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Effect of Adsorbed *Trichoderma harzianum* (G -18) and Different Doses of Lime on Biological Nitrogen Fixation Activity in Black Zone Soil of Republic of Tataristan (Russia)

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Abstract

The basic requirement for sustainable management of soil is to confirm that soil fertility is maintained in a productive state and condition so as to enable the soil to continue to provide viable economic yields with minimum degradation of soil quality and quantity. The biological nitrogen fixation activity of black zone soil of republic of Tataristan (Russia) was studied after inoculation of adsorbed *Trichoderma harzianum* (G-18) on different adsorbents like barely husk, saw dust, chick compost and different doses of lime. The total incubation period was ninety days. Results obtained showed that the biological nitrogen fixation is varied, depending upon the dose of lime, type of adsorbent and incubation time. The chick manure adsorbed *Trichoderma harzianum* showed the maximum nitrogen fixation 9.2 mg /kg/hr. at 5tons/hact.in 30 days ,the saw-wood adsorbed *Trichoderma harzianum* 5.6mg/kg/hr at 2 tons/hact, in 60 days and the barley husk adsorbed *Trichoderma harzianum*, 8mg/kg/hr at 2 tons/hact. of lime in 60 days incubation.

Keywords: Nitrogen fixation , Fungi *Trichoderma harzianum*, Black zone soil .

Introduction

The soil management system depends upon many factors such as soils, climate and accepted cultural practices. As in New Zealand, traditionally, apple orchards were grown under clean cultivation but this practice has recently been largely suppressed by grassing down as an under story vegetation of orchards in their early life, often with the use of a narrow herbicide strip of variable width along the row (Haynes, 1980). The importance of legumes as a source of nitrogen has been recognized (Goh and Malakouti 1992). The genus *Trichoderma harzianum* belongs to the filamentous Deuteromycetes. Generally it is present in all soils even in forest humus layer (Wardle, *et al.*, 1993). It is also present in agricultural and orchard soils (Chet, 1987, Roiger, *et al.*, 1991). Yet it has not been found as endophytes of living plants (Petrim 1986) *Trichoderma harzianum* is famous for biocontrol (Elad, *et al.*, 1995).

Biological fixation of atmospheric N_2 can be performed only by specific group of microorganism. All biological N_2 fixation depend upon the enzyme nitrogenase, which occurs in main types of microorganism such as Azotobacter, Clostridium, Rhizobium, which are different in the potential amount of N_2 that they can fix N_2 in temperate regions, Measurement of N_2 fixation (nitrogenase activity), can be observed by using acetyl reduction assay. Nitrogenase convert acetylene to ethylene, which may easily be measured by gas chromatograph (Giller *et al.*, 1995).

The quantification of biological N_2 fixation provides the opportunity to manipulate and apply agronomic practices for maximizing biologically N_2 fixation. The aim and object of our study was to measure the amount of biologically fixed N_2 with inoculation of absorbed *Trichoderma harzianum* with lime amendment in different doses in the black zone soil of

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Tataristan, because in last two decades the agricultural land of Tataristan did not provide the required production due to the development of acidity. *Trichoderma harzianum* possesses an oxidative system that is capable of degrading the organochlorine pesticide, Endosulfan Katayama and Matsumura, 1993; (Nigam, 1994). *Trichoderma harzianum* is disease suppressive, have greener turf, probably by enhanced root growth and reduced brown patch (*Rhizoctonia solani*), dollar spot (*Sclerotinia homeocarpa*), and Pythium blight (Lo *et al*, 1995).

Material and Methods

Culture: The culture of *Trichoderma harzianum* (G-18) was taken from the soil of Kazan's Republic of Tataristan (Russia) agriculture Farm.

Adsorbents

(Used as required media).

1. Barely Husk-From Kazan's Agriculture Farm.
2. Chick Compost - From Kazan's Chick Farm
3. Saw dust - From wood house of Kazan.
4. Lime ($CaCO_3$)

Inoculum

The above adsorbents were taken respectively in 3 liter round bottom flask and moistened with sufficient amount of water, sterilized at 15lb for 20 minutes. After sterilization the adsorbents were inoculated with suspension of *Trichoderma harzianum* (G-18) containing spores (10^9/gm) so the bluk of inoculum prepared, was transfered to lime contained soil. The dose of inoclulum 200gm/kg of soil was mixed with sterilized water up to 80% WV. The inoculated soil was incubated at $28C^0$ for ninety days. Analysis of the samples for Nitrogen were performed after every thirty days.

Methods

Nitrogen fixation was measured by the acetylene reduction assay (Marfenina, 1991). In this method 5g of each soil sample is mixed separately with 2% glucose (W/W) in a 10ml vial and moistened with approximately 8% of sterilized water. The vials were closed with cotton plugs and the contents were incubated at 28^0C for 24hrs. The cotton plugs were replaced by rubber cork and the vials were muffled, 0.5cc acetylene was then injected into vial and the vials were again incubated for 1hr.at same temperature. After incubation, 0.5cc of the gas sample from vials was injected into gas chromotogragh. The volume of ethylene produced was calculated from the peaks recorded, which is directly proportional to the volume/amount of nitrogen.

The control run was also performed. The potential activity of nitrogen fixation is expressed in mg which is fixasive nitrogen in 01kg of soil/hr. (mg. /1kg /1 hrs.)

Calculation of nitrogen fixation activity calculated from the out come of chromatographic analysis.

The general formula of nitrogen fixation of soil micro organisms is as under:

$Pa=2 \times 10^{-4} S \times (Vf - 0.7 Vp) \times (P-17.5)$ /STmg/Kg/hr.

VQ = Volume of bottle cm^3

VP = Volume of soil cm^3

0.7 = coefficient

St = Standard

S = sample

Results

Chick manure adsorbed *Trichoderma harzianum* and Lime

The results of chick manure adsorbed *Trichoderma harzianum* (G-18) with different doses of lime, N_2 production are shown in table - 1 and fig.1. The maximum production of N_2 was 9.2mg/kg/ hr at 5 tons/hact. of lime.The incubation period was 30 days.

Whereas on 60 days incubation, the maximum N_2 production was 3 mg /kg/hr at 4 tons / hact .of lime. and on 90 days incubation, maximum N_2 production was 0.7 mg/kg/hr.

Saw – dust adsorbed *Trichoderma harzianum* and lime result.

The results of saw-dust adsorbed *Trichoderma harzianum* (G-18) and different doses of lime. The N_2 production is shown in table – 2 and fig. 2. The maximum production of N_2 was 5.6.mg/kg/hr, at the 2 tons/hact. of lime, on 60 days incubation period but on 30 days incubation at 5 tons/hact. of lime, produced 5.2 mg/kg/hr of nitrogen and on 90 days incubation the maximum quantity of N_2 was 4 .7 mg/kg/hr. at 5 tons/hact. of lime.

Barley husk adsorbed *Trichoderma harzianum* and lime.

The result of barley husk adsorbed *Trichoderma harzianum* (G-18) with different doses of lime are shown in table - 3 and fig3. The maximum production of N_2 was 8 mg/kg/hr, on 60 days incubation period at 2 tons/hact. of lime. In others incubations at 30 days and 90 days, the maximum production of N_2 was 4mg/kg/hr at 5 tons of lime and 4.5 mg/kg/hr/ respectively.

Discussion

As biological nitrogen fixation provides not only a relatively cheap but also a renewable source of N to fruit trees, this source of N is sustainable means for maintaining soil fertility (Goh *et al.*,2004).

Results obtained in the present study showed that significant amount (9.2mg/kg/hr) of N_2 were fixed by chick manure adsorbed *Trichoderma harzianum* at 5 Tons/hact. of Lime, after 30 days incubation time. The second highest N_2 fixing quantity 8mg/1kg/hr was by barley adsorbed *Trichoderma harzianum* at 2 tons/hact. of lime containing soil after 60 days incubation time.

The chick manure absorbed *Trichoderma harzianum* has more nitrogenous substances which favor more N_2 fixation. Barley absorbed *Trichoderma harzianum* also showed the higher quantity of N_2 but incubation time is less, because the medium was more favorable for the growth of *Triochoderma harzianum*. How ever, the chick manure absorbed *Trichoderrma harzianum* at the 5 tons/hact. of lime, is more applicable for N_2 fixation.

It is clear that nitrogen fixation activity with association of fungi is many times more, from that *Trichoderrma harzianum* increase the nitrogen fixation activity three times in comparing to bacterial culture (Merchink, 1988).

Table 1: Showing nitrogen fixation in black zone soil introduced with chick manure adsorbed *Trichoderma harzianum* and lime

Dose of Lime	Incubation period	Volume of N_2 evaluated	ıncubation period	Volume of N_2 evaluated	Incubation period	Amount of N_2 evaluated
Control	30Days	0.5mg/kg/hr	60 Days	0.4 mg/ kg/hr	90 Days	1.2 mg/ kg/hr
0	30Days	2.5mg/kg/hr	60 Days	1.8 mg/ kg/hr	90 Days	0.1 mg/ kg/hr
2Tons	30Days	1mg/kg/hr	60 Days	3 mg/ kg/hr	90 Days	0.2 mg/ kg/hr
3Tons	30Days	1.5mg/kg/hr	60 Days	1.9 mg/ kg/hr	90 Days	0.7 mg/ kg/hr
4Tons	30Days	0.5mg/kg/hr	60 Days	1.9 mg/ kg/hr	90 Days	0.0 mg/ kg/hr
5Tons	30Days	9.5mg/kg/hr	60 Days	0.1 mg/ kg/hr	90 Days	0.1 mg/ kg/hr

Table 2: Showing nitrogen fixation in black zone soil introduced with saw-dust adsorbed *Trichoderma harzianum* and lime

Dose of Lime	Incubation period	Volume of N_2 evaluated	Incubation period	Volume of N_2 valuated	Incubation period	Amount of N_2 evaluated
Control	30 Days	3.8 mg/ kg/hr	60 Days	1m/ kg/hr	90 Days	4.9m/ kg/hr
O	30 Days	1.2 mg/ kg/hr	60 Days	3 m/ kg/hr	90 Days	1 m/ kg/hr
2 Tons	30 Days	0.5 mg/ kg/hr	60 Days	5.6 mg/ kg/hr	90 Days	3.8 mg/ kg/hr
3 Tons	30 Days	0.3 mg/ kg/hr	60 Days	3.8mg/ kg/hr	90 Days	4.1mg/ kg/hr
4 Tons	30 Days	0.2 mg/ kg/hr	60 Days	3 mg/ kg/hr	90 Days	2.5 mg/ kg/hr
5 Tons	30 Days	5.2 mg/ kg/hr	60 Days	1.2 mg/ kg/hr	90 Days	4.7 mg/ kg/hr

Table 3: Showing nitrogen fixation in black zone soil introduced with barley adsorbed *Trichoderma harzianum* and lime

Dose of lime	Incubation period	Volume of N_2 evaluated	Incubation period	Volume of N_2 evaluated	Incubation period	Amount of N_2 evaluated
Control	30 Days	0.0 mg/ kg/hr	60 Days	0.0mg/ kg/hr	90 Days	9.5m/ kg/hr
O	30 Days	0.1 mg/ kg/hr	60 Days	5.5 mg/ kg/hr	90 Days	4.5 mg/ kg/hr
2 Tons	30 Days	0.2 mg/ kg/hr	60 Days	8 mg/ kg/hr	90 Days	4.1mg/ kg/hr
3 Tons	30 Days	0.4 mg/ kg/hr	60 Days	6.9mg/ kg/hr	90 Days	4mg/ kg/hr
4 Tons	30 Days	0.1 mg/ kg/hr	60 Days	8.5 mg/ kg/hr	90 Days	3.1 mg/ kg/hr
5 Tons	30 Days	4 mg/ kg/hr	60 Days	0.0mg/ kg/hr	90 Days	0.0mg/ kg/hr

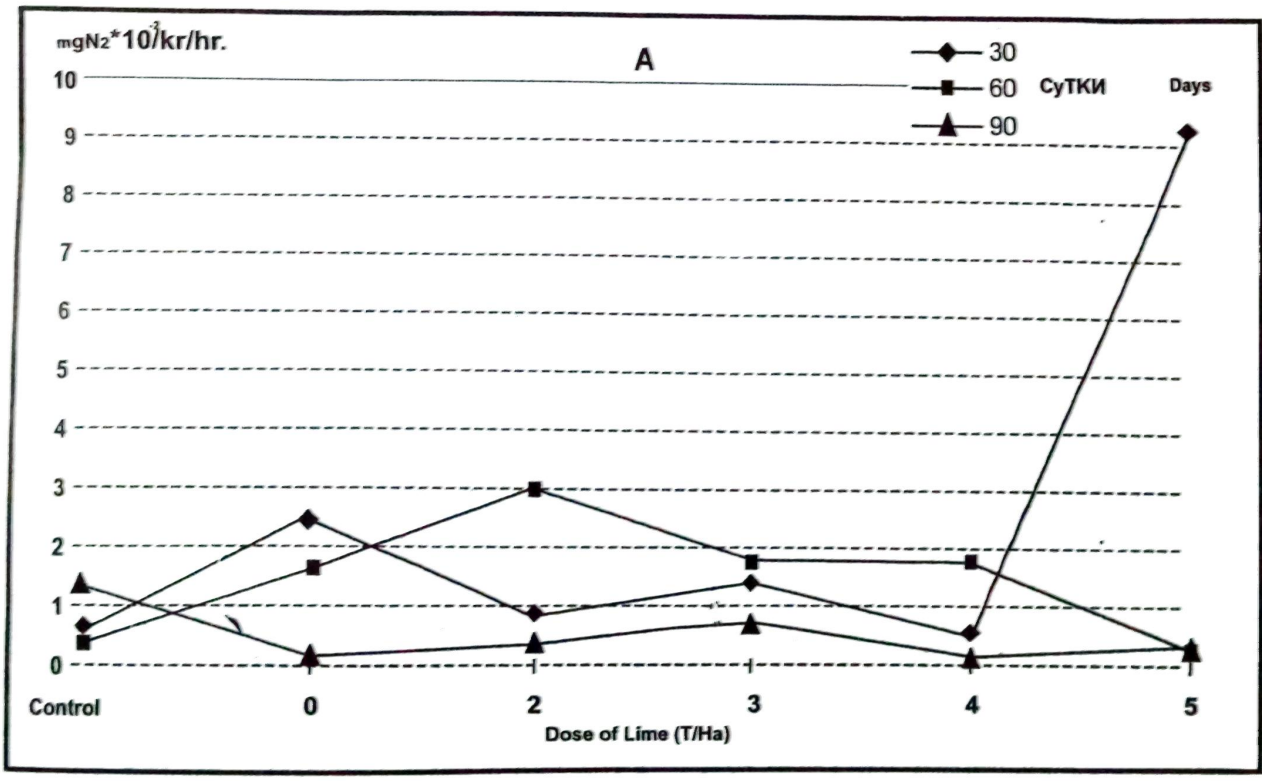

Fig. 1. Influence of lime and chick manure adsorbed *Tricherma harrzianum* -A on potential nitrogen fixation activity.

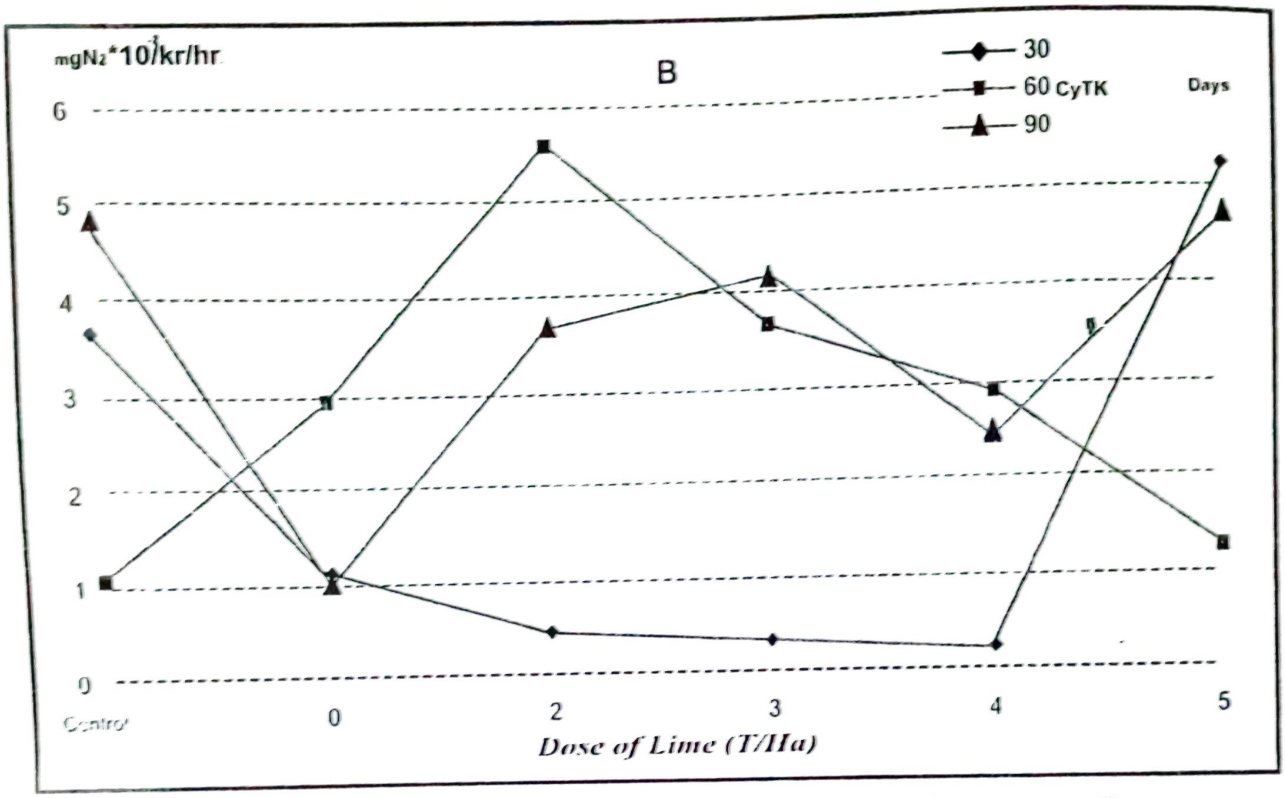

Fig. 2. Influence of lime and saw-dust- adsorbed *Tricherma harrzianum* - B on potential nitrogen fixation activity.

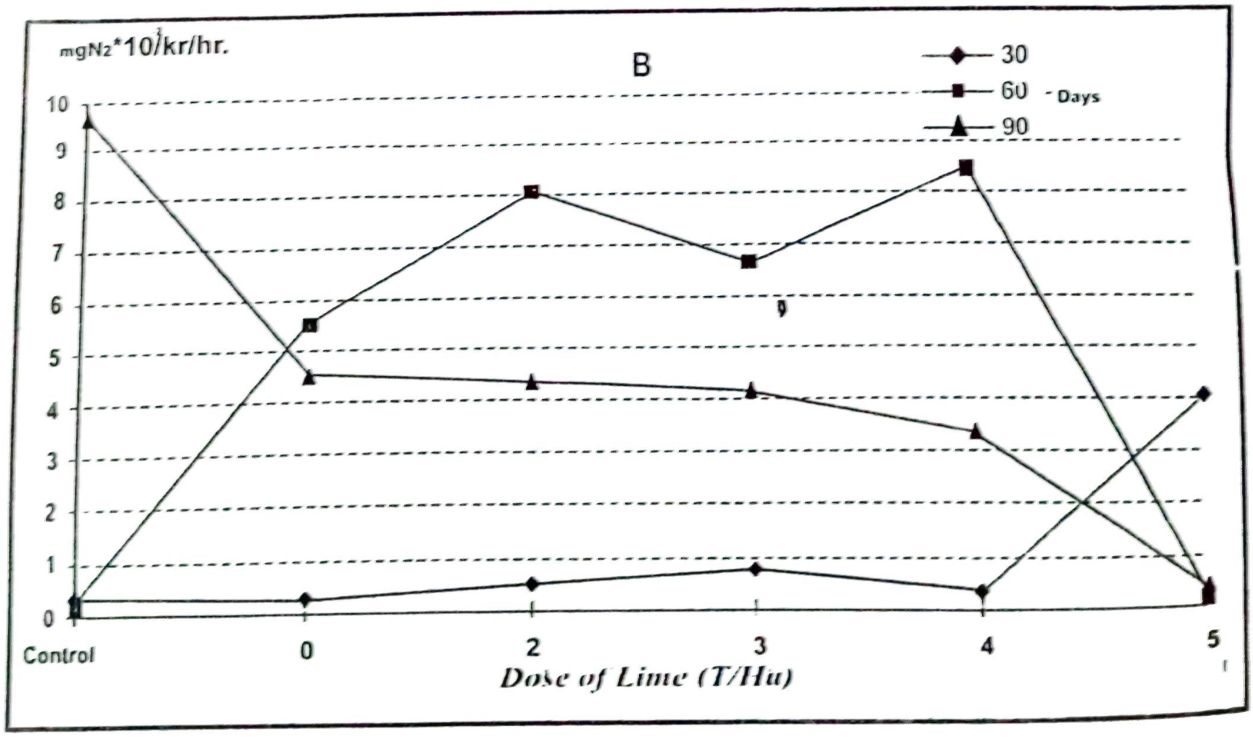

Fig. 3. Influence of lime and barley adsorbed *Tricherma harrzianum*-B on potential nitrogen fixation activity.

References

Chet.I. (1987) Trichoderma–application, mode of action, and potential as a biocontrol agent of soil born plant pathogenic fungi. In innovative approach as to plant disease control (ed.I. chet) PP. 137-160, John Wiley and Sons: New York.

Elad V. O' T. Neill, A. Cohen. and O. Sahllenberg. (1995) Factors in flouncing control of gray mould by means of Trichodex (*Trichoderma harzianum* T 39) under field conditions. Fifth International Trichoderma and Gliocladium workshop, Beltsville, April 1995, abstract.

Giller, K.E. and G. Cadisch (1995) Future benefits from biological nitrogen fixation: An ecological approach to agriculture. Plant Soil **174:** 255-277

Goh, K. M. and M. J. Malakouti. (1992) Preliminary nitrogen, phosphorus, potassium, calcium and magnesium DRIS norms and indices for apple orchards in Canterbury, New Zealand communication in soil science and plant analysis **23:** 1371-1385.

Goh . K.M., G.E. Ridgen and M .J.Daly (2004) Biological Fixation and biomass production in the under story vegetation of an organic apple orchard in Canterbury, New Zealand. Hort Research publication P.1-8.

Haynes, R. J. (1980) Influence of soil management practice on the orchard agri-ecosystem, Agro-Ecosystem **6**: 3-32.

Katayama, A. and F. Matsumera (1993) Degradation of organochlorine pesticide, particularly endosulfan,by *Trichoderma harzianum.* Environmental and Toxicological Chemistry **12**: 1059-1065.

Lo. C. T., E. B. Nelson and G. E.Harman (1995) Efficient biological control of turf diseases using granular and spray application of *Trichoderma harzianum.* Fifth International Gliocladium and Trichoderma workshop. Beltsville, MD April (1995) Abstract.

Marfenin O. E (1991) Microbiological activities in protected soil - M ezd - BO Moscow State University 1991-118C.

Nigam, P. (1994) Process selection for protein enrichment. Fermentation of sugar industry by – products, molasses and sugar beet pulp. Process Biochemistry **29**: 337-342.

Roiger, D. J. S. N. Jeffers, **8** R. W. Caldwell (1991) Occurrence of Trichoderma species in apple orchard and woodland soil. Soil Biology and biochemistry **23**: 353-359.

Wardle, D. A., D. Parkinson and J. E. Waller (1993) Inter specific competitive inter action between pairs of fungal species in natural substrates. Ocologia **94**: 165-172.

Petrim, O. (1986) Taxonomy of entophytes of aerial plats disuses. In microbiology of the Philosopher (ed. N. J. Fokkema and J. Vanden Heuvell, pp. 175-187 Cambridge University Press: Cambridge U. K.

Mirchink . T. G. (1988) Soil mycology-M: Ind-in Moscow State University, p. 22 0.