Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 4 ~ Issue 11 (2017) pp: 01-07
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Ecological Studies on VA-Mycorrhizal Fungal Spore Density in Rhizosphere Soil of Dutch white Clover Plant Community in Darjeeling Himalaya

[1*]Dr. Debabrata Das
Associate Professor of Botany, Ecology Laboratory, Lalgarh Govt. College, Lalgarh, Jhargram-721 516
(Formerly at Microbiology Laboratory, Post Graduate Department of Botany
Darjeeling Government College, Darjeeling, West Bengal, 734 101, India)
*Corresponding Author: *Dr. Debabrata Das*

Received 10 October, 2017; **A**ccepted 27 October, 2017 © The author(s) 2017. **P**ublished with open access at **www.questjournals.org**

ABSTRACT: *Community is dynamic with aid to fluctuate the composition of population due to change of environment by different means. It may be other factors totally which is posing to revert back or progress the ecological succession or to change the dynamics in hither to locate the other attributes that can trigger the behaviour of other population in a community. Clover of white kind in Darjeeling is common which is interesting as fodder as well as soil binder that have good ecological potential and good dominance to make a thick mat for sustenance of other populations. In meadows and in sub-moist soil it is found as thick mat and used as fodder plant for cattle with good nutritive value. As the plant is legume the roots in soil part having nitrogen fixing diazotrophs like Rhizobium along with VA-mycorrhizal fungi that can support the growth of the plant in such environment. In this communication some communities of clover plant have been studies along with seasonal variation of vesicular mycorrhizal fungal spore density in rhizospheric soils of Darjeeling Himalaya for potential use of those species in near future as mycorrhizal biofertilizers. The preliminary study aims to locate the source of specific VA-mycorrhizal organisms which could be new vistas for interactions study along with Rhizobia in near future. Highest spore density was observed in soils at Gorkhey under Singalila National Park (SNP) during post monsoon and lowest spore number at Meghma due to heavy fluctuation of altitude may be in Darjeeling of Eastern Himalaya. Here, highest spore diversity was observed in case of Glomus sp. followed by Gigaspora in the study area.*

I. INTRODUCTION

The Rhizosphere, volume of soil surrounding roots and influenced chemically, physically and biologically by the plant root, is a highly favourable habitat for the proliferation of microorganisms and exerts a potential impact on plant health and soil fertility (Sorensen, 1997). Root exudates rich in amino acids, monosaccharide and organic acids, serve as the primary source of nutrients, and support the dynamic growth and activities of various microorganisms within the vicinity of the roots. These root colonizing microorganisms could be free living, parasitic or saprophytic and their diversity remains dynamic with a frequent shift in community structure, and species abundance (Kunc and Macura, 1988). Dutch white clover is found heterogeneously all over the hilly tracts of Darjeeling Himalaya. On account of the excellent forage quality and high animal feed value, white clover (*Trifolium repens* L.) is the most important forage legume and cover crop, extensive grown in the temperate and the sub-tropical regions of the world (Majumder *et al*. 2004a).

White clover is agronomically high valuable because it adds enormous amount of nitrogen to the degraded soils using nitrogen fixed by the bacteria, *Rhizobia trifolii* in the roots (Marshall *et al*. 2002). There are many natural varieties and several bred-strains of *Trifolium repens* L., which are characterized by their leaf size. These can be divided in to three major groups: the small-leaved or wild white clover, the medium-leaved or Dutch white clover and the large-leaved or Ladino white clover (Quesenberry, 2002).

Among the varieties only Dutch white clover is found to grow as thick pasture vegetation in the Darjeeling Himalayan region (Lat. 27º 2´ 57´´ N and Lon. 88 º 15´ 45´´ E) of West Bengal, India (Anonymous, 1982) at 2248.20 metre at mean sea level. The bisexual flowers are pollinated by honey bees (*Apis dorsata*) and

thus found as excellent source of honey (Anderson *et al*. 1991). Vivipary, dependent on excessive moisture within fruits like lemon, oranges, tomatoes, melons etc. is often formed. Paddy grains also germinate on the mother plant if they have sufficient moisture (Gangulee *et al*. 1999). According to some views, the Vivipary in Duch white clover is noted repeatedly and is always favoured by extensive atmospheric moisture or wet condition experienced by the plant after seed-ripening (Majumder *et al*. 2004a). Blister beetle (*Mylabris pustalata* Thunb.) and the mollusc (*Macrochlamys tusgurium* Benson) were the most common consumers of leaflets of *Trifolium repens* (Majumder et al., 2004b).

II. BOTANY OF THE PLANT

The inflorescence head bears on an average 50 small white flowers, each containing 5 ovules. The maximum number of seedlings resulting from vivipary per inflorescence head is about 120; some seeds did not get germinate. Seed dormancy period is either very short or absent. From the last week of May, the inflorescence head of *Trifolium repens* begins to wither and turns brown. However, the peduncle remains erect and stout with the advent of monsoon from the first week of June, the moisture content of the atmosphere increases (93-96%). Previous report revealed that during continuous rainfall, the inflorescence head gets wet and the seeds begin to germinate inside the inflorescence head of almost all plants. Then the whole inflorescence head appears to hold the tuft of seedlings and displayed elongated hypocotyls with a pair of green leaves and a white radical.

III. MATERIALS AND METHODS

Soil samples were collected in post monsoon during 2014-2015 from 5 selected study sites. These were air dried at Laboratory for 15 days. Separation of VAM fungal spores from each soil sample was done by using wet sieving and decanting technique method proposed by Gerdemann and Nicholson, 1963 from 100 gm of collected and dry rhizospheric soils. Soil sample was taken and mixed with 1:1 of luke warm water in a large beaker until all the aggregates dispersed to leave a uniform suspension. The suspension that passed through 450 micrometer was decanted through 425, 300, 150 and 53 micrometer sieves consecutively. The residues in the respective sieves were collected in Petri dishes with about 10-20 ml water observed under a stereomicroscope for counting AM fungal spores. Spore density was calculated by counting the spores available in the 100 gm of rhizosperic soil sample. Then the spores were separated using wooden peg and segregated. The spores were mounted on clear glass slides using lacto phenol and or polyvinyl alcohol lacto phenol (PVL), covered with cover slips and sealed with DPX medium.

IV. IDENTIFICATION OF VAMF SPORES

Based on microscopic characters, spore morphology and available literature, INVAM site available in internet, some genera of the AM fungal spores were identified. For specific study in connection with identification, nomenclature, keys of Raman and Mohankumar, 1988; Schenk and Perez, 1990 were followed. The work based on colour, shape and size, hyphal attachment, structure and general nature of the spore contents. Soil physico-chemical properties like pH, moisture content, soil organic carbon as well as soil organic matter (SOM) were determined from collected rhizosperic soil samples in Microbiology Laboratory and in Plant Physiology Laboratory of Botany Department (PG), Darjeeling Govt. College, West Bengal, India.

V. RESULTS AND DISCUSSION

White clover, *Trifolium repens*, forms an important component of temperate pasture with a twofold benefit of providing high quality forage, and fixing atmospheric nitrogen in symbiosis with the bacterium *Rhizobium trifolii* Dangered, which promotes the growth of the grass in the sward (Meredith, 1995). It includes so many mycorrhizal species of fungi which give armature benefit to growth and potential development of the plant. As per the reference, VAMF are essential as they have the potency to work as biofertilizers (Ghosh and Verma, 2017). Va-mycorhizal fungi helps Clover plant in field even during germination of seedlings and onset of seedlings in a barren land too they help to accumulate nutrients. There are no such reports of extensive work on such foliage herb that have great ecological significance. Field investigations on mycorrhizal inoculation have been confined to soybean, groundnut and sunflower. In an alfisol soil, low in phosphorus, VAM inoculation with the VAM fungus *Glomus fasciculatum* and *Rhizobium* resulted in 20% increase in grain yield (Bagyaraj *et al*. 1979). Inoculation of VAM and *Rhizobium* was achieved by preparing alginate based granules which can be easily placed along with the seeds, both mechanically and manually. But for the same need local strains which is having good ecological potential. Therefore, spore density study and spore identification is required to fulfil the goal. In India pasture land, crops have been used traditionally to get the yield of quadrupeds, so to improve the yield of similar legumes inoculation is essential. The current investigation has revealed spore density of VAM fungi in rhizosphere soil. It varies from 111 to 212 with a variation of altitude in different places of Eastern Himalaya (Table 1). Overall, average number of VAMF spore per 100g soil was

recorded in October 144 (143.8) in Darjeeling Himalaya which may be a potential source to study colonization and VAM spore correlation in near future to develop VAM inocula for mycorrhizal biofertlizers. The present trial is a base line study to forfeit the yield in near future not only to improve the yield but also to develop the exact strain for mycorrhizal biofertilizer production from locally available resistant species. Hope that all the VAM-inoculated plants have shown increased plant growth, dry matter production, NPK and sulphur levels, micro-nutrients, seed production and protein content over non-mycorrhizal plants. This depends upon the spore density and distribution pattern in high altitude line of Darjeeling Himalaya with variations of altitude and other climatic factors.

Table 1. Vesicular Arbuscular Mycorrhizal (VAM) spore density in Rhizosphere soil of five study sites at Eastern Himalaya (Darjeeling)

Sl. No.	Name of the Study sites	Spore Density per 100g Rhizosphere soil
1	Lamaidhura	148
2	Meghma	111
3	Chitrey	127
4	Tonglu Gorkhey	121
5	Gorkhey	212
	Average spore density in October	143.8

Note: All are made during favourable season *i.e* after monsoon when weather was sunny

VI. CONCLUSION

Among the five study sites Gorkhey of Singalila National Park, Darjeling showed highest spore density in rhizosphere soil while lowest in soil of Meghma area in which less or least population of white clover have been found. The variation of such type may be due to alterations of variations from site to sites. These need further study to make a comprehensive status of spore density on white clover (Fig. 1). Among the spore studied maximum spore was under the genus **Glomus** (Fig.12, 13) followed by **Gigaspora** (Fig.11). The soil composition showed varied degree of spore numbers as it perhaps due to the high variation of soil organic matter (SOM) which ranging between 0.98 to 1.74 in the entire study site.

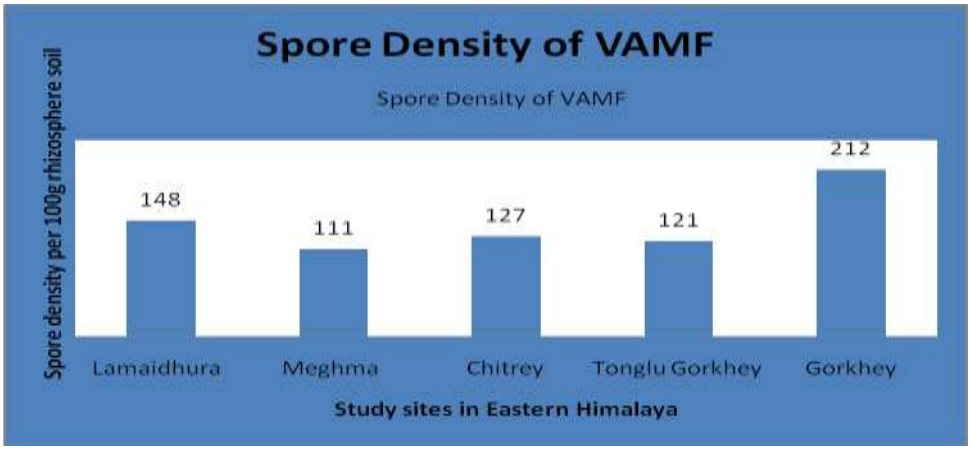

Fig. 1 Status of VAMF spore density in 5 study sites during Post monsoon in E. Himalaya (Darjeeling)

More study is essential to establish a final conclusion on VAM study in Eastern Himalaya. Colonization and soil property study throughout the season is essential to establish a standard conclusion on VAM research in India which is no need scientific programme round the year using different populations and co-populations too along with other Lupin group (Fig. 6). Hope that researchers could be available to do the work in near future.

VII. ACKNOWLEDGEMENTS

The author is thankful to Dr. Biswajit De, Head of Cytology Laboratory to get information of previous kind on Dutch white clover. Dr. Prajjwal Chandra Lama, Officer-in-Charge of Darjeeling Govt. College is well acknowledged for his co-operation and permission to visit field at Lamaidhura, Meghma, Chitrey, Tonglu, Gorkhey etc. in Darjeeling Himalaya. For accompanying in field visit, Dr. Biswajit Maiti, Head, Physics Department, Govt. General Degree College, Kharagpur-II (Formerly Darjeeling) is well acknowledged. I convey my sincere thanks to my University Professor, Prof. Dr. N. K. Verma (Retired), Dept. of Botany & Forestry, Vidyasagar University, Midnapore for his kind suggestion and help as and when required during my

Darjeeling tenure. Smt. Pampi Ghosh, Assistant Professor & Head, Department of Botany, Seva Bharati Mahavidyalaya, Kapgari, Jhargram (Formerly Paschim Medinipur) is well acknowledged for valuable suggestions and species of VAM spores identification. Dy. Librarian Vidyasagar University, Midnapore, West Bengal and Librarian, Darjeeling Govt. College, Darjeeling is acknowledged for use of literature. In-charge, Tea Science Department, North Bengal University, Siliguri is well acknowledged for physical and chemical properties of soil analysis. Divisional Forest Officer (DFO), Silviculture (Hills), Darjeeling, is well acknowledged for the permission to work at Singalila National Park, Gorkhey for MPCA research in addition to my interest work on VAM study from Post Graduate Department of Botany, Darjeeling Govt. College, Darjeeling.

PHOTO PLATES

Fig. 2 Author capturing landscape with *Trifolium repens* at Chitrey

Fig. 3 Group leader watching plant population at Chitray during Tonglu trip

Fig. 4 Trifolium repens-White clover at Meghma

Fig. 5 Grazing animals at Duch white Clover field

Fig. 6 *Lupinus* sp. an important Legume plant available in Hills

Fig 7 Dr. Biswajit Maiti studying in field during Tumling Journey

Fig. 8 Author showing use of Biofertilizers (Hill people are much reluctant about biofertilizer use)

Fig. 9 Gorkhey area during Post monsoon in SNP area

Fig. 10 Research group along with author and forest official at Singalila National Park (SNP) area

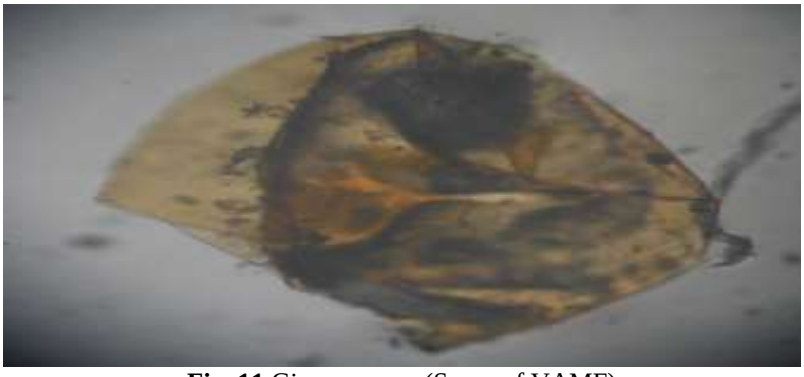

Fig. 11 Gigaspora sp. (Spore of VAMF)

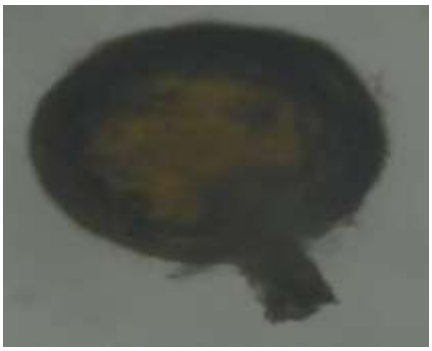

Fig. 12 Glomus sp.,

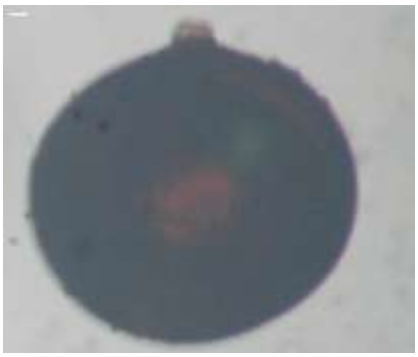

Fig. 13 Glomus sp. (Spore of VAMF)

REFERENCES

[1]. Majumder, S; Banerjee, S and De, K. K. 2004a. Vivipary in white clover (*Trifolium repens* L.), Current Science, 86(1): 29-30

[2]. Majumdar, S; De, K K and Banerjee, S. 2004b. Influence of two selective factors on Cytogenesis polymorphism of *Trifolium repens* L. in Darjeeling Himalaya, Journal of Plant Biology, 47(2): 124-128

[3]. Anonymous. 1982. Wealth of India (ed. Chadha, Y.R). Publication of Information Directorate, CSIR, New Delhi, 1982, Vol. X; p. 296.

[4]. Quesenberry, K H. 2002. Proceedings of the 57th Southern pasture and forage Crop improvement conference, Athens, GA, 23-25 April

[5]. Marshall, A H; Michaelson-Yeates, TPT; Abberton, MT; Williams, A and Powel, HG. 2002. Variations for reproductive and agronomic traits among *T. repens* x *T. nigrescens* third generation backcross hybrid in the field, Euphytica, 126: 195-201.

[6]. Anderson, J A; Taylor, N.L. and Williams, E G. 1991. Cytology and Fertility of the inter-specific hybrids *T. ambiguum* and *T. repens* and backcross populations, Crop Science., **31**: 683-687.

[7]. Gangulee, H. C; Das, K. S and Dutta, C. 1999. College Botany, New Central Book Agency Private Ltd., Kolkata, Vol.-I, pp.45.

[8]. Chhettri, S and Das, D. 2015. Standardization of pre treatment techniques for the study of Arbuscular mycorrhizal fungal association with an important medicinal plant of Darjeeling, North Face: Academic Journal of Darjeeling Government College, **1**(1): 98-103.

[9]. Sorensen, J. 1977. The Rhizosphere as a habitat for soil microorganisms, In J. D. Van Elsas, J. T. Trevors and E M H Welington (Eds.), Modern Soil Ecology (pp 21-46), New York: Marcel Dekker. Inc.

[10]. Kunc, F and Macura, J. 1988. Mechanism of adaptation and selection of microorganisms in the soil, In V. Vencura & F Kunc (Eds.), Soil Microbial Associations (pp. 281-299), Elsevier, Amsterdam.

[11]. Bagyaraj, D.J; Manjunath, A and Patil, R.B. 1979. Interactions between Vesicular Arbuscular Mycorrhiza and *Rhizobium* and their effects on soybean in field. New Phytol, **82**: 141-145.

[12]. Meredith, M. R., Michaelson-Yeates, T.P.P; Ougham, H.J and Thomas, H.J. 1995. *Trifolium ambiguum* as a source of variation in the breeding of white clover, Euphytica, **82**: 185-191.

[13]. Ghosh, P and Verma, N. K. 2017. Arbuscular Mycorrhizal Fungal (AMF) inocula as Potent Bio-fertilizers, IJSART, **3**(9):660-665.

[14]. Gerdemann J. W. And Nicolson T. H. 1963. Spores of mycorrhizal Endogone Species extracted from soil by wet sieving and decanting, Trans. Br. Mycol. Soc., **46**:235-244.

[15]. Schenck, N. C. and Perez, Y. 1990. Manual for the identification of VA mycorrhizal fungi. INVAM, University of Florida, Gainesville, Fk, pp: 245.

[16]. Raman N. and Mohankumar, V. 1988. Techniques in mycorrhizal research. University of Madras, Madras. Pp: 279.

*Dr. Debabrata Das. "Ecological Studies on VA-Mycorrhizal Fungal Spore Density in Rhizosphere Soil of Dutch white Clover Plant Community in Darjeeling Himalaya." Quest Journals, Journal of Research in Agriculture and Animal Science, vol. 03, no. 06, 2017, pp. 12–18.