Sindh Univ. Res. Jour. (Sci. Ser.) Vol: 39 (2) 65-70 (2007)

Sindh Univ. Res Jour.

SURJ

The study of adsorbed *Trichoderma harzianum* (G-18) on Vbasal (without substrate induced) and Vsir (Substrate induced) Microbial respiration in black zone soil of *Tataristan* (Russia)

G. A. Mako, and F. K. Alimova*

Institute of Pharmacy University of Sindh, Jamshoro, Pakistan

(Received 13th May 2007 and Revised 26th August 2007)

Abstract

The soil microorganisms are the causative agents of Fungistasis, with their action mediated either by available carbon limitation (nutrient deprivation) or production of antifungal compound. To obtain evidence for either of these actions, soil respiration was measured. The Vbasal and VSIR microbial respiration were studied after inoculation of adsorbed *Trichoterma harzianum*_ G-18 grown on different adsorbents such as saw wood, barley husk, chicken compost and animal manure. The culture of *Trichoderma harzianum* was isolated from green house of Kazan Republic of Tataristan (Russia) and implication of microbial respiration was studied at black Zone soil of *Tataristan* Kazan (Russia), the results of induced respiration with barley husk adsorbent were higher than non induced respiration.

Keywords: Mircobial soil respiration, *Trichoderma harzianum*, substrate induce respiration, adsorbents, black zone soil of *Tataristan*.

Introduction

Soil respiration is the measure of the net CO_2 production and O_2 consumption of a soil. It is one of most frequently used indicator for measuring microbial soil activity. The majority of natural soils suppress the germination and growth of fungi to a certain extent (Lock,1977). The process called as fugistasis. (Dobbs and Hinson. 1993). The intensity of fungistasis is dependent on the physical and chemical soil properties as well as soil microbial activities. (Alabouvette,1999). Fungistasis development was measured as restriction of the ability of the fungi *chactomium globsum, Fusarium culmorum, Fusarium oxysporum and Trichodrema harzianum*. (Patrick verheggen 2003). alone, or in combination with other *Trichoderma* species or chemical adjuvants was used in control of several disease. (Chet 1987). For instance the Rhizoctonia damping-off in radish (Lifshitz, *et al.* 1985). Grey-mould on tomato (Migheli *et al.* 1994). Grapes, and straw berry (Elad *et al.* 1995, Harman *et al.* 1995). In general, the microbial respiration measurement in the laboratory is used to observe the microbial activity and microbial biomass, using the controlled condition of appropriate moisture, temperature and respiration sieved having size (2-2.6mm) (Dahlin. 1997). During the past 25 years, in the structure of agricultural and of Tatraristan the rate of its acidity grew and that resulted in the decrease of phytosuppression and development of fungistatic characteristics of the soil. This deteriorates the phytosanitization conditions in the region. Chemically treated seeds therefore do not produce required results, (Popkova, 1998), but on the contray, it promotes the diseases of plants.

*Department of Microbiology the Kazan State University. Kozan 420008 Russia

CamScanner

This deterioration effect is reduced by using the appropriate microorganisms. That may not produce the disease.

In this study, the antagonistic effect of the fungi-*Trichodema harzianum* was biotechnically implicated to suppress the acidity of soil and it's performance. The inoculation of *Trichodema* in soil produces wide toxins. It shows high antagonistic activity, against phytopathogenic microorganism. It is non toxic to plants, and thrive well in its own habitate and within short time improved the phytosanitization conditions.

Materials and Methods

All the chemical used were of analytical grade.

Cultures: The culture of *Trichoderma harzianum*_G-18 was taken from the soil of kazan's Agriculture farm.

Glucose Mineral Mixture

Contain 10% glucose and calcium % solution.

Adsorbents

Selected for study (Required media).

a. Barely husk: from kazan farm.

b. Chick compost: From Kazan Chick farm.

c. Manure: from animal farm of Kazan.

Saw Wood: from wood house of Kazan.

3. Preparation of Inoculum and soil samples of various adsobents.

The known adsorbents were taken respectively in 2 liter round bottomed flask and moistened with appropriate amount of distilled water and sterilized at 15 lb for 20 minutes. After sterilization, the required media was inoculated with supension of *Trichodrema harzianum*_spores T-18.

The count of spores was 10^9 /gm/ml. The bulk of incolum so prepared, was transferred to various desired soils. the dose of inoculum 200 g/kg of soil and mixed with sterilized water up to 80% w/v. Incubation of the inoculated soil 28^0 C for ninety days. Analyses of the samples were determined after every thirty days.

Method: Vbasal Method

Two gms of incubated black zone soil of Tataristan were taken in 3 vials and moistened with 0.1ml of sterilized water and the vials then were closed with rubber stopper and then muffled to stop gas leakage, and the various sample contents were incubated at 28^0C for 24 hrs, 0.5CC gas ample was taken out with the help of syringe for CO_2 determination using Gas *Chromatograph*, as reported in Table.1 and Fig.1.

CamScanner

Table.1. Showing respiratory results of Vbasal. By taking 2g. sample of black zone soil of Tataristan, without glucose mineral mixture (10 mg/g of soil) moistened with 0.1ml sterilized water. The contents were incubated at 28°C for 24hrs. The evaluation of CO_2 was determined by Gas Chromatography.

Types of adsorbents		Incubation Period (Days)	Volume of CO_2 evolved
1.	Chicken Compost	30	0.9 mg
2.	Saw wood	30	2 mg
3.	Animal Manure	30	1 mg
4.	Barely Husk	30	1.5 mg
5.	Control(without absorbent and G.M.M)	30	1 mg
1.	Chicken Compost	60	1.5 mg
2.	Saw wood	60	4.3 mg
3.	Animal Manure	60	1 mg
4.	Barely Husk	60	3.8 mg
5.	Control(without absorbent and G.M.M)	60	2 mg
1.	Chicken Compost	90	8.8 mg
2.	Saw wood	90	9 mg
3.	Animal Manure	90	5.5 mg
4.	Barely Husk	90	9.9 mg
5.	Control(without absorbent and G.M.M)	90	8 mg

Calculation of CO_2

PCO_2=0.012xCO_2x(Vf-0.7xVp)xR-17.5)mg/k/hr.

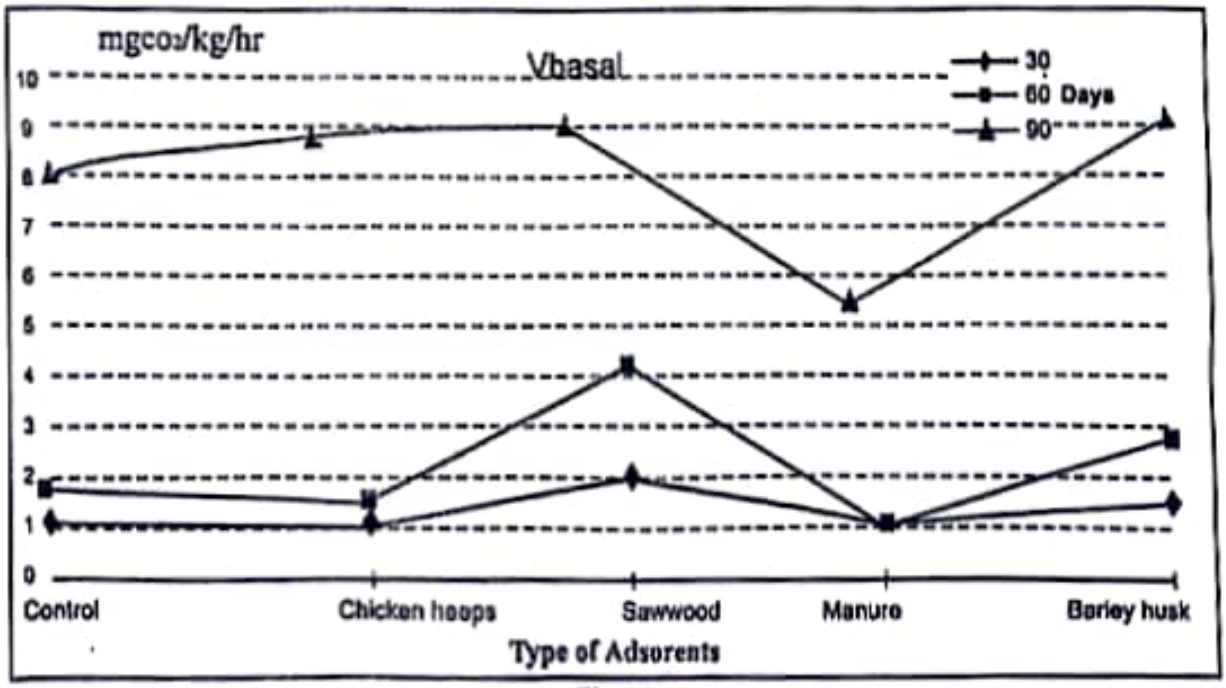


Fig. # 1
The Effect of adsorbed T.Harzianum
on without substrate induces soil mircobial respiration.

(i) Vsir Method: After the Vbasal determination, the vials were opened and the glucose mineral mixture as free carbon source. (10mg/gm of soil). Diluted up to 60% and incubated closed vials for 3-4hrs. at 25°C. After incubation, the sample of 1cc was taken out and determined by gas chromatography as reported in table. 2. The amount of CO_2 produced was calculated using the formula.

$PCO_2 = 0.012 x co_2 (Vf-0.7xVp) xR-17.5)$ mg/k/hr.

Vf = Volume of vial.
Vp = Volume of soil
R = atmospheric pressure.
$CO_2 = ?$

Results and Discussion

The results showed in tables and graphs.

Vbasal : The results of Vbasal respiration determined after 90 days incubation of soil sample with barely husk adsorbed *Trichoderma harzianum* T-18 showed in fig.No.1. The absorption was 9mg CO_2/Kg/hr whereas chicken maximum production of CO_2 9.9mg CO_2/Kg/hr, and at the second position the saw wood compost resulted 8.8mg CO_2/Kg/hr. and the manure absorbent respiration was observed 5.5 Co_2/Kg/hr, against their respective control samples. Table.1 Fig.1.

ii). The other observations of Vbasal process of respiration with barely husk, saw wood, animal manure and chicken compost at 60 days and 30 days incubation were proportionally in decreasing order respectively, as reported in Table.1 and Fig.1.

Vsir: After the 90 days of incubation period, the Vsir results the maximum CO_2 – (i.e.) by barely husk adsorbent. (60mg CO_2/Kg/hr) production. Chicken compost adsorbent produced 50 mg CO_2/kg/hr, the animal manure adsorbents 40 mg/kg/hr, and the saw wood adsorbent result's 30 mg CO_2/kg/hr, against their respective controls. Results have been showed in Table 2 and Fig. 2.

Table. 2. Showing respiratory results of Vsir by taking the 2g. sample of black zones soil of *Tataristan* mixed with glucose mineral mixture (10 mg/g of soil) moistened up to 60% and incubated closed Viats for 3-4hrs at 28°C. The evaluation of CO_2 was determined Gas Chromatography.

S. No	Types of adsorbents	Incubation Period (Days)	Volume of CO_2 evolved
1.	Chicken Compost	30	10 mg
2.	Saw wood	30	12 mg
3.	Animal Manure	30	18 mg
	Barely Husk	30	18 mg
	Control (without absorbent and G.M.M)	30	18 mg
1.	Chicken Compost	60	30 mg
2.	Saw wood	60	30.5 mg
3.	Animal Manure	60	28 mg
4.	Barely Husk	60	40 mg
5.	Control (without absorbent and G.M.M)	60	40 mg
1.	Chicken Compost	90	50 mg
2.	Saw wood	90	30.0 mg
3.	Animal Manure	90	40 mg
4.	Barely Husk	90	60 mg
5.	Control (without absorbent and G.M.M)	90	60 mg

Calculation of CO_2

$PCO_2 = 0.012 x CO_2 x (Vf-0.7xVp) xR-17.5)$ mg/k/hr.

CamScanner

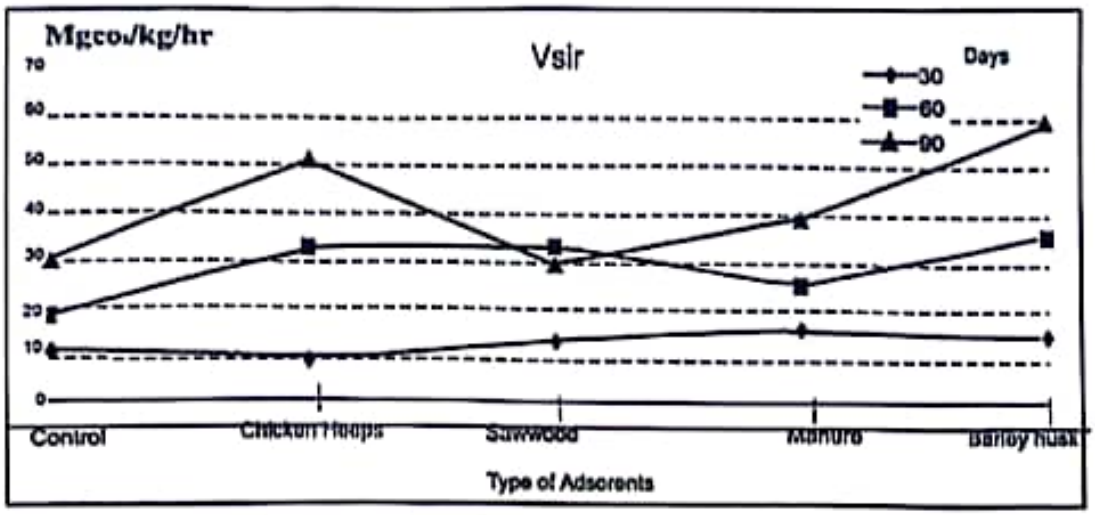


Fig. # 2
The Effect of absorbed T.Harianum
on substrate indues soil mircobial respiration.

Sy, the results vary with 60 days and 30 incubation periods with their respective adsorbents and the respiration.

Coefficient result (Q).

The coefficient is described as the ratio of the Vbasal and Vsir respiration under same time and temperature condition of the incubation.

The (Vsir) substrate induced and without substrate induced (Vbasal) effect of *Trichoderma harzianum* G-18 on respiration in black zone soil of Tataristan under different adsorbents, such as saw wood barley husk, chicken compost and animal manure were tried and compared. It was found, that after 90 days incubation the results with induced *Trichoderma harzianum* were higher than non induced with barley husk in comparison to other adsorbents. This suggests that more cellulosic (hemicellose) structure was favorable substrate for the growth of *Trichoderm harzianum* G-18. However, the others substrate used for instance animal manure and chicken compost of more nitrogenous substrate were observed less favorable for *Trichodedrma harzianum* G-18 growth and less respiratory effect Never the less the other effect of poisonous materal can not be rule out such as physical and chemical properties of and soil microbial activities have the great role in intensity of Fungigtatis (Mondal, S.N. *at el.* 1998).

The growth of fungi is inhibited by suppressed soil (lock 1986). Plant pathogenic fungi appear more sensitive to fungistasis then saprophytic fungi (De Boer, *et al.* 1998).

The most popular explanation for microbial cause of fungistasis is that strong competition by soil microorganism limits carbon availability to the germinating spores or inveding hyphae (Arora, D. K. *et al.* 1983).

Psudomonads produce antifungal compounds under nutrient limiting condition. (Ellis, R. J. *et al.* 2000).

References:

[1] Arora, D.K, A.B. Filonow, and Lock (1983) Exudation from ^{14}C Labeledfungal propegules in presence of specific microorganisms.Can. J. Microbial 29: 1487-1492.

CamScanner

[2] Alabouvette, C. (1999) Fusarium wilt suppressive soils: an example of disease-suppressive soil. Australal. Plant Pathol: 28: 57-64.

[3] Chet, (1987) Trichoderma-application, mode of action, and potential as a biocontrol agent of soilbrone plant pathogenic fungi. In *Innovative Approaches to plant Disease Control* (ed, I. Chet),137-160. John wiley and sons: New York.

[4] Dobbs, C. G., and W. H. Hinson.(1993) A wide spread fungiistasis in soil. *Nature* (London) 172: 197-199.

[5] De Boer, W. P. J. A. Klein Gunnewiek, and J. waidendlop (1998) Suppression of hyphial growth of soil-borne fungi by dune soils from vigorous and declining stainds of Ammophila arenaria. New Phytol. 138: 107-116.

[6] Dahlin, S. (1997) the soil microbial biomass and environmental stress. Acta Universitatis Agriculturae Sueciec Agraria 25: 161-167.

[7] Elad, Y. O., T. Neill, A. Cohen, G, O. Schllenber (1995) Factors influencing control of gray mold by means of Trichodex (Trichoderma harzianum T39) Under field conditions. *Fifth International Trichderma and Gliocladium workshop, Beltsville*, (April 1995), Abstract.

[8] Ellis. R.J, T.M., Timms-wilson and M. J. Bailey. (2000) Identification of conserved traits in fluorescent pseudomonads with antifugal activity. Environ Microbiol 2: 274-284.

[9] Lifshitz, R., S. Lifshitz, and R. Ba... (1985) Decrease in incidence ... Rhizoctonia preemergence dampin... by the use of integrated and chem... contro. *Plant Disease* 69: 4341-4344.

[10] Lock (1977) Fungistasis in soil. Biol, Re... 52: 1 43pp.

[11] Lockwoo, J. L. (1986) Soil borne plant pathogen: concept and connections. *Phytopathology* 76: 20-27.

[12] Migheli, Q., Herrera. A. Estrella, M. Avataneo, and M. L. Gullino, (1994) Fate of transformed *Trichoderma harzianum* in the phylloplane of tomato plants. *Molecular Ecology* 3, 153-159.

[13] Mondal, S.N., and M. Hyakumach (1958) Carbon loss and germinability, viability, and virulence of chlamadospore of fusarium solani of Sp. Phased (after exposure to soil at different Ph levels, temperature and matric potentials. Phytopathology 88: 148-155.

[14] Patrick verheggen (2003) Microbial community Composition affects soil fungistasis:*Applied environmental Microbiology*, Vol. 69, No. 2: 835-844.

[15] Popkova (1988) The method of seeds mycoflora determination. In *agriculture phytopathogen, Agropromizdat*, Moscow, Russia, 311-316.

CamScanner