

Management of Root-Knot Disease in *Phaseolus vulgaris* Using Potassium Fertilizer and Biocontrol Agents

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Abstract

The experiment was conducted to determine the effect of different doses of potassium fertilizer (K_2O) along with the biofertilizers, *Trichoderma harzianum*, and *Pochonia chlamydosporia*, in the management of the root-knot disease caused by the root-knot nematode (*Meloidogyne incognita*) and on the growth and physiological parameters of *Phaseolus vulgaris*. From the result it was observed that the application of potassium along with biofertilizers in the treatment T-7 in which plants were treated with double dose of potassium along with both fungal biocontrol agents and root-knot nematode, improved all the growth as well as biochemical parameters viz. chlorophyll, protein, nitrate reductase, nitrogen and phosphorus contents and reduced the number of galls per root system in comparison to the control and other treatments.

Keywords: *Phaseolus vulgaris*; Potassium oxide; Root-knot Nematode; *Trichoderma harzianum*; *Pochonia chlamydosporia*; *Meloidogyne incognita*

Introduction

The red kidney bean (*Phaseolus vulgaris*) is one of the most important leguminous plants worldwide and is the highly relished pulse grain in Northern India. The bean seeds contain an important source of dietary protein amounting to 22% of the total seed weight. The root-knot nematode causes great damage, to more than 300 plant species [1], and is one of the devastating pathogen of the common bean in temperate and tropical regions [2,3].

The nematode infection directly or indirectly affects the plant host physiology and total yield by changing the elemental concentration, (Melakeberhan et al.,) [4] resulting in changes in nutritional value of the crop. Therefore, it is necessary to control this nematode in order to reduce the crop losses. Several chemical pesticides and nematicide are being used for controlling the nematodes, but now a day's management by chemicals methods is not recommended because of risk of human being and the environment. Besides this, the biological control agents are used to control plant pathogens as alternative control strategies such as potassium nutrition and /or biocontrol agents are needed. Several fungal antagonists against the plant parasitic nematodes have been identified and applied [5-7]. *Trichoderma* sp. widely used for nematode control can survive in the soil in the presence of compost around the rhizosphere, and has a high nematicidal property against the nematode [8]. Direct parasitism of egg and larva through the increase in chitinase and protease activities and inducing plant defense response are the two mechanism of action of *Trichoderma* spp. which are thought to be responsible for controlling nematode. Biocontrol activity of *Trichoderma* sp against the *M. incognita* in soil has been reported by Sharon et al., [9]. *Trichoderma* can promote the plant growth, increase phosphate solubility and availability of micronutrients in the soil [10]. *Pochonia chlamydosporia* is another nematophagous biological control agent, which can infect the nematode eggs, larvae and ingest adult, and is used for egg parasitism of *Meloidogyne* sp [11]. It acts as true endophyte, colonizing the plant root of many crops such as tomato and barley [12,13]. Endophytic colonization by *P. chlamydosporia* provides the protection of host plant against different soil pathogen such as nematode and fungi, also promote the plant growth by facilitating the soil nutrient uptake [14,15].

In plant disease management use of commercial fertilizers in combination with biofertilizers can be implemented. Melakeberhan, et al. [4] reported significant decrease in the photosynthetic rate and crop yield with increasing nematode inoculums level and duration of infection, in the bean plant infected with *Meloidogyne incognita*. The disease was associated with leaf chlorosis, premature abscission and with changes in the nutrient elemental concentration [16]. Potassium an essential element for plant nutrition affect the plant growth and crop production by making plant resistant to disease, producing hard and strong stem, reduces lodging, increases performance and transfer of starch, sugar and fat and make the plant resistant to the frost [17]. It also plays an important role in host/pathogen relationship as defense mechanism [18]. Application of potassium increases the plant resistance by increasing the epidermal cell wall thickness and increase the plant growth [19]. Gupta and Mukhopadhyaya [20] reported that increase level of potassium have significantly reduced the number of galls of *M. javanica* in tomato.

The objective of this work was to study the effect of different doses of potassium in combination with biofertilizers (*Trichoderma harzianum*, and *Pochonia chlamydosporia*) on the growth of plant infected with root-knot nematode.

Material and Methods

The root-knot nematode, *Meloidogyne incognita* was selected as the test pathogen; *Trichoderma harzianum* and *Pochonia chlamydosporia* as the test biocontrol agents, which were added into the soil together with the potassium fertilizers for the control of root-knot nematode on kidney bean, *Phaseolus vulgaris* the experiment was performed in glass house.

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Culturing of nematode

Pure culture of *Meloidogyne incognita* from single egg mass was maintained on brinjal plants (*Solanum melongena*) in the green house for obtaining sufficient number of second-stage juveniles.

Nematode inoculum

For obtaining second-stage juveniles of the nematode, *Meloidogyne incognita* infected brinjal plants were uprooted and washed gently under tap water. The egg masses were carefully removed from galled roots and placed in 10 cm diameter, 15 mesh coarse sieves in which crossed layers of tissue papers were placed. The sieves were kept in petridishes containing sufficient water with lower part partially submerged in water. The petridishes were covered and kept in an incubator at 25°C. After 24 h onwards second-stage juveniles were collected and stored for later use, and fresh water was added. The number of juveniles was counted using counting dish.

Culture of fungus

Pure cultures of both the fungi *T. harzianum* and *P. chlamydosporia* were obtained from IARI, New Delhi. These were grown and maintained on the Richards medium at 25 ± 1°C. 10 ml of suspension contained one g of mycelium [21].

Potassium fertilizer, potassium oxide (K₂O) was used as a fertilizer where K1, K2 and K3 doses were evaluated at 50, 100 and 150 mg /pot.

Maintenance of Test Plant

The seeds of *Phaseolus vulgaris* procured from the Indian Institute of Pulse Research (IIPR), Kanpur were sterilized by treating with 1% sodium hypochlorite (NaOCl), and sown in 30 cm earthen pots filled with autoclaved soil having mixed compost. After one week of emergence of seedlings thinning was done to retain only one seedling per pot. Each pot was given treatment differently,

C= control, T1=1,000 J₂,

T2= K1+ Th (80 ml) + 1,000J₂, T3= K1+ Pc(80 ml) + 1,000J₂, T4=K1+ Th (80 ml)+ Pc(80 ml) + 1,000 J₂, T5= K2+ Th (80 ml) + 1,000 J₂, T6= K2 + pc (80 ml) + 1,000 J₂, T7= K2 + Th (80 ml) + Pc (80 ml) + 1,000 J₂, T8=K3+ Th (80 ml) + 1,000 J₂, T9= K3 + pc (80 ml) + 1,000 J₂, T10= K3 + Th (80 ml) + Pc (80 ml) + 1,000 J₂,

K1= 50 mg/pot, K2= 100 mg/pot, K3= 150 mg /pot, J₂= Second stage juvenile of *Meloidogyne incognita*, Th= *Trichoderma harzianum*, Pc= *Pochonia chlamydosporia*.

All the treatments were replicated five times. The plants were watered regularly and pots were arranged in a completely randomized design. The plants were harvested after 60 days of sowing. After harvesting plant-growth parameter such as, plant height, leaf area, number of pods, number of seeds, seed weight and number of galls were statistically analyzed.

Biochemical tests were performed 15 days after nematode inoculation. The leaf protein content was estimated by the method of Lowry *et al.*, [22] (Figure 1I). The chlorophyll content in fresh leaves was estimated by the method described by Arnon [23]. Nitrogen and phosphorus contents in the leaves were estimated by the method of Linder [24] and Fiske and Subbarow [25], respectively. Nitrate reductase activity (NRA) was measured by adopting the methodology of Jaworski [26].

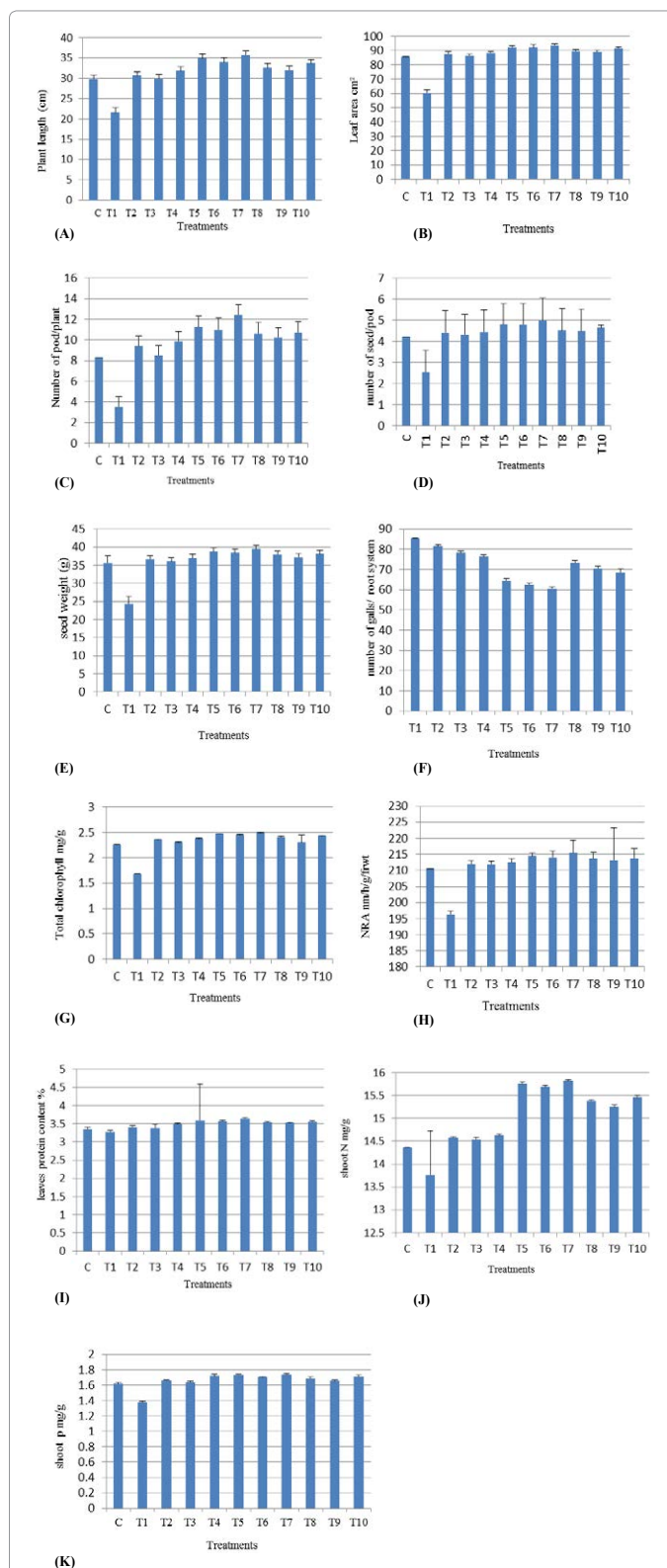


Figure 1: Figures represent the combined effect of Potassium fertilizer along with *T. harzianum* and *P. chlamydosporia* on plant length (A), Leaf area (B), number of pods/plant (C), number of seed/pod (D), seed weight (E), number of galls/root system (F), total chlorophyll content (G), NRA content (H), Leaves protein content (I), Shoot N content (J) and Shoot P content (K), of *M. incognita* infested *P. vulgaris*, and error bars represent the mean ± SD of replicates.

Statistical analysis

Data was analyzed by one-way analysis of variance and Least Significant Difference was calculated at $P = 0.05$ to test for significance. The analysis was performed with the software R (R Development Core Team, 2011).

Result and Discussion

Data presented in the (Table 1), revealed that combined application of *Trichoderma harzianum*, *Pochonia chlamyosporia* and potassium (K2) significantly ($P=0.05$) increased plant height in all the treatments except (T1), over the control (C). Highest increase in plant length (Figure 1A) was recorded in T7 (35.83 cm) as compared to uninoculated control, due to sufficient dose of K2 and both biofertilizers, which had given best response in comparison to other two doses of potassium. Combined application of NPK fertilizer and biofertilizers and organic manure increased the root length of *B. campestris* [27]. *Trichoderma* improved nitrogen fixation improved absorption efficiency of solubilized micronutrients such as Fe, Mn, and Cu etc. and improved the plant growth [10].

Melakebarhan *et al.*, (1988) reported that physiological and growth response of *M. incognita* infected bean (*Phaseolus vulgaris*) can be improved by applying KNO_3 to the soil. Long term application of organic manure and biofertilizers were reported to increase the soil nutrients such as organic carbon, nitrogen, phosphorus, potassium and also soil health [28,29]. Significant ($P=0.05$) increase in the leaf area was observed in all the treatments except T2 and T3, where non-significant increases were encountered (87.38, 86.56 cm^2 respectively) (Figure 1B). Maximum increase was observed in T7 (93.56 cm^2) over the control plants. In nematode inoculated plants (T1), significant reduction at ($P=0.05$) was recorded (60.28 cm^2), when compared with the control. Foliar application of K_2PO_3 positively affected parameters of cowpea, grown under salinity stress conditions such as plant height, number of green leaves per plant and both fresh and dry weight [30]. Mineral fertilizers such as ammonium nitrate, potassium nitrate, potassium sulphate, superphosphate and triple phosphate reduced the population of *M. javanica* [31]. Number of pods per plant and number of seeds per pod decreased significantly (3.50, 2.54) respectively, in nematode inoculated plant (T1), when compared with the control. However, maximum increase was observed in T7 (12.41, 5.00) respectively on comparing with un-inoculated control (Figure 1D). T1 plants exhibited significant ($P=0.05$) decrease in seed weight (24.37) whereas significant increases ($P=0.05$) were recorded in T5, T6, T7, T8, T10 (Figure 1E). However, the non-significant increase was observed in T2, T3, T4, and

T9 plant over the control. Sa *et al.*, [32] reported significant difference in the number of pods per plant in French bean after application of different doses of various fertilizers. A number of researchers have recorded increase in potato tubers yield as a result of the increasing level of potassium (K) fertilization [33,34]. Weir [35] suggested that application of foliar potassium on cotton plant, at the beginning of two weeks after first bloom, increased in the lint yield but later applications in the season had little response.

The number of galls was reduced significantly at Probability =0.05 level in all the treatment, while the maximum reduction was observed in T7 (60.34), over the control (Figure 1F). It was also observed that *P. chlamyosporia* along with K2 further reduced number of galls than *T. harzianum* due to its nematicidal activity, however, the combined application of both biofertilizers and potassium fertilizers (K2) give the better response in controlling the nematode. The increase in the plant growth may be ascribed to increase the nutrient availability and the reduction of nematode population might be due to toxic compound. Applications of potash in combination with phosphorus or nitrogen or potash alone check the reniform nematode multiplication on okra to a great extent [36]. Omaima *et al.*, (2012) reported that application of K_2O along with Bio-Nematon enhanced the plant growth, yield and improved the plant defense against fungal root rot and *M. incognita* infection.

Total chlorophyll content in leaves was reduced significantly (1.68 mg/g) in T1, and maximum chlorophyll content was observed in T7 (2.49 mg/g) treated with both fungal biocontrol agents (*T. harzianum* and *P. chlamyosporia*) along with potassium at the time of nematode inoculation, over the control (Table 2) (Figure 1G). El-Brammy *et al.*, [37] reported that application of potassium in soil and as a foliar spray increased the plant height, number of branches, leaf chlorophyll number of pods/plant, 100 grain weight (g) and grain yield of faba bean plant infected with chocolate spot and rust disease. Barber *et al.*, [38] found that chlorophyll content of cotton plants decreased under the potassium deficiency leading to marked reduction in the rate of photosynthesis close to 95% (Figure 1C). Nitrate reductase activity and protein content were significantly lower (196.35 nm/h/g/frwt, 3.28% respectively) in T1, in which the plants were inoculated with $1,000J_2$ of *M. incognita* alone without fungal biocontrol agents and potassium fertilizer in comparison to control. However, the maximum increases in NRA and protein content were recorded in T7 plants (215.50 nm/h/g/frwt, 3.65%, respectively) (Figure 1H), where the plants were treated with potassium (K2) along with both fungal biocontrol agents at the time of nematode inoculation as compared to the control (Table-2).

Treatments	Plant length (cm)	Leaf area (cm^2)	Number of pods/plant	Number of seed/pod	100 seed weight (g)	Number of galls/root system
C	29.88	85.57	8.30	4.23	35.61	0
T1	21.58	60.28	3.50	2.54	24.37	85.34
T2	30.65	87.38	9.41	4.42	36.66	81.35
T3	30.00	86.56	8.52	4.33	36.12	78.20
T4	31.75	88.34	9.87	4.45	37.00	76.34
T5	34.94	92.34	11.29	4.80	38.74	64.56
T6	34.00	92.00	11.00	4.78	38.42	62.44
T7	35.83	93.56	12.41	5.00	39.48	60.34
T8	32.58	89.55	10.64	4.52	37.92	73.41
T9	32.00	89.00	10.25	4.50	37.20	70.59
T10	33.68	91.32	10.75	4.65	38.00	68.37
LSD= 0.05	1.73	2.31	1.70	1.56	2.07	2.04

Table 1: Combined effect of biofertilizers (*T. harzianum*, *P. chlamyosporia*) and different doses of Potassium (K2) on the growth of *Phaseolus vulgaris* infested with *M. incognita*.

Treatments	Total chlorophyll mg/g	NRA nm/hg fr wt	Leaves protein%	Shoot N mg/g	Shoot P mg/g
C	2.25	210.54	3.35	143.6	1.62
T1	1.68	196.35	3.28	13.76	1.38
T2	2.35	212.00	3.40	14.58	1.66
T3	2.30	211.85	3.38	14.54	1.64
T4	2.38	212.50	3.49	14.62	1.72
T5	2.47	214.90	3.60	15.76	1.73
T6	2.45	214.00	3.58	15.68	1.70
T7	2.49	215.50	3.65	15.82	1.74
T8	2.41	213.68	3.54	15.38	1.69
T9	2.30	213.20	3.52	15.25	1.65
T10	2.43	213.75	3.56	15.45	1.71
LSD=0.05	0.03	6.19	0.06	0.49	0.02

Table 2: Combined effect of biofertilizers (*T. harzianum*, *P. chlamydosporia*) and different doses of Potassium on the biochemical parameters of *Phaseolus vulgaris* infested with *M. incognita*.

The shoot N and P content was reduced significantly in T1 (13.76 mg/g and 1.38 mg/g, respectively) in comparison to control. However, the treatment T7 exhibited the maximum increased in shoot N and P content over the control (Table 2) (Figure 1J and 1K).

Application of potassium along with *P. chlamydosporia* for controlling root-knot nematode was not reported earlier. From the present study it was evident that of *P. chlamydosporia* with potassium were compatible in controlling the nematode. Potassium plays an important role in enzyme activation involved in ATP production, in regulation of photosynthetic rate that improves the plant growth and yield [39]. Potassium is associated with disease reduction, it provide resistance to the plant by suppressing the disease caused by the pathogen. Increased level of Potassium showed the 80% reduction in root-knot nematode population in tomato plants [20].

Fungal biocontrol agents which we were used in our experiment having the nematicidal activity, they parasitized the egg, larvae of the root-knot nematode and reduced the nematode population. The findings from the experiments clearly showed the increased plant growth parameters with decreased nematode infestation. Nematophagous fungi may increase plant growth by participation in nutrient uptake or by modification of plant growth regulators (hormones or related compounds). Improvements in uptake of nutrients and growth due to application of *Trichoderma* were also noticed [40-42]. *T. harzianum* has strong capacity to mobilizes and take up of soil nutrients [39]. Up take of K was found to be higher than N and P in sugarcane as also reported by Shukla et al. [42]. *Trichoderma* strains colonise the plant roots, establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology and may result in the improvement of abiotic stress resistance, nitrogen fertilizer uptake, and resistance to pathogens and photosynthetic efficiency. Application of *P. chlamydosporia* successfully established in tested plants, and suppressed the nematode growth, resulted in growth enhancement on the application with *Trichoderma* or alone. Attraction of the fungus *Pochonia* to a richer source of energy (e.g. carbohydrates) such as plant rhizo deposits may support the hypothesis that nutrition is one of the factors involved in switching from saprophytic to parasitic behavior and the fungi *P. chlamydosporia* should translocate nutrients across the mycelial network. According to Kerry [43] root exudates from tomato infected by root-knot nematodes contain more water soluble and several metal ions which support more colonization of *P. chlamydosporia* than healthy roots. It has been noted that maize

supports a higher degree of fungal growth, even in the presence of high nematode populations compared to tomato (Bourne and Kerry) [41].

From our previous findings it had been suggested that *Trichoderma viride* and *Pochonia chlamydosporia* along with urea as a nitrogen fertilizer improved the plant growth of red kidney bean infested with *M. incognita* (Sharf et al.,) [42]. Biofertilizers (*T. harzianum*, *P. chlamydosporia*) along with K2 gave better response in controlling the nematode and also improved the plant growth and yield of *Phaseolus vulgaris* [43-50]. Our results suggested that application of potassium along with *Trichoderma harzianum* and *P. chlamydosporia* improved the plant growth, by increasing plant nutrient uptake properties and reducing the nematode population by their nematicidal activity [51].

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