *Helicotylenchus indicus*; Rot, *Rotylenchulus reniformis*; Try, *Tylenchorhynchus brassicae*; Tyl, *Tylenchus filiformis*; Mel, *Meloidogyne incognita;* Hem, *Hemicriconemoides mangiferae*; Pra, *Pratylenchus coffeae*; Dor, *Dorylaims,* viz *Longidorus elongatus*, *Xiphinema basiri* and *Trichodorus mirzai* organic soil than conventional one. Number of other antagonists such as bacteria, saprozoic is likely to exhibit a positive relationship with nematode suppression in soil.

Rhizobacterial strain significantly elevated the growth and yield of mungbean in addition to the production of toxic compounds which may be antagonistic to phytopathogens. Induced resistance caused by such type of bacteria is termed 'systemic acquired resistance' (Durrant and Dong 2004). The presence of *Pseudomonas* improved plant growth and subsequently reduced damage caused by soil-borne diseases, including root-knot nematodes. Root galls caused by *M.incognita* on pepper and muskmelon were reduced by some strains of *Pseudomonas fluorescens* (Kokalis-Burelle *et al.* 2002). Burr *et al*. (1978) found improved growth of potatoes with the application of *P. fluorescens* and *P. putida*. *Pseudomonads* may also improve plant growth by reducing parasitic root pathogens (Oostendrop and Sikora 1989) through the production of biologically active substances (Gamtlel and Katan 1993) or the conversion of unavailable minerals and organic compounds into such form that are available to plants (Siddiqui and Mahmood 1999). *Pseudomonads* can synthesize enzymes that can modulate the level of plant hormones, may limit the available iron via siderophores production and can also kill the pathogens with antibiotics (Siddiqui 2006). The use of *P. fluorescens* with organic matter was better than the use of *P. fluorescens* singly with untreated plants. Organic soil amendments with oilseed cakes results in several benefits such as better soil structure which provides a more suitable medium for plant growth. Organic matters supply nutrients for the plant and also help to build-up antagonistic organisms. The combined use of oilseed cakes with *P. fluorescens* resulted in build-up of high bacterial population which adversely affected the nematode populations and thereby improved plant growth. In addition, an induced systemic resistance by *Pseudomonads* is also considered to be a mechanism for the biocontrol of pathogens (Gazalakshmi and Abbasi 2004).

It appears from the present investigation that the use of a *P. fluorescens* along with oilseed cakes shows antagonistic activity against nematodes. This could be developed into a valuable crop management tool to reduce the deleterious impact of plant-parasitic nematodes on plant growth. The information available on the use of organic amendments along with *P. fluorescens* for nematode control should lead to a promising area for organic farming in the third world. The concept of organic agriculture is receving much attention and organic food market expanding rapidly in our country. Such organic amendment could serve the purpose of alternative sources of nutrition supply in crop production.

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