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# Combined Effect of Biofertilizers and Fertilizer in the Management of *Meloidogyne incognita* and Also on the Growth of Red Kidney Bean (*Phaseolus vulgaris*)

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## ABSTRACT

The experiment was conducted to determine the combined effects of two biofertilizers (*Trichoderma viride* and *Pochonia chlamydosporia*) and the nitrogenous fertilizer (urea) in the management of the root-knot disease caused by the nematode (*Meloidogyne incognita*) and on the growth and the biochemical parameters of red kidney bean (*Phaseolus vulgaris*). From the results it was evident that combined application of the biofertilizers and the nitrogenous fertilizer in the treatment T-8 improved all the growth parameters as well as biochemical parameters viz., chlorophyll, protein, nitrate reductase, nitrogen and phosphorus contents in comparision to control as well as in comparision to other treatments. The number of egg masses and the number of galls per root system were significantly reduced in all the treatments, however, maximum reduction was observed in the treatment T-8.

**Key words:** *Phaseolus vulgaris*, biofertilizers, *Meloidogyne incognita*

## INTRODUCTION

Red kidney bean is a major source of protein and is an important component for human nutrition. Like other leguminous crops it is also sensitive towards saline conditions such as yield is reduced at salinity level below 2 $dSm^{-}$ (Subbarao and Johansen, 1994). The yield and quality of red kidney bean are affected by several diseases caused by various plant pathogens (Habish and Ishag, 1973; Mahdi, 1993). Red kidney bean is also damaged by plant parasitic nematodes, specially different species of the root-knot nematode (*Meloidogyne* spp.) which cause heavy yield losses (Ngundo and Taylor, 1974). Root-knot nematode *M. incognita* is the single most damaging plant parasitic nematode with host range of about 3000 plant species (Ehlers *et al*., 2002; Trudgill and Block, 2001). Continuous cultivation around 3-4 year in the same field is the major reason of high incidence of root-knot nematode (Wahundeniya and Kurukulaarachchi, 1999). The root-knot nematode has been reported to cause 10-100% yield loss depending on the crop and locality; and 5-30% yield loss with other pathogens and also suppresses the nodulation in legumes (Jenkins and Taylor, 1967; Trudgill *et al*., 2001; Taha and Samie, 1993). Highest inoculum level of *M. incognita* increase the number of galls and decrease the plant weight in tomato (Singh and Khurma, 2007). Chemical pesticides are generally used for the control of plant pathogen due to their easy applicability but these pesticides have adverse effect on soil health and environment (Diwedi and Diwedi, 2007). Maareg *et al*. (2005) reported some nitrogenous fertilizers to manage the population density of the root-knot nematode. Certain fungal biocontrol agents were found

colonizing near the plant roots that grew on roots, provided physical barrier against the nematode and enhanced plant growth (Wickramaarachchi and Ranaweera, 2008). *Trichoderma* sp., a widely studied fungus that showed antagonistic activity towards the nematodes and soil borne fungal pathogens. *Trichoderma viride* can survive in the soil with compost and plant rhizosphere and had a very high nematicidal activity to the nematode (Liu *et al*., 2007). Khan *et al*. (2004) recorded the parasitic activities of *Trichoderma* against the nematode. Nematicidal activity of *T. viride* was suggested to increase in chitinase and protease activities. (Sharon *et al*., 2001). In addition to *Trichoderma* nematophagous fungi has also been used for the control of root knot nematodes. Nematophagous egg parasitic fungus penetrated in to the egg reduced the nematode population by colonizing the rhizosphere without affecting the plant growth (De Leij and Kerry, 1991). The fungus also colonized the root of host plant (Lopez-Llorca *et al*., 2006). Endophytic colonization of the root by *P. chlamydosporia* provided protection to the plant against various pathogens (Macia-Vicente *et al*., 2009; Monfort *et al*., 2005).

Biofertilizers are known to induce resistance against nematodes resulting in improvement of plant growth (Durrant and Dong, 2004). Inoculation of biofertilizers together with the organic and inorganic nitrogen increased the plant growth and yield due to beneficial effect of biofertilizers that fixed nitrogen, produced phytohormones like substances and increase in the uptake of nutrients (Govindan and Purushothaman, 1984). Inoculation of microorganism such as PSB, *Azatobactor*, *Trichoderma viride*, *Azospirillum* and vermicompost along with FYM and inorganic nitrogen significantly enhanced the growth parameters (Kalidasu *et al*., 2008).

The following experiment was carried out to investigate the effects of bicontrol agents (*T. viride* and *P. chlamydosporia*), antagonistic to *M. incognita*, at different doses of nitrogen fertilizer on red kidney bean.

## MATERIALS AND METHODS

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

**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 = Mi(1,000 $J_2$)
T2 = N20+Pc(80 mL)+Mi(1,000 $J_2$)
T3 = N40+Pc(80 mL)+Mi(1,000 $J_2$)
T4 = N20+Tv(80mL)+Mi(1,000 $J_2$)
T5 = N40+Tv(80 mL)+Mi(1,000 $J_2$)
T6 = N20+Tv(80 mL)+Pc(80 mL)+Mi(1,000 $J_2$)
T7 = N40+Tv(80 mL)+Pc(80 mL)+Mi(1,000 $J_2$)
T8 = N20+Pc(80 mL)

T9 = N40+Pc(80 mL)
T10 = N20+Tv(80 mL)
T11 = N40+Tv(80 mL)

Uninoculated plant served as a control. All the treatments were replicated five times. Pots were arranged in a completely randomized design and the plants were watered regularly. The plants were harvested after 60 days of sowing.

**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 *Meloidogyne incognita*, brinjal plants infected with *M. incognita* were uprooted and washed gently under tap water. The egg masses carefully removed from galled roots were 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. viride* 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 (Riker and Riker, 1936).

Urea [Co(NH2)2] was used as fertilizer; where one gram urea was equivalent to 460 mg of nitrogen (Lindquist *et al*., 2010). N20 and N40 values were evaluated as 107.29 and 215.51 mg urea $kg^{-1}$ soil. Biochemical tests were performed 15, 20 and 25 days after nematode inoculation.

The leaf protein content was estimated by the method of Lowry *et al*. (1951). The 20 mg of oven dried red kidney bean leaves were ground by adding of 1 mL of 5% trichloroacetic acid. The absorbance was read at 660 nm using spectrophotometer. The total protein content was calculated by comparing the absorbance of each sample with a calibration curve plotted by taking known graded concentration of bovine serum albumin.

The chlorophyll content in fresh leaves was estimated by the method described by Arnon (1949). The reading was taken at 663 and 645 nm on spectrophotometer, against a blank reagent. Nitrogen and phosphorus contents in the leaves were estimated by the method of Lindner (1944) and Fiske and Subbarow (1925), respectively. Phenol content in the leaves was estimated by the method of Swain and Hillis (1959). NR was measured by adopting the methodology of Jaworski (1971).

**Statistical analysis:** Data was analysed 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).

## RESULTS AND DISCUSSION

Data presented in the Table 1 revealed that the combined application of *T. viride* and N40 significantly (p = 0.05) increased the plant height and the plant fresh weight over the control as

Table 1: Effect of *T. viride, P. chlamydosporia* and urea on the growth parameters of red kidney bean infested with *M. incognita*

| Treatments | Plant height (cm) | Plant weight (g) | No. of pods plant$^{-1}$ | Leaf area (cm$^2$) | No. of galls root$^{-1}$ system | No. of egg masses root$^{-1}$ system |
|---|---|---|---|---|---|---|
| Control | 27.33 | 10.26 | 7.10 | 80.06 | 0 | 0 |
| T1 | 22.00 | 06.46 | 3.90 | 62.53 | 83.36 | 48.06 |
| T2 | 28.48 | 10.80 | 8.65 | 82.66 | 62.42 | 31.71 |
| T3 | 29.68 | 11.00 | 8.78 | 83.51 | 57.78 | 30.79 |
| T4 | 31.03 | 11.48 | 9.00 | 84.72 | 65.21 | 36.35 |
| T5 | 33.58 | 11.81 | 9.13 | 87.05 | 69.18 | 34.40 |
| T6 | 35.56 | 12.27 | 10.19 | 89.52 | 55.98 | 28.30 |
| T7 | 36.88 | 12.66 | 10.35 | 91.39 | 52.50 | 27.35 |
| T8 | 38.00 | 13.57 | 11.56 | 92.72 | 0 | 0 |
| T9 | 38.82 | 13.81 | 11.65 | 94.64 | 0 | 0 |
| T10 | 39.15 | 14.46 | 12.10 | 96.55 | 0 | 0 |
| T11 | 40.52 | 14.89 | 12.60 | 97.49 | 0 | 0 |
| LSD = 0.05 | 3.5086 | 2.7202 | 1.76 | 5.1452 | 3.6799 | 2.9493 |

well as other treatments. Maximum increase in plant height and weight was recorded in T10 (40.69 cm and 14.89 g, respectively). There was significant improvement in the leaf area in all the treatments except T1, T2 and T3 where improvement was found to be non-significant (p = 0.05). The similar findings have been reported by Shamalie *et al.* (2011) in which *T. viride* incorporated compost and inorganic fertilizers significantly increased the plant growth parameters and reduced the root galls in the plant gotukola. The length of nematode infected plant significantly (p = 0.05) increased in the presence of bio and chemical fertilizers. Haque *et al.* (2010) reported that certain doses of NPK and 50% *Trichoderma*/compost show the better performance on the growth, dry matter accumulation and yield of mustard. *Trichoderma* can promote the plant growth by increasing phosphate solubility and availability of micronutrient in the soil (Altomare *et al.*, 1999). *Trichoderma* sp. significantly suppressed the root-knot disease in maiz plant. (Windham *et al.*, 1989). Sikora (2008) reported that *Trichoderma* has been extensively used for controlling plant parasitic nematodes. Enhancement of plant growth by *Trichoderma* might be due to production of secondary metabolites which may act as auxin like compound (Vinale *et al.*, 2008a, b). *Trichoderma* sp., also increased nutrient uptake through enhancement of root growth (Harman *et al.*, 2004).

Application of nitrogen fertilizer in the soil was more effective in suppressing nematode population due to excessive metabolism of nitrogen leading to toxicity (Rodriguez-Kabana, 1986) and also increase the nodulation in plant (Harper and Cooper,1971). Farmers and agricultural scientists have found the favourable response of urea in the form of nitrogen as compared to $NH_4OH$ (Lahav *et al.*, 1976). Westermann and Kolar (1978) reported the total nitrogen uptake were related to the seed yield of different dry beans. Maareg *et al.* (2000) found that mineral fertilizers such as ammonium nitrate, potassium nitrate, potassium sulphate, superphosphate and triple phosphate reduced the population of *M. javanica*. *Pochonia chlamydosporia* along with urea improved the plant growth which not only acted as nematophagous fungus but along with chemical fertilizers improved the plant growth and reduced the nematode population. The biofertilizers can be used alone and with chemical fertilizers for the management of root-knot disease. Significant (p = 0.05) reduction in the number of galls and egg masses on the plants with combined application of *P. chlamydosporia* and urea, also confirmed the compatibility of bio and chemical fertilizers and

its effect on nematode population. However, maximum reduction in the number of galls and the number of egg masses were observed in the treatments T6 and T7 due the combined application of *T. viride*, *P. chlamydosporia* and urea. Direct parasitism of eggs, increase in extracellular chitinase activity, activation of plant defense mechanism leading systematic resistance are the two possible mechanism for the suppression of nematodes (Sahebani and Hadavi, 2008). Olowe (2012) found that nitrogen resulted in better performance in the plant yield and other biomass parameters which also reduced the *M. incognita* infection and galling; the combined treatment of NPK significantly improved all the parameters.

The number of pods plant$^{-1}$ increased significantly in all the treatments over the control except T2 where the increase was non-significant ($p = 0.05$). Maximum number of pods/plant was recorded in the treatment T11 due to the combined application of bio and chemical fertilizers. Sa *et al.* (1982) reported significant difference in the number of pods per plant in French bean after application of different doses of various fertilizers. Significant increase in the yield of *B. campastris* by the application of chemical, bio fertilizers and compost manure was reported by Datta *et al.* (2009).

Nitrogen, the most important element required for plant growth, it is a major constituent of protein, enzymes, chlorophyll and growth regulators (Schwartz and Corrales, 1989). Growth of many leguminous plants and cereals affected by various nitrogen sources. (Ryle *et al.*, 1978; Trung and Yoshida, 1983; Dale 1977; De Mooy *et al.*, 1973). Unavailability of nitrogen acts as critical limiting factor (Vance, 2001). Leguminous plants require more nitrogen than any other nutrients, because reserve food material is stored in the form of protein in this seeds (Schwartz and Corrales, 1989). Long term application of organic manure and biofertilizers were reported to increase soil nutrients such as organic carbon, nitrogen, phosphorus potassium and also soil health (Bhunia *et al.*, 2006; Kumar *et al.*, 2009) Combined application of bio and chemical fertilizers significantly increased the nitrogen and phosphorus content in the leaves of red kidney beans in all the treatment (Fig. 1). Maximum concenteration of N and P was recorded in T-11 with combined application of *T. viride* and N40 (Fig. 1 and 2). *Trichoderma* improved the nitrogen fixation used efficiency and solubilized micronutrients such as Fe, Mn and Cu etc., improving plant growth and development Altomare *et al.* (1999). Data pertaining the total chlorophyll content in the leaf depicted that the treatment increased the total chlorophyll content in the leaves of red kidney bean. Highest value in chlorophyll content were recorded in T-11 probably due to higher dose of bio and chemical fertilizers (Fig. 3).

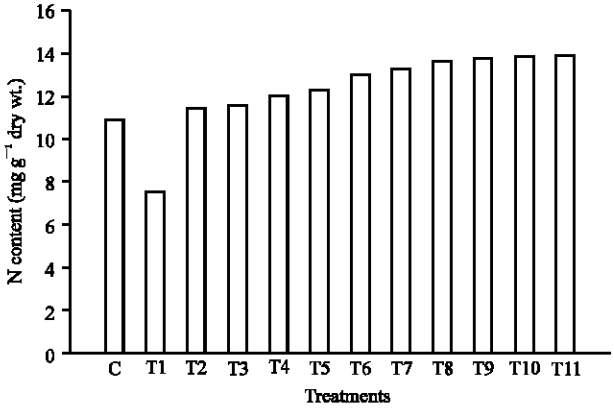


Fig. 1: Variation occurred in N content in leaves of kidney bean

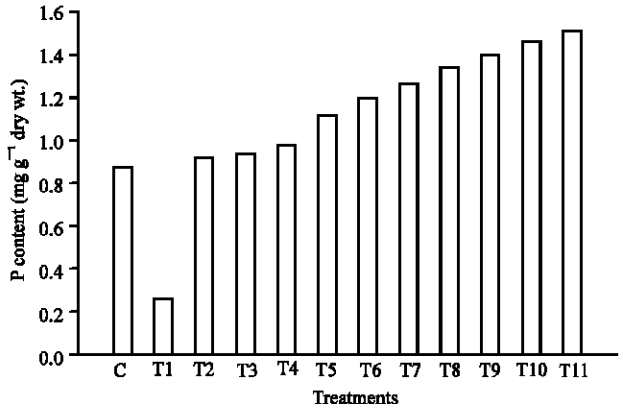


Fig. 2: Phosphorus content in leaves of kidney bean

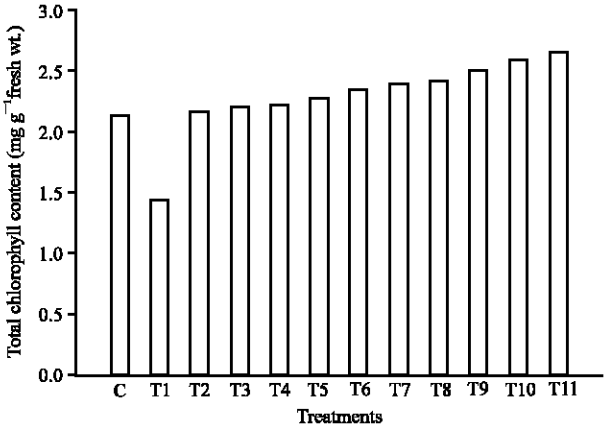


Fig. 3: Chlorophyll content in the leaves of kidney beans

The nitrate reductase plays a key role in regulation of assimilatory reduction of nitrate. It can be exist in active and inactive form, the conversion is governed by redox mechanism. The inter conversion may have a great significance in plant productivity regulating the ratio of carbohydrates to protein (Aparicio and Maldonado, 1977). From this data it is evident that in the leaves of red kidney bean NR showed significant increase in the treatment T11 over the control and the T1 (Fig. 4). Combined application of bio and chemical fertilizers exhibited the better effect on the phenol content of leaves of red kidney beans. The phenol content in the leaves of red kidney beans significantly ($p = 0.05$) increased in all the treatments except T2, T3, T4 and T5. However *P. chlamydosporia* showed less significant result than *T. viride* with and without nematode infestation (Fig. 5).

Protein is the most important constituent in the legume grains increased significantly in the leaves of red kidney beans in the treatments T8,T9, T10 and T11 while in other treatments increase was non-significant ($p = 0.05$) due to nematode infestation. Highest protein content was recorded in T11 having higher dose of N40 and *T. viride* (Fig. 6). Datta *et al.* (2009) found that root length of *B. campestris* was increased by combined application of NPK fertilizer, Azophos (biofertilizer) and organic manure. It has been reported that sole application of *Trichoderma* spp. increased both root and shoot growth of corn (Bjorkman *et al.*, 1994).

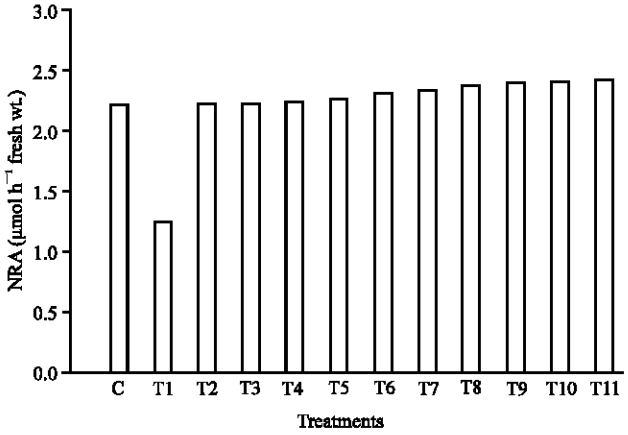


Fig. 4: Variation occurred in NRA in the leaves of kidney beans

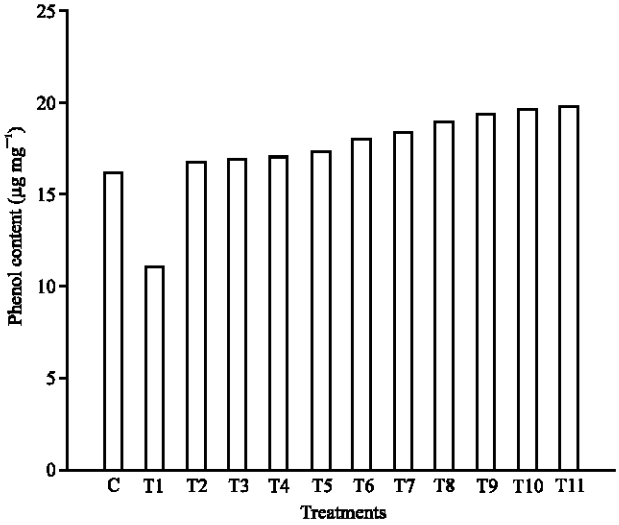


Fig. 5: Phenol content in the leaves of kidney beans

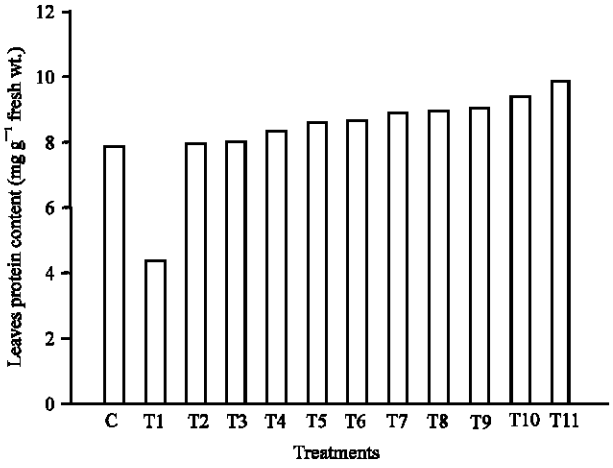


Fig. 6: Leaves protein content in the kidney beans

From the result of above experiment it can be concluded that Biofertilizers (*T. viride*, *P. chlamydosporia* ) and nitrogen fertilizer (Urea) could be utilized for improving the crop yield under field conditions.

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