ISSN No:-2456-2165

Bio-Fertilizer: Possibilities and Scope in Nepal-A Review

Saraswoti Kandel[1*]
[1]National Soil Science Research Centre
National Agricultural Research Centre (NARC)
Khumaltar, Lalitpur, Nepal

Pratiksha Sharma[2]
[2]National Plant Pathology Research Centre
National Agricultural Research Centre (NARC)
Khumaltar, Lalitpur, Nepal

Reena Sharma [3]
[3] National Potato Research Program
Nepal Agriculture Research Council (NARC)
Khumaltar, Lalitpur, Nepal

Suraj Chaudhary[4]
[4]Central Department of Biotechnology
Tribhuvan University (TU)
Kritipur, Nepal

Prakriti Sapkota[5]
[5]Central Department of Botany
Tribhuvan University (TU)
Kritipur, Nepal

Abstract:- A biofertilizer is a biological material that contains active or dormant microbe cells (bacteria, actinomycetes, fungi, algae) that help fix atmospheric N or solubilize/mobilize soil nutrients while also secreting growth-promoting chemicals to boost growth and yield. This paper reviews research and development regarding bio-fertilizers in Nepal. The usage of biofertilizers can be very beneficial for maintaining the agricultural systems. A number of research groups and organizations have been working on biofertilizer research and development to improve the usage of biofertilizers in Nepal's agriculture. In Nepal, Azotobacter and Rhizobium strains are used in the production of bio-fertilizers. However, there have been fewer attempts to identify and classify associative N fixers. If Nepal works more on N fixers along with standard production of biofertilizers, it would help in the increment of agricultural yield by improving soil quality.

Keywords:- *Azotobacter, Rhizobium, Soil, Yield.*

I. INTRODUCTION

A bio-fertilizer is a material containing living, productive microorganisms that, when given to soil, seeds, or plant surfaces, colonize the plant's rhizosphere and foster development by increasing the host plant's supply of necessary nutrients. They are also known as plant growth-promoting rhizobacteria (PGPR). These PGPR work as biofertilizers, aiding plant growth. Biofertilizer is mostly produced by microorganisms, particularly nitrogen fixers, phosphate solubilizers, and mycorrhiza. Bacteria of *Bacillus* are commonly used for biofertilizers. Biofertilizers, with the exception of Azolla, which is used as green manure, are not fully dependent on the nutrients that are already in them. Bio-fertilizers are more environmentally friendly than chemical fertilizers (Rana et al, 2012) and are fuel-dependent, easily available (Umesha et al, 2018) and require less capital, technology and labor force (Raimi et al, 2017). They are made up of microorganisms that can produce phytohormones, mineralize phosphate, fix nitrogen, and govern biological processes that are vital to plant growth for non-leguminous and soil fertility (Naveed et al, 2015).

Biofertilizer production and use for both leguminous and non-leguminous crops is now prevalent in both the developed and developing nations. The economy of Nepal is heavily reliant on agriculture and other related industries. The Nepalese economy is mostly based on agriculture, making up around one-third of the country's Gross Domestic Production (GDP). Bio-fertilizer is a necessity for Nepalese farmers because the country pays millions of dollars each year to buy chemical fertilizers from abroad. Integrated usage of chemical fertilizer, organic resources, and bio-fertilizer must be given increased priority in order to address these issues. Legumes were thought to fix about 30,000 t N yearly in Nepal, worth about US$ 15 million (Maskey et al, 2002). Anabaena and Nostoc, two symbiotic Cyanobacteria, are significant biofertilizers for increasing rice output. The technology of rhizobium inoculation can greatly increase the production of legume crops in Nepal. Since 1985, NARC has manufactured and distributed thousands of microbial inoculant packets, while NARC, NAST, and TU have been responsible for research, development, and training operations. Wheat and millet yields have improved dramatically as a result of mycorrhizae research and development. Azolla, compost, and vermicompost are a few examples of organic fertilizers that significantly increase agricultural output in Nepal (NAST 2007).

Biofertilizers are carrier or liquid-based products containing living or dormant microbes (i.e., bacteria, fungi, algae, and actinomycetes) that help fix atmospheric nitrogen or solubilize various soil nutrients while also producing substances that promote crop growth and yield, according to Dinesh Kumar et al, 2018. Nitrogen fixing *Azospirillum* and P solubilizing bacteria are two examples of microorganisms that make nitogen and phosphorous available to plants (Saraswoti and Sumarno, 2008).

The microbes in biofertilizers provide plants with direct access to air nitrogen. They aid in the uptake of other plant nutrients like potash and encourage the solubilization and mineralization of phosphates. They contribute to the restoration of the soil's natural nutrition cycle and enhance soil organic matter through better hormone synthesis and availability of vitamins, auxins, and other growth-promoting substances. They also enhance plant development. Numerous organisms, including various species of Rhizobium, *Azobacter*, and Azolla, have been found at NSSRC as having the potential to fix gaseous nitrogen to plant-usable form (*Azolla Pinnata*).

II. IMPORTANCE OF BIO-FERTILIZERS

- Enhance soil quality.
- Guard against pathogens on the plant.
- Prevent environmental pollution and eliminate potentially dangerous compounds in soil.

III. SOME IMPORTANT TYPES OF BIOFERTILIZERS AVAILABLE IN NEPAL

- *Symbiotic Nitrogen-Fixing Bacteria:*

Rhizobium is the most important symbiotic nitrogen-fixing bacteria, and it thrives and multiplies inside the nodules of many genera of Leguminaceae plants. Rhizobium helps plants by providing free nitrogen. Legumes with rhizobium inoculation aid in raising the soil's nitrogen fertility. In comparison to the non-inoculated control at the research farm and farmer field, Rhizobium inoculation improved the production of soybean (15-62%), groundnut (16-34%), lentil (13-25%), black-gram (49%) and broad bean (67%) (Bhattarai, 1994).

- *Symbiotic Nitrogen-Fixing Cyanobacteria:*

The native free-floating fern *Azolla pinnata* can be found all over Nepal. Symbiotic nitrogen-fixing cyanobacteria called Anabaena are released when Azolla plant breakdown occurs. The leaf chambers of the fern contain Anabaena. The rice plants can utilize the nutrients released when the fern plants break down. When Azolla is int to paddy fields, its ability to fix di-nitrogen (N2) in conjunction with *Anabaena azolla* aids in lowering the needed dosage of chemical fertilizer (Kandel, et. al., 2020). With the same amount of P and K fertilization, Azolla pinnata application (non-incorporation) yielded roughly 14% greater grain production than the control plot (Adhikari et al, 2015)

- *Free-Living Nitrogen-Fixing Bacteria:*

Azotobacter is a soil bacterium that fixes nitrogen. If wheat is inoculated with efficient strains of *Azobactor*, the amount of chemical fertilizer containing nitrogen can be reduced by 15%. (Karki and Baral, 1977). There are reports of a 10–30% increase in wheat, rice, maize, and barley yield when *Azotobacter* is used (Bhattarai and Shah, 1988). In regions were, chemical fertilizer cannot be delivered, it is advised to use *Azotobactor* (soil application or seed inoculation) to boost maize productivity (Adhikary et al, 2011).

Table 1: Common nitrogen fixing grain legumes and Pasture species in Nepal and their *Rhizobium* strains

Common name	Botanical name	Associated *Rhizobium* strains
Mungbean	Vigna radiata	*Bradyrhizobium*
Lathyrus	*Lathyrus odoratus*	*Rhizobium leguminosarum*
Blackgram	*Vigna mungo*	*Rhizobium aegyptiacum*
Chickpea	*Cicer arietinum*	*Rhizobium spp*
Cowpea	*Vigna unguiculata Vigna sinesis*	*Rhizobium spp*
Siratro	*Macroptilium atropurpureum*	*Rhizobium spp*
Lab lab	*Lablab purpureus*	*Bradyrhizobium, Mesorhizobium*
Rice bean	*Phasiolus calcaratus*	*Rhizobium spp*
Cetrosema	*Centrosema pubscens*	*Rhizobium spp*
Broad bean	*Vicia faba*	*Rhizobium leguminosarum*
Lentil	*Lens culinaris*	*Rhizobium leguminosarum*
Stylo	*Stylosanthes species*	*Rhizobium spp*
Soybean	*Glycine max*	*Bradyrhizobium japonicum*
Ipil Ipil	*Leucaena leucocephala*	*Rhizobium spp*
Pigeon pea	*Cajanus cajan*	*Rhizobium leguminosarum*
White Clover	*Trifolium repens*	*Rhizobium trifolii*
Berseem	*Trifolium alexandrium*	*Rhizobium trifolii*
Fenugreek	*Trigonella foenumgracum*	*Rhizobium spp*
Vetch	*Vicia villosa*	*Rhizobium leguminosarum*
Common bean	*Phaseolus vulgaris*	*Rhizobium leguminosarum*

IV. DEVELOPMENT OF RHIZOBIUM INOCULANT TECHNOLOGY IN NEPAL

From a historical point of view, Legumes are cultivated in Nepal traditionally. These legume crops are grown in conjunction with other non-leguminous crops in mixed cropping, intercropping, and crop rotation system. The area planted with pulses was expected to be at 316,010 ha in 2003–2004, according to the statistics that are currently available and were provided by the Ministry of Agriculture and Cooperatives in 2005. According to the HMG-NARC study from 2001/2002, pulse output in 2003/04 was 265,360 Metric Tons with a yield of 840 kg/ha, which is poor when compared to the average yield of pulses in Asian regions. Due to inadequate research, farmers' lack of understanding of soil fertility management, and the abundance of insects, pests, and diseases, Nepal's low yield of pulses can be explained.

Inoculant development has become the primary method for the production of bio-fertilizers in Nepal. *Rhizobium* culture is kept as an inoculant on the carrier or inert support, usually peat. Liquid inoculum is also used.

However, there is no effort to isolate and characterize the associative N fixers in Nepal till date.

V. PRODUCTION AND DISTRIBUTION OF BIOFERTILIZERS IN NEPAL

NARC's Division of Soil Science & Agriculture Chemistry has been producing *Rhizobium* and *Azotobacter* inoculants for cereals, lentils, peas, soybeans, clover, and Berseem over the past ten years (SSD, 2006).

The efficient strains of Rhizobium and *Azotobacter* are isolated from the soil and roots of several legume pastures tree and crops, including cowpea, lentil, chickpea, groundnut, and soybean. Since 2065, the National Soil Science Research Centre has been engaged in a continuous process of identification, characterization, maintenance, and production of bio-fertilizer from these inoculants for various food and pasture legumes and non-legumes, as well as distribution in response to demand from researchers, students, and various stakeholders.

*Azotobacte*r, blue-green algae and Frankia inoculants are also some of the frequently produced inoculants for bio-fertilizers. Similarly, NAST and NARC both are involved in research on mycorrhiza too. Ectomycorrhiza and Endomicorrhiza are produced. Green fertilizer (Azolla), organic compost, and vermicompost are also produced in both NARC and NAST.
The production and distribution of biofertilizer by NSSRC has increased over the years and started to decrease after covid-19 situation (Figure 1).

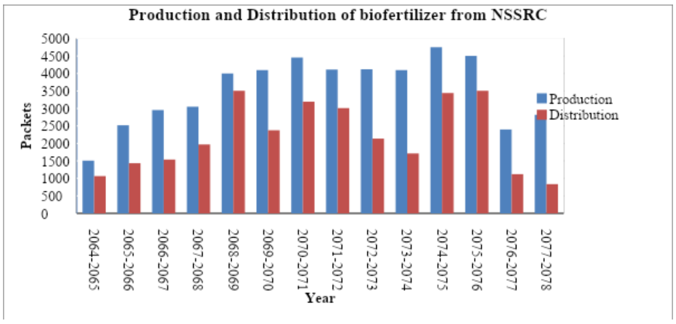

Figure 1: Bar diagram showing production and distribution of biofertilizer from NSSRC

VI. INSTITUTES INVOLVED IN RESEARCH AND DEVELOPMENT OF BIOFERTILIZER INOCULANT TECHNOLOGY IN NEPAL

- Division of Soil Science and Agriculture Chemistry (DSSAC), Nepal Agricultural Research Council (NARC) - active in biofertilizer inoculum research and production
- Nepal Academy of Science & Technology (NAST) – involved in research only
- Central Department of Botany, TU – human resource development

Rhizobium inoculants and Azotobacter inoculants, in particular, are produced in enormous quantities and can even be shipped to adjacent SAARC nations.

Most of the data were collected from the annual reports of National Agriculture Resource Centre dating back to 1998 till now. In case of legumes, inoculated yield was increased in most cases. Mixed inoculum and chemical fertilizer also increased the legumes productivity (Table 2).

Only few researches were found in case of crop like rice and wheat. Azolla strains were used in case of rice. Inoculated sample gave high yield in most of the cases (Table 3).

Table 2: Yield and yield attributing characters for legumes

Crop	Location	Year	Average Grain yield (kg/ha)			Increased yield (%)	
			Control	Inoculated	Inoculated+ chemical fertilizer	Inoculated	Inoculated+ chemical fertilizer
Soybean	Khumaltar,Lalitpur	1998/1999	508.30	916.70	-	80.35	-
Soybean	Khumaltar,Lalitpur	1998/1999	765.6	859.38	828.13	12.25	7.55
Soybean	Bhairahawa	1998/1999	246.95	239.12	273.78	-3.17	9.80
Soybean	Panchkhal	1998/1999	1.63	1.62	1.00	-0.61	-63.00
Soybean	Khumaltar,Lalitpur	1999/2000	353	363	-	2.83	-
Soybean	Khumaltar,Lalitpur	2000/2001	541.2	562.0	-	3.84	-
Rajma bean	Khumaltar, Lalitpur	2006/2007	375.0	-	600	-	37.50
Lentil	Rampur, Chitwan	2010/2011	119.13	104.20	75.98	-12.53	-56.79
Lentil	Parwanipur, Bara	2012/2013	104.22	169.89	142.97	63.01	27.10
Lentil	Parwanipur, Bara	2013/2014	73.242	91.04	118.23	24.30	38.05
Lentil	Tarhara, Sunsari	2020/2021	883	937	1303	6.12	32.23

Table 3: Yield and yield attributing characters for Crops

Crop	Location	Year	Average Grain yield (kg/ha)			Increased yield %	
			Control	Inoculated	Inoculated+ chemical fertilizer	Inoculated	Inoculated+ chemical fertilizer
Rice azolla	Khumaltar	2015/16	5.24	-	5.08	-	-0.01
Rice azolla	Parwanipur, Bara	2015/16	2.1	-	1.8	-	-0.01
Rice azolla	Dhanusa, Janakpur	2015/16	2.15	-	3.13	-	0.03
Wheat	Parwanipur, RARS	2015/16	1.641	2.278	3.299	38.82	0.05
Rice azolla	Khumaltar	2014/15	6.97	-	8.07	-	0.09
Rice azolla	Parwanipur Bara	2014/15	2.68	-	2.71	-	0.00
Wheat	Khumaltar	2000/2001	1416.66	1958.33	-	38.24	-
Wheat	Khumaltar Agronomy farm	1999/2000	800	1100	--	37.50	-
Wheat	Khumaltar	1998/99	667.6	604.2	-	-9.50	-

VII. LIMITATIONS OF BIOFERTILIZERS

- Biofertilizers are likely to minimize the demand for chemical pesticides and fertilizers while also improving soil health and sustainability.
- Specific fertilizer for specific crops is required for symbiotic biological nitrogen-fixing.
- Because microorganisms are light sensitive, continuous exposure to sunlight can kill of biofertilizer inoculants. Furthermore, the self-life of bio-fertilizer is temperature dependant.
- A variety of parameters such as soil moisture, pH, temperature, the amount of organic matter in the soil, and the presence of other microorganisms can all have an impact on biofertilizer performance. The application of bio-fertilizers is significantly hampered by compatibility, stability, and survival issues in varied soil settings. One frequent issue in the process of producing bio-fertilizer is contamination during mass culture.

VIII. FUTURE PROSPECTS AND RECOMMENDATIONS

- Numerous field tests of legume inoculation with rhizobia should be conducted in Nepal's various agroclimatic conditions.
- To lessen reliance on imported chemical fertilizers, research should be done on a holistic or integrated strategy employing biofertilizers.
- In order to accelerate the decomposition of cellulose at higher altitudes, where the cold temperature acts as the main barrier to decomposition, research on the isolation and development of effective cellulolytic microbes should begin.

IX. CONCLUSION

Due to Nepal's reliance on imported chemical fertilizers and pesticides, use of biofertilizers may be a suitable option. We can utilize biofertilizer to reduce a considerable amount of chemical fertilizer and pesticides while maintaining productivity and yield. Biofertilizer can also be used for sustainable organic farming. As a result, we may infer that Nepal has a significant need and potential for biofertilizer inoculum research and development.

REFERENCES

[1]. Adhikary BH, J Shrestha and BR Baral. 2011. Efficacy of Azotobacter in maize production in acid soils of Rampur, Chitwan. Rampur Campus, IAAS (TU). Plant Breed J.6:36-41.

[2]. Adhikary BH, SK Bajracharya, R Adhikary, KP Bhurer and SP Vista. 2015. Efficacy of Azolla pinnata in rice (Oryza sativa L.) production in the central region of Nepal. Pp.273-280. In: Proc. Of the 2nd National Soil Fertility Research Workshop, organized by Soil Science Division (SSD), NARC; CIMMYT, Nepal; International Rice Research Institute (IRRI), Nepal and Soil Management Directorate, DoA, Ministry of Agriculture, Nepal held at NARI, NARC, khumaltar, Lalitpur, Nepal on March 24-25, 2015.

[3]. Bhattarai S.1994. Use of Rhizobium inoculants: On-farm experience in Nepal in linking biological nitrogen fixation in Asia. Pp. 13-16. In: Proc. of the Asia Working Group on Biological Nitrogen Fixation in Legumes, 6-8 Dec. 1994. ICRISAT Asia Center, India.

[4]. Dineshkumar R, Kumaravel R, Gopalsamy J, Sikder M. N. A, and Sampathkumar P. (2018). Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity. *Waste Biomass Valor.* 9: 793–800. https://doi.org/10.1007/s12649-017-9873-5

[5]. Karki A. B. and J. R. Baral. 1977. Status of bio-fertilizer in Nepal- A Review. J. Inst. Agric. Anim. Sci. 1:155-169.

[6]. Maskey SL, S Bhattarai, MB Peoples, and DF Horridge. 2002. On-farm measurements of nitrogen fixation by winter and summer legumes in the Hill and Terai regions of Nepal. Field Crops Research, 70: 209-221.

[7]. Naveed M, Mehboob I, Shaker M. A, Baqir Hussain M, & Farooq, M. (2015). Biofertilizers in Pakistan: Initiatives and limitations. International Journal of Agriculture and Biology, 17(3), 411–420. https://doi.org/10.17957/IJAB/17.3.14.672

[8]. Raimi A, Adeleke R, and Roopnarain A. 2017. Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa. *Cogent Food & Agriculture*. **3**(1): 1–26. https://doi.org/10.1080/23311932.2017.1400933
[9]. NSSRC,2021. Annual Report-2020/21(2077/78). National Soil Science Research Centre, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[10]. Rana A, Joshi M, Prasanna R, Shivay Y. S, and Nain L. 2012.Biofortification of wheat through inoculation of plant growth-promoting Rhizobacteria and cyanobacteria, *European. Journal Soil Biology*. 50: 118–126.
[11]. Saraswati R, and Sumarno. 2008. Application of soil microorganisms as a component of agriculture technology. *Iptek. Tan. Pangan*, 3: 41–58
[12]. SSD, 2000. Annual Report-1998/99(2055/56). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[13]. SSD, 2001. Annual Report-1999/2000(2056/57). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[14]. SSD, 2002. Annual Report-2000/2001(2057/58). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal
[15]. SSD, 2006. Annual Report-2004/2005(2060/61). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal
[16]. SSD, 2008. Annual Report-2006/07(2062/63). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[17]. SSD, 2012 Annual Report-2010/11(2066/67). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal
[18]. SSD, 2014 Annual Report-2012/13(2068/69). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal
[19]. SSD, 2015. Annual Report-2013/14(2070/71). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[20]. SSD, 2015. Annual Report-2014/15(2071/72). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[21]. SSD, 2016. Annual Report-2015/16(2072/73). Soil Science Division, National Agricultural Research Institute (NARI), Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.
[22]. Statistical information on Nepalese agriculture time series information- HMG-Ministry Of Agriculture and co-operatives; agro-business promotion and statistics division; astrostatistics section. Singhadurbar, Kathmandu (June, 2005)
[23]. Umesha S, Singh P. K, and Singh R. P. 201). Microbial biotechnology and sustainable agriculture. In Ram Lakhan Singh, Sukanta Mondal (Eds.), *Biotechnology for sustainable agriculture*, pp. 185– 205. Woodhead Publishing. https://doi. org/10.1016/B978-0-12-812160-3.00006-4
[24]. Yami KD. 2009. Status and Prospects of Biofertilizers in Nepal Nepal Academy of Science and Technology(NAST). Proceedings of “National Seminar on Present Status and Future Prospects of Biotechnology Development in Nepal”. Technical papers/Agricultural Biotechnology Sector. Pp:17