ORIGINAL RESEARCH PAPER

Volume-8 | Issue-8 | August - 2019 | PRINT ISSN No. 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSESSMENT OF NITRATE REDUCTASE ACTIVITY IN A FORMULATED ORGANIC SOIL MEDIA; A BIOMATERIAL (JAIV-SAMVARDHAN) AND ANALYSIS BY 'TWO-WAY ANOVA'

Botany

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ABSTRACT

Nitrogen (N) is one of the most abundant elements in plants and constitutes 0.5–5% of the plant dry weight (8-10% in the case of microalgae). (Lindblad P., Guerrero M.G.). The molecular atmospheric nitrogen is assimilated by Biological and Abiological process. Nitrogen available in the soil is regulated by bacterial association with plants initiating the availability of Nitrogen from the surroundings. Nitrate supports the activity for fixation of N_2 by converting it to Nitrite and at the end to ammonia. For this action the reductase enzyme is required and hence the research attempt is carried out to study "Nitrate reductase activity" in Formulated Organic Soil media: A biomaterial named as Jaiv-Samvardhan, using an enzyme source of 'Bryophyllum leaf'.

The research successfully concludes the competence of Formulated organic biomaterial against an enzyme source of bryophyllum leaf used as a standard. The statistical analysis by a 'Two-way ANOVA' specifies Nitrate reductase activity to be interned Formulated Organic Biomaterial required for a development of plant.

KEYWORDS

Nitrate reductase Activity(NRA), N_2 cycle, Two-way ANOVA, Bryophyllum leaf, Organic biomaterial.

INTRODUCTION

The atmosphere contains almost 80% nitrogen in the form of dinitrogen gas (N_2). (Lindblad P., Guerrero M.G.). Only certain prokaryotic organisms are able to utilise this N_2 by a process called biological nitrogen fixation - amounting to about 10^8 tonnes of N_2-N per year. (Lindblad P., Guerrero M.G.). For most plants in their natural environment, nitrate is the primary source of nitrogen. (Lindblad P., Guerrero M.G.). The assimilation of N involves the nitrate reductase (NR) enzyme, and its activity seems to be dependent on N supply. (Jairo Osvaldo Cazetta, Luciana Cristine, Vasques Villela). Molybdenum (Mo) is also important because it is a cofactor of NR. (Jairo Osvaldo Cazetta, Luciana Cristine, Vasques Villela).

Atmospheric nitrogen is converted into ammonia (NH_3) with the help of "Abiological" and "Biological" process called as Nitrogen fixation (N_2-fixation). During lightning storms, a molecular Nitrogen present in an atmosphere combine with oxygen to form an oxides of nitrate. The reaction given below is as follow;

$N_2 + O_2 \rightarrow 2NO$

$2NO + O_2 \rightarrow 2NO_2$

However, Biological N_2 fixation happens in a presence of living cells with the help of Nitrogenases enzymes. The molecular N_2 converted into ammonia is processed for further metabolic activity. In a phase of Biological N_2 fixation the nitrate compound is of greater importance; since, it initiates activity of fixing molecular nitrogen. A compound NO_3^- (Nitrate) converted to NO_2^- (Nitrite) is catalysed by an enzyme 'Nitrate reductase'. This enzyme contains constituent such as FAD, Cytochrome, NADPH/NADH and Molybdenum. Later, NO_2^- converted to ammonia with the help of an enzyme "Nitrite reductase". The whole process involves acceptance of electrons from sources like NADH, NADPH and $FADH_2$.

Different pathways of plant metabolism lead to an increase in NR activity or decrease in NR activity. A factor such as light also affects the NR activity. However, some research papers contain experimentations on utilisation of pathways for a study of NR activity. Nitrate reductase activity develops to a maximum in a Crassulacean acid metabolism (CAM) plant canopy before either ribulose 1,5-bisphosphate carboxylase, or phosphoenolpyruvate carboxylase, or CAM. (Nam Kee Chang, H. Max Vines, Clanton C. Black). Hence, one may use the leaf of plant following CAM pathway as an enzyme source for nitrate assimilation. The genus Bryophyllum; a succulent species, sometimes been included within a genus Kalanchoe in Nepal may be used as an enzyme source for studying Nitrate assimilation.

The current attempts are made to study 'Nitrate reductase' activity in a Formulated enriched soil media: An Organic sustainable biodegradable material; namely **"Jaiv- Samvardhan"**. It's Richness to sustain the soil with zero harmness and pure natural growth for Organic Farming aspiration suits best to name it as "Jaiv-Samvardhan". (Dr. Urmila Sarkar, Sensei Surendra Sawardekar). Jaiv-Samvardhan is found to be wealthier for an *organic matter: Carbon* as stated by an author Dr. Urmila Sarkar and Sensei Surendra Sawardekar.

An effort to study Nitrate reductase activity in a formulated soil media successfully concludes stable and increased NR activity against an enzyme source used during study along with a soil sample. The experimentation specifies microbial action; initiating a support for biological N_2 fixation.

MATERIAL AND METHOD

Six samples of two parameters each considered for an experiment is as given below;

1.1 Untreated soil sample without Bryophyllum leaf disc.
1.2 Untreated soil sample with Bryophyllum leaf disc.
1.3 Treated soil sample without Bryophyllum leaf disc.
1.4 Treated soil sample with Bryophyllum leaf disc.
1.5 Formulated biomaterial sample without Bryophyllum leaf disc.
1.6 Formulated biomaterial with Bryophyllum leaf disc.

The formulated dry mixture i.e. Soil: Organic mixture added in a proportion of 3:2. (Dr. Urmila Sarkar, Sensei Surendra Sawardekar). The treated soil sample was utilised as per above mentioned statement viz. 1gm soil: 0.75gm formulated biomaterial soaked overnight in 10 ml of distilled water was filtered and 1gm of solution was used as a sample. While the one without any addition of formulated biomaterial was considered to be an untreated soil sample. Each sample with 0.5mm diameter of 5 leaf discs weighing 50 mg of '*Bryophyllum pinnatum'* was used as enzyme source.

2ml of phosphate buffer of pH=7 with 0.1ml of 0.1MKNO_3 was added to all filtered samples (treated and untreated with and without leaf discs). The incubation of all samples were kept at 28^0C for 30 minutes. The solution was transferred by separating leaf discs with further addition of 1ml of 1% Sulphanilamide in 8% HCl. 1ml of 0.02% of NEDD [N-(1-napthyl) ethylenediamine-dihydrochloride] was added to all the sample solutions. Upon addition of NEDD the diazotization and formation of azo dye was colorimetrically measure at 540nm. A triplet of each sample was used during experimentation.

RESULTS AND DISCUSSION

The readings analysed by a spectrophotometer for a study of NR activity is shown in ***Table 1.1***; while its graphical representation is expressed in ***Figure 2.1***.

The research statistical analysis **"Two-way ANOVA"** utilized for **0.05** level of significance is given in ***Table 1.2*** and **0.01** level of significance presented in ***Table 1.3***.

Table1.1

	Untreated soil sample	Treated Soil sample	Formulated biomaterial
Without (enzyme source) leaf disc	0.12	0.17	0.13
With (enzyme source) leaf disc	0.13	0.14	0.13

Table 1.2

Two-way ANOVA (0.05 level of significance)					
Source of Variation	*SS*	*df*	*MS*	*F* ***	*P-value*
Rows**	0.000267	1	0.000267	2.285714	0.269703
Columns**	0.001033	2	0.000517	4.428571	0.184211
Error	0.000233	2	0.000117		
Total	0.001533	5			

Table1.3

Two-way ANOVA (0.01 level of significance)					
Source of Variation	*SS*	*df*	*MS*	*F* ***	*P-value*
Rows**	0.000267	1	0.000267	2.285714	0.269703
Columns**	0.001033	2	0.000517	4.428571	0.184211
Error	0.000233	2	0.000117		
Total	0.001533	5			

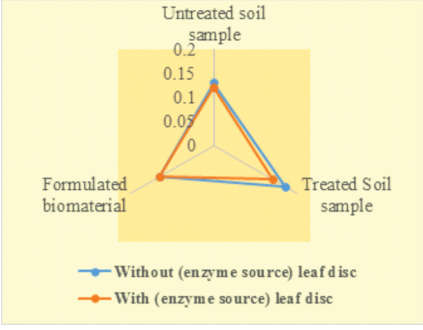

Figure 2.1

CONCLUSION

The research study by a 'Two-way ANOVA' reveals that at 0.05 and 0.01 level of significance the effect of formulated Organic Biomaterial has subsequent effect of increase in Nitrate reductase activity against an enzyme source of Bryophyllum leaf used as an initiator in different samples. Hence, Alternate hypothesis $\mathbf{H_1}$ is accepted as $P < 0.05$ and $P < 0.01$; while Null hypothesis $\mathbf{H_0}$ viz. that there is no effect of Formulated Organic Biomaterial in increase of Nitrate reductase activity is rejected.

The ***F-calculated value at 0.05 and 0.01 level of significance within the samples and In-between the samples of rows and columns such as **Rows and **Columns are found to be significant. Therefore, an experimental study holds a firm ability of 'Formulated Organic Soil Media; A Biomaterial' supporting Nitrate reductase activity for conversion of Nitrate into Nitrite during N_2metabolism.

REFERENCES

1. Dr. Urmila Sarkar, Sensei Surendra Sawardekar. (2019). Formulation of Organically Enriched Soil Media and Analysis of Organic matter (Carbon): A Biomaterial for Sustainable Agricultural Practices. Int. Journal of Science and Research. ISSN:2319-7064. 8(2):1564-1566.
2. Jairo Osvaldo Cazetta, Luciana Cristine Vasques Villela. (2004). NITRATE REDUCTASE ACTIVITY IN LEAVES AND STEMS OF TANNER GRASS (Brachiaria radicans Napper). Sci. Agric. (Piracicaba, Braz.). 61(6):640-648.
3. Lindblad P., Guerrero M.G. (1993) Nitrogen fixation and nitrate reduction. In: Hall D.O., Scurlock J.M.O., Bolhàr-Nordenkampf H.R., Leegood R.C., Long S.P. (eds) Photosynthesis and Production in a Changing Environment. Springer, Dordrecht.
4. Nam Kee Chang, H. Max Vines, Clanton C. Black. (1981). Nitrate Assimilation and Crassulacean Acid Metabolism in Leaves of Kalanchoë fedtschenkoi Variety Marginata. American Society of Plant Biologist (ASPB). Plant physiology. 68: 464-468.