Our Nature | December 2018 | 16 (1): 43-47

ISSN: 1991-2951 (Print)
ISSN: 2091-2781 (Online)

Our Nature

Journal homepage: http://nepjol.info/index.php/ON

Substitution of chemical fertilizer nitrogen through *Rhizobium* inoculation technology

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Abstract
Nitrogen is one of the most essential elements for plants growth and development. Urea is commonly used as a substitute for chemical nitrogen. *Rhizobium* inoculation technology for legume crop was evaluated in a number of field experiments comparing with 80kg urea per hectare application. The inoculation and urea application trial showed almost similar biomass accumulation, nodule number and nodule dry weight compared to un-inoculated control. The symbiotic effectiveness with inoculated and urea application showed similar results. The inoculant strains isolated locally from *Mucuna pruriens* (velvet bean) were found suitable for inoculants production. The bio-technology of inoculation can be a promising and cheap alternative of urea for the legume crops.

Key words: Legume crops, Biomass, Inoculant, Strains, symbiotic effectiveness, *Mucuna pruriens*.

DOI: http://dx.doi.org/10.3126/on.v16i1.22121

Manuscript details: Received: 09.05.2018 / Accepted: 02.08.2018

Citation: Paudyal, S.P. and V.N.P. Gupta 2018. Substitution of chemical fertilizer nitrogen through *Rhizobium* inoculation technology, *Our Nature* 16(1): 43-47. DOI: http://dx.doi.org/ 10.3126/on.v16i1.22121

Introduction

The leguminous plants have a unique advantage in agriculture because they possess the potential capability to fix atmospheric nitrogen by establishing symbiotic relationship with root nodule bacterium called *Rhizobium*. A nodule on the roots of legume plants is the real site of nitrogen fixation. They are induced by compatible *Rhizobium* species available in the soil. Artificial inoculation with specific rhizobial species to a particular legume in the soil (lower fertility status containing lower levels of nutrient nitrogen) enhances nodulation process and in turn fixes nitrogen required for plants growth and development. This type of practice, as reported in many cases, leads towards the nitrogen independency of the legumes. At the same time soil becomes enriched with nutrient nitrogen by sloughing off of the older nodules or leaching out of fixed nitrogen which gets mixed up with the soil. Tian *et al*. (2000) experimented on the legume crops and found that the nitrogen uptake of the maize crop is higher following cover crops than after maize or other natural grasses. The different legume cover crops used in their experiments could potentially save 50-100 kg N/ha. The symbiosis between *Rhizobium* and *Bradyrhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crops and pasture production than the application of fertilizer N. The introduction of legumes into the pastures is seen as the best strategy to improve the nitrogen nutrition of the grasses.

It has been speculated that the non-renewable energy boxes will be emptied after a few decades. When the energy boxes finish up, the present practice of industrial production of N-fertilizer will suffer. Thus, it leads to the scarcity of chemical fertilizer, which in turn, reduces crop productivity. To stop such a disaster, legume *Rhizobium* bio-technology may be properly modulated to meet the demand of crop production on a sustainable basis.

It is widely accepted that the capacity of nitrogen fixation by a nodulating legume is influenced, at least in two ways by mineral nitrogen in the soil. First, relatively low levels of available nitrate or ammonia may promote the process of nodulation, higher concentration of which almost always suppresses nodulation (Davidson and Robson, 1986; Eaglesham, 1989). Second, the rate of nitrogen fixation by an actively growing and well nodulated legume is always suppressed by NO_3^- ions (Herdina and Silsbury, 1989; Xia *et al*., 2017). It is being established that soil nitrate inhibits root infection (Abdel-Wahab *et al.*, 1996), nodule development (Atkins *et al*., 1984; Ismande, 1986) and nitrogenase activity (Purcell and Sinclair, 1990; Sanginga *et al*., 1996; Arreseigor *et al*., 1997).

Rhizobial inoculant is relatively less expensive. It would take at least US $87 worth of urea to produce a soybean yield comparable to that possible only US $ 3 worth of inoculants (Somasegaran *et al*., 1992). Moreover, increased use of fertilizer may lead to health problems apart from the degradation of soil properties as well as soil erosion. The huge increase of population in different developing countries compelled to take necessary steps to increase crop productivity by alternate means. In addition, the nitrogenous compounds used as fertilizers are rather expensive and in many instances beyond the reach of poor farmers. Use of composite biofertilizers can increase fertility status of the soil; it also imparts crop management and the addition of the major nutrients for the plants.

During the present study, the effect of inoculation on the plant biomass accumulation, nodulation, plant height attained have been compared with the application of urea fertilizer. The effect of urea application and inoculation on the nodule initiation, biomass accumulation and the ratio between dry matter production in inoculated and urea applied plants of *Mucuna pruriens* have been evaluated.

Materials and methods

Seeds of *Mucuna pruriens* were collected from the wildly growing plants during the months of January/February from sal forest at the foothills of Sunsary district in eastern Nepal. Morphologically healthy-looking seeds were selected and were sterilized according to Vincent (1970). The sterilized seeds were soaked in sterile water overnight and grown in the field plots of $1m^2$ area. Two sets of seeds: a. inoculated and b. un-inoculated were sown in different plots.

The bacterial isolate used for the present study was *Rhizobium meliloti* MPR-8 isolated locally from *Mucuna* plants. The test strain MPR-8 was grown in YM broth (Vincent, 1970) in a thermostatic shaker at 28 ± 1^0C. Broth culture with 2.5×10^8 cells ml^{-1} was used as inoculants. Adequate amount of inoculum was added with wood charcoal powder as the adsorbant and gum Arabic (1.5%) as a sticker to impregnate the seeds. Methods used for seed inoculation was followed according to Somasegaran and Hoven (1985). The inoculated seeds were analyzed for the cfu (colony forming unit) count of rhizobial cells on the seed surface by dilution plate technique.

Field experiment was done taking 4 different treatment conditions as according to Brockwell (1980). Fertilizer urea 80kg/ha was used for applied nitrogen treatment. The treatment conditions are as follows:

Rh^+N^- (Inoculated seeds without applied chemical nitrogen)

Rh^-N^+ (Un-inoculated seeds with applied chemical nitrogen)

Rh^+N^+ (Inoculated seeds with applied chemical nitrogen)

Rh^-N^- (Un-inoculated seeds without applied chemical nitrogen)

Plants were grown for a total period of 50 days. Two plants from each treatment condition were uprooted very carefully after every 10 days and the parameters like biomass, nodulation and symbiotic effectiveness were analyzed.

Results and discussion

For the improvement of soil quality by legume cultivation it is necessary that there should be the presence of compatible population of rhizobia in the soil. All soils may not have a native population of effective rhizobial strains and therefore, tested strains of the inocula were used to inoculate legume seeds to get desirable response in the enrichment of soil nitrogen.

The total biomass in inoculated without chemical nitrogen (Rh^+N^-) and un-inoculated with urea (Rh^-N^+) treatments were found almost similar impacts (Fig. 1). Similar results were obtained by Clayton *et al*. (2004). Seneviratne *et al*. (2000) and Tahir *et al*. (2009) reported that both fertilizer application and inoculation increased plant growth and seed yield as compared to control. Cruz *et al*. (1997) found that mineral nitrogen addition is not required if the soybean plants are inoculated with proper rhizobia for dry matter production. The maximum plant biomass was accumulated on the 40th days of growth. The sequence of treatment at the maximum biomass accumulation was found in the order of Rh^+N^-=Rh^-N^+>Rh^+N^+>Rh^-N^- (LSD=2.46; P>0.05).

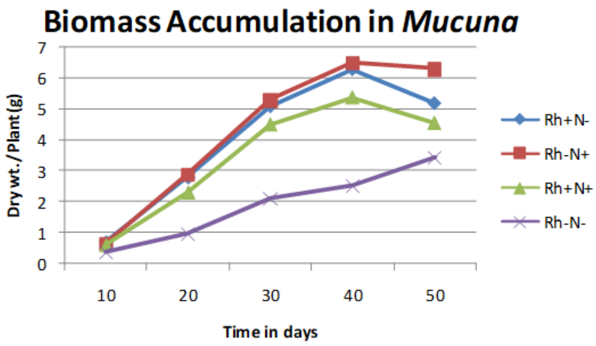

Figure 1. Biomass production of four treatment conditions in *Mucuna pruriens*

The nodulation in *Mucuna* in different treatment conditions indicated that the soil condition was poor in specific and native rhizobial population showing no nodulation under controlled conditions (Rh^- N^-) (Fig. 2). Nitrogen application in the form of urea resulted into the suppression of nodulation while under similar situation with no applied nitrogen but with rhizobial inoculation gave highest score of effective nodules. Adeyeye *et al*. (2017) noticed that the use of fertilizers provided sufficient nitrogen which played a role in antagonistic effect for rhizobial population to form the nodules and nitrogen fixation. Similar results were also obtained by Hoque (1993), Abdel Wahab *et al*. (1996), Aslam *et al*. (2000), Li *et al*. (2009), and Xia *et al*. (2017). Hoque, reported that the inoculation treatments with *Bradyrhizobium* markedly increased nodule number, nodule mass, shoot weight and yield of the crop as compared to un-inoculated control and chemical nitrogen (urea) treatment. In his experiment it has been found that inoculation increased yield 113% over control and 49% over the urea treatment. However, in our experiment the yield was increased by 150% on inoculation and 157% over the urea treatment as compared to control. Various workers (Vincent, 1974; Islam *et al*., 1987; Hoque *et al*., 1988) have reported similar beneficial effects of inoculation with *Rhizobium* and *Bradyrhizobium* on legume crops.

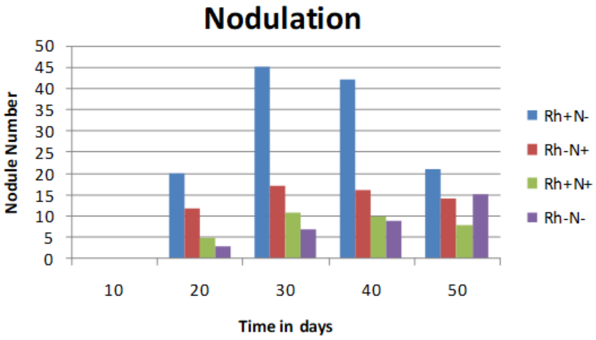

Figure 2. Nodulation in *Mucuna pruriens* in different treatment conditions

Plant biomass ratio of Rh^+N^-: Rh^-N^- and R^-N^+: R^-N^- were analyzed to derive the comparative assessment of rhizobial inoculation and applied nitrogen (Fig. 3). Interestingly, the effectiveness of biomass accumulation was found to be similar in both the treatments (P>0.05). It has been reported that the nitrogen application reduced the nodule number and mass of the *glycine max* and *Phaseolus vulgaris* (Abaidoo *et al*., 1990; Aslam *et al*., 2000; Muniz *et al*., 2017). Application of urea (90kg/ha) to soybean plants suppressed nodulation by curtailing the enrichment of *Bradyrhizobium* species on the host plant (Thies *et al*., 1995). Belanger and Richards (2000) observed that the application of nitrogen fertilizer increased the shoot nitrogen concentration. Chase *et al*. (1975) also achieved similar results.

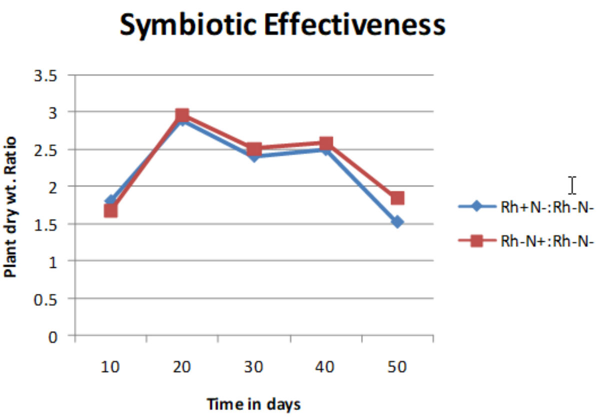

Figure 3. Symbiotic effectiveness of inoculants MPR-8.

Thus, this study concluded that *Mucuna pruriens* essentially requires rhizobial inoculation in the soils. Inoculant *Rhizobium* (MPR-8), showed adequate nodulation and affect soil nitrogen fertility equal to the application of 80kg urea/ha as has been shown by the biomass accumulation. In terms of biomass productivity inoculation or urea application gave almost similar results. So, the use of inoculation practice is far better in terms of nature conservation and the economic perspectives for the small and developing countries like Nepal.

Acknowledgements

We are very much thankful to the Department of Botany, Trichandra Multiple College, Ghantaghar, Kathmandu for providing us the essential support for the completion of the study. My colleagues as well as the non-teaching staffs of the department are highly acknowledged.

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