EFFECT OF MYCORRHIZA AND RHIZOBIUM ON PHASEOLUS VULGARIS L

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Abstract: Study on effectiveness of *Rhizobium leguminosarum* (bv. *phaseoli*) inoculum and mycorrhiza on red kidney bean (*Phaseolus vulgaris* L.), locally called Rajma was carried out in pot experiment at Nepal Academy of Science and Technology (NAST) in 2008. The parameters evaluated to study the effectiveness of inoculum application were number of nodules, biomass, number of pods, root length, shoot length and dry seed weight. The study consisted of four treatments, *Rhizobium*, Mycorrhizal spores, fertilizer (DAP) and control which were replicated 4 times. *Rhizobium* inoculated plants showed higher dry biomass, longer root and shoot length, higher number of pods and maximum nodule formation of red kidney bean in comparison to other treatments which was statistically significant ($P< 0.05$). Those treatments which were treated with *Rhizobium* inoculum and fertilizer showed nearly the same weight of dry seed than other treatments which is followed by mycorrhiza and control. Hence *Rhizobium* and mycorrhza can be used as biofertilizer which is economically and environmentally friendly.

Key words: *Rhizobium*; Mycorrhiza; Inoculum; Biofertilizer.

INTRODUCTION

Grain legumes provide protein rich food and most people in the world fulfill their required protein from legumes. Increasing the productivity of legumes depends on supply of nitrogen that can be obtained from symbiotic nitrogen fixation in the presence of effective native soil *Rhizobia* or through inoculation. It is estimated that biological nitrogen fixation (BNF) on a global scale may reach a value of 175 million metric tons of nitrogen per year of which 80% comes from symbiosis involving leguminous plants and different strains of *Rhizobium.* World wide legumes fix about 90 million tones of nitrogen per year. Soils of most parts of Nepal do not contain appropriate strains of bacteria. Due to this, legumes crops do not obtain sufficient nitrogen from soil like other non-leguminous plants. So, inoculation of legume seed with specific species of *Rhizobia* ensures better growth of the host plant and effective nitrogen fixation by the nodule organisms (Roughley 1976).

Rhizobium and mycorrhiza are economically and environmentally friendly microorganisms used as biofertilizers. Biofertilizers from microorganisms are good for improvement of soil properties, and supply nutrient for longer period of time without leaving negative effect on soil, produce high yield and quality crops (Yami and Khanal 1997).

The nitrogen fixing bacteria *Rhizobium* are fast growing and acid producer, infective on temperate legumes. It is gram negative, non-sporulating, aerobic, motile, rod shaped bacteria of width 0.5-0.9 and length 1.2-3.0μm is capable of establishing symbiosis in the leguminous plants, directly fixing nitrogen from atmosphere. The *Rhizobium* legumes symbiosis is the most significant source of nitrogen in the soil. Three genes nod, nif and hup play important role in biological nitrogen fixation. Hup gene has ability to recycle H_2 which saves the energy loss as H_2 gas at the time of conversion of N_2 to NH_3.

Mycorrhiza are biotrophic in nature which improve nutrient status of plants by symbiotic association with the roots system of living plants. In this mutualistic symbiotic relationship between fungal and living roots of plants, both partners provide the plant with phosphorus and other nutrient in exchange of photosynthate (Smith and Read, 1997). It has been estimated that at least 80 to 90% of the world's higher plants form mycorrhiza except few members belonging to cruciferae, cyperaceae, chenopodiaceae, brassicaceae, junaceae and proteaceae (Smith and Read, 1997).

Red kidney bean (*Phaseolus vulgaris* L.) a kind of annual leguminous crop, native to China is locally called Rajma in Nepal. It is cultivated as a crop or intercropped with other

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crops, especially maize (*Zea mays* L.). Using of minimal technical inputs and low fertility can gain more production of Rajma. It is also nitrogen fixing crop with symbiosis of *Rhizobium* i.e. *R. leguminosarum* bv. *phaseoli* form nodulation and fixes atmospheric nitrogen symbiotically. The main objective of the present research was to develop appropriate *Rhizobium* inoculum which increases the fertility of soil, productivity and biomass formation of the red kidney bean through biological nitrogen fixation.

MATERIALS AND METHODS

Collection of Materials

Root nodules of red kidney bean were collected from farmer's field of Bhaktapur district, Kathmandu valley, seeds of red kidney bean and chemical fertilizer were purchased from local market.

Isolation, Identification, Multiplication and Acid Alkali Test of *Rhizobium*

Rhizobium strain was isolated in pure form from the root nodules. These nodules were thoroughly washed with tap water and surface sterilized with 95% ethanol, finally washed with series of sterile distilled water and then crushed to form solution and subjected for Gram staining. Then the extract was observed under a high power microscope with oil immersion and also streaked on yeast extract manitol agar (YEMA) medium (Vicent, 1970) and congo red (Somasegaren and Hoben, 1994) for isolation. This isolate was identified according to Bergey's manual 1994. A loopfull pure culture of *Rhizobium* inoculated in *Rhizobium* broth which was free from congo red and agar and incubated for 24 h at 28±1 ^{0}C for multiplication of the *Rhizobium*. For alkali and acid tests, both solid and liquid YEMA- bromo thymol blue (BTB) medium were employed. Pure culture of *Rhizobium* was streaked on the plates containing YEMA-BTB medium and also inoculated in broth and incubated at 28 ±1 ^{0}C for their growth and finally color change was noted.

Infectivity Test of Rhizobium

Before applied to the pots, the infectivity test of inoculum was done on sterile sand and paper pouch method, using Jensen's N-free medium and yeast extract manitol broth (YMB) of rhizobial isolates (Somasegaren and Hoben, 1994; Arryo *et al.*, 1998) with the help of germinated Rajma seedlings.

Isolation of Mycorrhiza

Mycorrhiza spores were isolated following wet sieving and decantation method adapted by Gerdemann and Nicolson (1963) from the agricultural soil of Kirtipur, Kathmandu .

Trials and Doses of Inoculums and Fertilizer

Clean pots were filled with 6 kg non-sterilized soil and sand in 2:1 ratio. Fresh seven to eight rajma seeds were sown at equidistance in each pot, watered and after one week seedlings were thinned and maintained five in each pot. Four treatments T1 (control), T2 (*Rhizobium* inoculum), T3 (mycorrhiza) and T4 (fertilizer) were replicated four times. Each plant of T2 was treated with 1ml of *Rhizobium* broth. Each pot of T3 was treated with 10 spores of mycorrhiza. Diammonium phosphate (DAP) fertilizer was applied @ 2 g/ pot (@100 kg/ha). The pots were watered 2-3 day's interval.

Data Collection and Analysis

At the time of flowering, required plants were uprooted from all treatments and different parameters such as nodule number, fresh and dry biomass, length of roots and stems were recorded. After maturation, the plants were harvested the number of pods formation and yield of dry seed were recorded. The collected data were analyzed with the help of SPSS program.

RESULTS AND DISCUSSION

The blue colour of Bromothymol (BTB) was changed to yellow after the significant growth of *Rhizobium* in YEMA-BTB medium plates within 5 days and in liquid medium when *Rhizobium* inoculated the p^H of the broth lowered i.e. acidic, so this *Rhizobium* is acidic in nature.

From authentication test, the seedlings treated with *Rhizobium* inoculum on sterile sand showed nodules formation while paper pouch method did not show any traces of nodules.

The plants treated with fertilizer, mycorrhiza and *Rhizobium* showed better growth and yield in comparison to the control. Plants treated with *Rhozobium* showed higher dry biomass, length of roots and shoots and number of pods formation in comparison to other treatments which were statistically

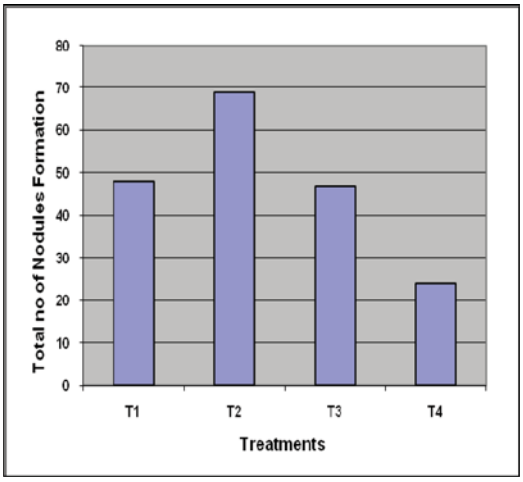

Fig 1: Total number of nodules formation under different treatments.

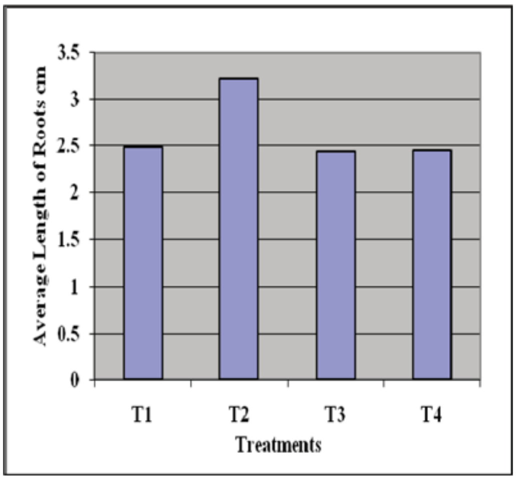

Fig 2: Average length of roots formation under different treatments.

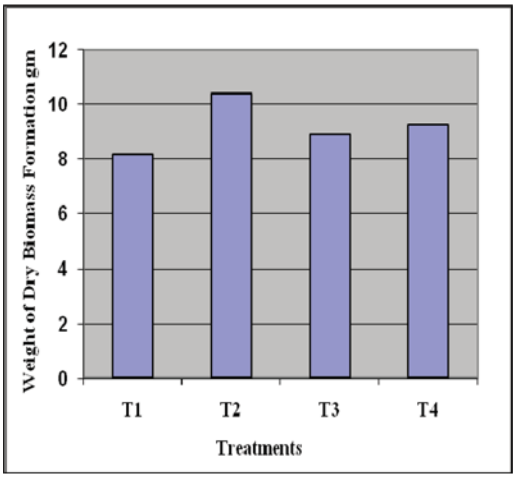

Fig 3: Total dry biomass formation under different treatments.

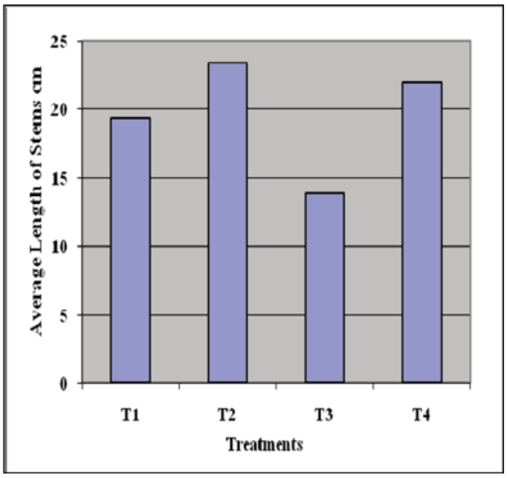

Fig 4: Average length of stems under different treatments.

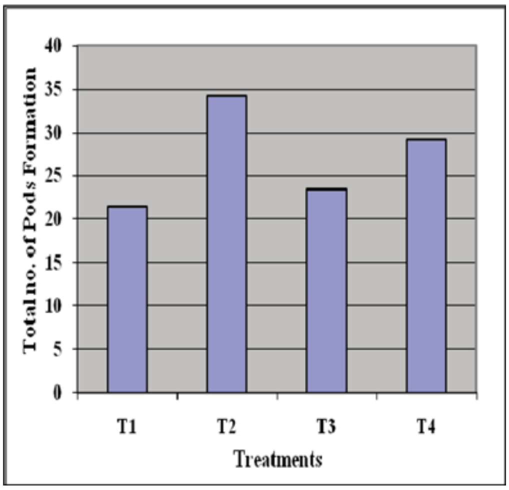

Fig 5: Total pods formation under different treatments.

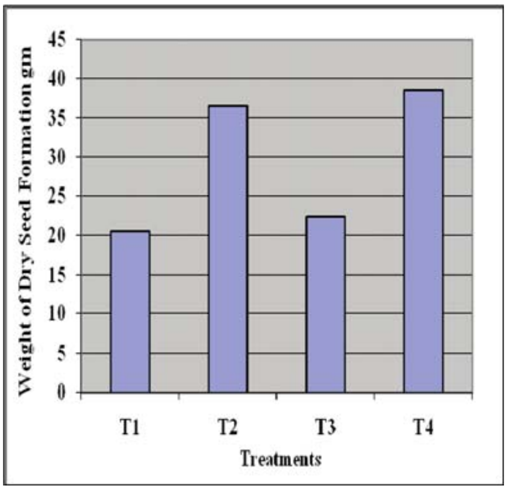

Fig 6: Total dry seed formation under different treatments.

significant (P< 0.05). *Rhizobium* performed better than other treatments. *Rhizobium* and fertilizer yielded nearly the same weight of dry seed than other treatments (Fig 6).

Rhizobium treated (T2) plants showed higher number of nodules formation (Fig.1) and average length of roots (Fig.2) than other treatments; that means it was effective for establishing symbiosis to the plants and also statistically significant(P<0.05) which was similar to the work of Pathak and Khurana (1993) or Yami and Shakya (2005).

Plants treated with chemical fertilizer formed least number of nodules (Fig. 1) than other treatments. They utilized the nutrients for increasing the plant height, biomass formation and also inhibited the nodules formation. The treatments with mycorrhiza and control showed few and nearly same number of nodules formation. It means that there was presence of local *Rhizobium* strain in the unsterilized soil but it was not effective for symbiosis. The treatment with mycorrhiza was gave lower number and smaller roots (Fig. 2) than other treatments.

The plants treated with *Rhizobiuim* increased the weight of dry biomass significantly (P<0.05) which was followed by chemical fertilizer, mycorrhiza and control (Fig. 3). This finding was in agreement with the works of Bagyaraj *et al.*, (1979), Islam and Ayanaba (1981), Yami and Shakya (2005).

Rhizobium treated plants had maximum length of stem (Fig. 4) which was followed by fertilizer, control and mycorrhizal treatments. It was close to the work of Yami and Shakya (2005). This output was statistically insignificance (P>0.05). The treatment with *Rhizobium* showed higher biomass and longer roots reveals the effective symbiosis of *Rhizobium* with plants.

Plants treated with *Rhizobium* inoculum formed maximum number of pods (Fig. 5) than other treatments and followed by fertilizer, mycorrhiza and control. This finding was close to the work of Singh and Singh (2005).

It was found that plants treated with chemical fertilizer produced maximum weight of dry seed (Fig. 6) and statistically significance (P<0.05), followed by *Rhizobium*, mycorrhiza and control which was in agreement with the work of Bagyaraj *et al.*, (1979).

Rhizobium and mycorrhiza were found to be effective and well symbiotic to the Red Kidney Bean. The symbiosis of *Rhizobium* and inoculation of mycorrhizal fungi enhanced the growth of plant by supplying two major nutrients Nitrogen and Phosphorus through biological nitrogen fixation and solublizing the insoluble soil phosphate. In sustainable agricultural systems, the role of beneficial microorganisms or microbial inoculants (Yami and Khanal, 1999) in maintaining soil fertility and bio-control of plant pathogens may be more important than in conventional agriculture where their significance has been marginalized by high inputs of agrochemicals (Shrestha *et al.*, 2010). Alternative soil management strategies are therefore needed for resource-poor farmers to increase yields of crops, e.g. adding lower amount of inputs such as expensive phosphate fertilisers (Shrestha *et al.* 2009).

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