Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Tech. 94 (2016) 40343-40346

ISSN: 2229-712X

Studies on the changes of microbial population in different soils of Sundarbans and its requirement for efficient use in field application of biofertilizer

Sisir Kumar Si

Vivekananda Institute of Biotechnology, Sri Ramakrishna Ashram, P.O.-Nimpith Ashram, South 24-Parganas (Sundarbans) West Bengal, India, 743338.

ARTICLE INFO

Article history:

Received: 31 March 2016;
Received in revised form:
9 May 2016;
Accepted: 13 May 2016;

Keywords

Temperature,
Soil properties,
Microbial population.

ABSTRACT

Surface soils representing a wide range of pH, EC and OC in Sundarbans were studied to determine the relationships between microbial growth efficiency and soil properties besides temperature changes. The field study shows that bacterial and fungal growth efficiency are maximum (100 %) in November and in January and minimum (9 -21 %) in April and May months respectively. Regression analysis indicates that pH, EC and OC besides temperature accounted for most of the variability in efficiency of microbial population over 85%. Multiple regression analysis predicted the growth efficiency of bio inoculates in different soil conditions in Sundarbans. Further field study shows that the application of Azotobacter increases tomato yield 2.5-20.4 % and chilli yield 2.1-16.1 % over balanced 75% NPK doses. These percentage of increases yield of tomato and chilli are highly statistical significant (r= 0.975 and r= 0.961 respectively) with bacterial growth efficiency. Increases of percentage of tomato and chilli yield on Azotobacter application based on bacterial growth efficiency depending on soil properties in Sundarbans is also predicted from regression equation. Soil and temperature requirements for microbial growth efficiency are tabulated according to Sys *et al.* (1993) from regression equation.

Introduction

Biofertilizer is a natural product carrying living microorganisms derived from the root or cultivated soil. Besides their role in atmospheric nitrogen fixation and phosphorous solubilisation, these microorganisms also help in stimulating the plant growth hormones providing better nutrient uptake and have no any ill effect on soil health and environment. Azotobacter is the most important non-symbiotic N-fixing bacteria in non-leguminous crops. The positive role of bacterial and fungal inoculates in many vegetables and spice crops has been recorded by different authors Okon and Labendera Gonzalez 1994, Okon and Itzigshohn 1995, Rao 1986 and Narula and Gupta 1986 in different soils and climatic regions .

Tomato and chilli are important vegetables and spice crops worldwide. Due to their food values, these crops are gaining importance both in developing and developed countries and efforts are being for the quality and quantity production of this commodity (Mahajan and Singh 2006). The microbial population which is depended on different soil environment plays a dominant role in biological efficiency in nitrogen fixation in crop production under INM system. There is a lack of information on how different soil properties and temperature affect the microbial population and their activities at the time of crop production.

The present investigations were therefore undertaken to study the microbial growth efficiency based on variation of soil and temperature and consequently increases of crops yield on application of bioinoculate in different sites of Sundarbans.

Materials and Methods

To study the changes occurring in the microbial population under month wise weather changes, field was selected at Radhakantapur village (Mathurapur II block), South 24 Parganas district, West Bengal during the year 2014-15. The land under field studies were silt-clay in texture medium to high in land situation, neutral pH, normal EC and high in OC contents. The surface (0-15 cm) soils were collected shortly before the weekly determination of bacterial and fungal populations (Chhonkar *et al.* 2002). Month wise their average values of microbial population were considered to relate with the effect of monthly average temperature in Sundarbans region (Table 2).

Month wise microbial growth efficiency (Table 2), is calculated as

$$= \frac{\text{Average microbial population}}{\text{Maximum average microbial population}}$$

To study the changes in the microbial population that assessed by the influence of soil properties in fourteen farmers' fields of wide range of pH, EC and OC in different locality of Sundarbans were selected. The soil samples were collected in month of October, 2015 and a part of soil samples were air dried and ground to pass through a 2 mm sieve before use for chemical analysis and other part of refrigerator kept samples are used for microbial analysis. For chemical analysis of soils, pH and EC were measured following methods described by Jackson 1973 and organic carbon was determined by the methods of walkley and Black (Basak 2000).

E-mail address: sisirkrsi@gmail.com

The population of bacteria, and fungi in soil was determined by serial dilution pour plate using Thornton's medium for bacteria, and Martin's Rose-Bengal streptomycin agar medium for fungi (Chhonkar *et al*. 2002) and consequently microbial growth efficiency are calculated as afore mentioned procedure. Regression analysis of microbial growth efficiency against temperature and soil characteristics is done manually and these equations are presented in table 4.

Field trials were also conducted in selected 10 farmers' fields of wide range of soil pH, EC and OC (Table 5). Each farmer's field was divided into four equal plots of approximate size 2 kata (133 m^2) for application of four fertilizer treatments (Tables 1) two for tomato and two for chilli crops and rest field is cultivated both tomato and chilli crops with 100% NPK doses(Ali 2005). Separate soil test based recommended 75% NPK doses (Tt_1 & Tch_1) were applied to both tomato and chilli paddy as urea, single superphosphate and murate of potash (Ali 2005) and 75% NPK + Azotobactor treatments (Tt_2 & Tch_2) were used for another two chosen plots of same field growing tomato and chilli respectively. Average yield of tomato and chilli per plant in four plots (considering 5 plants of each crops in each plot) are recorded and percentage of yield increases on Azotobactor application in treatments (Tt_2 & Tch_2**)** with respect to treatments (Tt_1 & Tch_1) is calculated and computed in table 5 along with bacterial growth efficiency of respective land based on soil properties calculated from regression equations, sl.no. 9, table 4.

Table 1. Treatment combinations used in tomato and chilli crops.

Treatments	Treatments detail
Tt_1	Soil test based recommended 75%NPK doses for tomato (Ali 2005)
Tch_1	Soil test based recommended 75%NPK doses for chilli(Ali 2005)
Tt_2	75% NPK + Azotobactor for tomato
Tch_2	75% NPK + Azotobactor for chilli

Soil informations of suitability and non suitability classes for biofertilizer in crop production suggested by Sys et al.(1993) was given in table 6 which was prepared by putting percentage of microbial growth efficiency 100, 95, 85, 60, 40 and 25 on regression equations (Sl. No. 1 to 8 in tables 4).

Results and Discussion

The month wise variation of average temperature and average rainfall in Sundarbans along with variation of bacterial as well as fungal population and their respective growth efficiency are given in table 2. Table 2 shows that the temperature rises up to 39.3^{0}C during peak summer (April) and comes down to as low as 13.6^{0}C during winter (January). The average temperature among different month varies from 19.7^{0}C to 33.2^{0}C (Table 2). The last 5 years average annual rainfall of this area is 1886.14 mm and the highest average rainfall lies in 493.46 mm (July) and lowest in 7.04 mm (December). Range of bacterial and fungal population and their average value are recorded month wise in table 2. Table 2 reveals that highest average microbial population is found in November and February month (>0.90 bacterial growth efficiency) lowest average value is found in April and May (<0.10 bacterial growth efficiency), whereas the average fungal population is found in December and January month (>0.95 fungal growth efficiency) lowest in April and May month (<0.21 fungal growth efficiency). The average microbial population both **bacteria and fungi are** comparatively low (varies from 0.58 to 0.38 microbial growth efficiency) in months of June and September through their mean temperature is not so far from October and February. It may be due to water saturation of soil during higher rainfall (>300mm) (Alexander 1983) On the other hand the average temperature of this region lies 19.7-33.2^{0}C but comparatively lower average microbial population around in summer (April & May) is due to higher daytime temperature(>35^{o}C) and low moisture (Motsara *et al*.1995).

The soil characteristics along with microbial population of the fourteen selected locality in Sundarbans are presented in table 3. Scrutinizing the table 3, it is found that the soils of the these localities are low to high in OC content (varied from 0.28 to 0.99%), acidic to neutral in reaction (pH varied from 4.43 to 7.27), and saline to normal in nature (EC lies between 0.23 and 1.73 dSm^{-1}).Besides these bacterial population ranged from 1.4 to 48.0 x 10^5g^{-1} dry soil and **fungal** population ranged from 22.0 to 120.0 x 10^3g^{-1}dry soil are recorded. Bacterial growth efficiency (varies from 0.03 to 1.0) and fungal growth efficiency (lies between 0.18 and 1.0) are calculated and also presented in table 3.

Regression analysis of the microbial growth efficiency both bacteria and fungi with temperature and soil parameters besides with the combinations of soil parameters (pH, EC & OC) are showed in table 4 . The table 4 shows that 80.58 and 90.55 % of the variability in the bacterial and fungal growth efficiency were accounted for by the average temperature and their respective regression equations were also given in table 4. While for bacterial growth efficiency against pH, EC and OC separately accounted 88.74%, 93.09% and 87.75% of the variability and for fungal growth efficiency against pH, EC and OC separately accounted 85.98%, 86.83% and 84.86% variability and their regression equations is recorded in table 4.

Table 2. Month wise variation of microbial population in soil with changing weather.

Months	Temperature (O^0C)		Average Rainfall (mm)	Bacterial population X 10^5g^{-1} dry soil		Fungal population X 10^3g^{-1} dry soil		Microbial growth efficiency	
	Max.–Min.	Average		Range	Aver.	Range	Aver.	Bacteria	Fungi
Jan	25.8-13.6	19.7	10.20	40.0-46.0	42.25	118.0-128.0	123.50	0.88	1.00
Feb	29.5-16.5	23.0	11.70	39.0-49.0	43.75	95.0-109.0	101.25	0.91	0.82
Mar	34.3-21.5	27.9	22.70	26.0-34.0	29.25	38.0-46.0	42.00	0.61	0.34
Apl	39.3-26.0	32.7	35.90	2.0-6.0	4.25	23.0-31.0	26.00	0.09	0.21
May	38.8-27.5	33.2	102.00	3.0-7.0	4.75	17.0-26.0	21.00	0.10	0.17
June	34.1-26.7	30.4	310.41	20.0-29.0	24.00	55.0-64.0	59.25	0.50	0.48
Jly	32.5-26.3	29.7	493.46	22.0-30.0	26.00	52.0-62.0	56.75	0.54	0.46
Aug	32.0-26.2	29.2	343.95	19.0-25.0	22.00	43.0-51.0	47.00	0.46	0.38
Sept	31.2-25.1	28.1	315.25	25.0-31.0	27.75	45.0-54.0	49.50	0.58	0.40
Oct	30.2-22.9	26.6	185.88	31.0-39.0	34.50	48.0-56.0	52.00	0.72	0.42
Nov	29.5-18.4	24.0	47.65	44.0-51.0	48.00	95.0-104.0	98.75	1.00	0.80
Dec	26.0-14.2	20.1	7.04	37.0-46.0	40.75	112.0-121.0	117.25	0.85	0.95

Table 3. Chemical and biological properties of soils in fourteen sites of Sundarbans.

Sites	pH	EC ($dS m^{-1}$)	OC (%)	Bacterial population X $10^5 g^{-1}$ dry soil	Fungal population X $10^3 g^{-1}$ dry soil	Microbial growth efficiency	
						Bacteria	Fungi
1	7.01	0.46	0.99	41.0	110.0	0.85	0.92
2	6.05	0.97	0.51	31.0	65.0	0.45	0.54
3	5.26	1.12	0.39	11.0	44.0	0.23	0.37
4	6.85	0.86	0.70	27.0	90.0	0.56	0.75
5	4.82	1.73	0.36	2.6	35.0	0.05	0.29
6	5.12	1.65	0.32	1.4	22.0	0.03	0.18
7	7.27	0.23	0.91	48.0	120.0	1.00	1.00
8	5.41	1.60	0.47	8.3	42.0	0.17	0.35
9	6.61	0.78	0.75	23.0	62.0	0.48	0.52
10	5.44	1.48	0.54	7.3	26.0	0.25	0.40
11	5.61	1.20	0.62	17.0	80.0	0.35	0.47
12	4.74	1.55	0.40	5.0	36.0	0.10	0.30
13	6.42	0.80	0.82	19.0	55.0	0.54	0.61
14	4.43	1.39	0.28	4.3	32.0	0.09	0.27

Table 4. Temperature and soil properties predicting the bacterial and fungal growth efficiency.

SL. No.	Characteristics	Regression equations	Contribution:R^2*100	'r'value
1	t^0C (B)	Y_b=0.059(t^0C) + 2.193	80.58	-0.897
2	t^0C(F)	Y_f =0.060(t^0C) + 2.153	90.55	-0.951
3	pH(B)	Y_b=0.307(pH) + 1.41	88.74	0.942
4	EC(B)	Y_b= - 0.618 (EC) + 1.066	93.09	-0.965
5	OC(B)	Y_b=1.227 (OC) – 0.339	87.75	0.937
6	pH(F)	Y_f =0.251(pH) – 0.952	85.98	0.927
7	EC(F)	Y_f = - 0.495 (EC) + 1.057	86.83	-0.932
8	OC(F)	Y_f =1.00(OC) – 0.078	84.86	0.921
9	PH,EC,OC (B)	Y_b= - 0.06 – 0.23(pH) + 1.6 (EC) + 2.7(OC)	99.9	…
10	PH,EC,OC (F)	Y_f = 0.29 + 1.6(pH) – 2.5(EC) – 10.57(OC)	75.02	…

Y_b= Bacterial growth efficiency; Y_f= Fungal growth efficiency; t^0C= Average temperature; B=Bacteria and F= Fungi

A combination effect of soil pH, EC and OC accounted for 99.9% variability in bacterial growth efficiency and accounted for 75.02% variability in fungal growth efficiency and their multiple regression equations are given in table 4. Data in table 4 reveals that temperature, pH, EC and OC have significant effects on both bacterial as well as fungal growth efficiency.

In another study of soil test based fertilizer recommendation with or without *Azotobacter*, the percentages of tomato and chilli yield increase in farmers' fields are given in table 5. The percentage of bacterial growth efficiency in each farmer's fields is calculated putting soil pH, EC and OC values on regression equation (Sl. No. 9 in table 4) and are also presented in table 5. The bacterial growth efficiency is highly correlated with percentage of tomato yield increases (r=0.975) and percentage of chilli yield increases (r=0.961).The regression equation between bacterial growth efficiency and percentage of tomato and chilli yield increases are also computed in table 5 respectively. These regression equations are helpful ultimately to predict the percentage of tomato as well as chilli yield increases on Azotobacter application knowing bacterial growth efficiency based on soil pH, EC and OC.

The range of soil parameter and temperature ^0C within suitability (S_0, S_1, S_2, S_3) and non suitability (N_1 and N_2) of microbial growth efficiency classes suggested by Sys *et al.* (1993) were calculated from the regression equations (Sl. no. 1 to 8 in tables 4) and tabulated in table 6. Analyzing the table 6, it is found that 20 to 30^0C is suitable for microbial growth both bacteria and fungi.

Above 30^0C and below 19^0C temperature microbial growth rate reduced and soil go to non suitable range (Sys *et al.* 1993) for biological efficiency of biofertilizer. On the other hand, table 6 shows that suitable range of soil pH, EC and OC for bacterial **inoculates** are pH 5.8 to 7.8, EC below 1.1 dSm^{-1} and OC above 0.6 % and for fungal **inoculates** are pH 5.5 to 7.8, EC below 1.3 dSm^{-1} and OC above 0.5 % respectively.

Table 5. Percentage of crops yield increases on Azotobacter application over 75% NPK treatment.

Farmers' fields	Soil test values			Bacterial growth efficiency*	Percentage of yield increases		Bacterial growth efficiency predicting the % of crops yield increases
	pH	EC (dSm^{-1})	OC (%)		Tomato	Chilli	
1	5.80	0.80	0.69	0.60	16.5	11.6	**Y(T)= 25.914(bacterial growth efficiency) – 0.466 Contribution :R^2*100=95.09 r=0.975
2	6.20	1.01	0.76	0.76	20.4	16.1	
3	6.61	1.41	0.64	0.37	10.7	10.3	
4	6.40	0.75	0.78	0.69	15.1	14.6	
5	5.70	0.96	0.65	0.54	12.6	13.7	
6	5.31	1.68	0.46	0.23	5.2	5.8	***Y(Ch)= 20.874(bacterial growth efficiency) +0.361 Contribution :R^2*100=92.37 r=0.961
7	4.51	0.52	0.43	0.15	2.5	2.1	
8	4.25	0.94	0.37	0.11	3.3	3.1	
9	7.85	1.20	0.79	0.45	11.8	8.4	
10	7.28	1.53	0.67	0.32	6.6	6.0	

*Bacterial growth efficiency calculated from regression equations (sl.no. 9, table 3) **Y (T) =% of tomato yield increases; ***Y (Ch) = % of chilli yield increases

Table 6. Temperature and soil requirements for bio- inoculates.

Microbes	Particulars	Microbial growth efficiency (%); Suitability(S) and non suitability (N) classes					
		100-95 %	95-85%	85-60%	60-40%	40-25%	25-0%
		S_0	S_1	S_2	S_3	N_1	N_2
Bacteria	Mean temp.(^{0}C)	20.2-21.0	21.0-22.7	22.7-26.9	26.9-30.3	30.3-32.9	32.9-37.1
Fungi		19.1-20.0	20.0-21.6	21.6-25.8	25.8-29.1	29.1-31.6	31.6-35.8
Bacteria	pH	7.77-7.61	7.61-7.29	7.29-6.48	6.48-5.84	5.84-5.35	5.35-4.55
	EC (dSm^{-1})	0.11-0.19	0.19-0.35	0.35-0.76	0.76-1.10	1.10-1.32	1.32-1.73
	O C (%)	1.09-1.05	1.05-0.97	0.97-0.76	0.76-0.60	0.60-0.48	0.48-0.28
Fungi	pH	7.80-7.60	7.60-7.20	7.20-6.20	6.20-5.40	5.40-4.80	4.80-3.80
	EC (dSm^{-1})	0.12-0.22	0.22-0.43	0.43-0.94	0.94-1.35	1.35-1.65	1.65-2.16
	O C (%)	1.08-1.03	1.03-0.93	0.93-0.68	0.68-0.48	0.48-0.33	0.33-0.08

Temperature and soil suitability are calculated from equations (SL. No. 1-8) in table 4.

Beyond this limit, the microbial activities are quickly slow down below 40% efficiency limit. These results are agreements with the reports given by Biswas and Mukherjee 1987 and Alexander 1983. Within suitability range (S_0 to S_3) the tomato and chilli yield increases between 10-25 percentages on Azotobactor application where as within non suitable (N_1–N_2) range the yield increases below 9.0 percentage, which are remarkable low. So there are more soil constrains in respect pH, EC and OC in non suitable classes for field application of biofertilizer.

Conclusions

The microbial population both bacteria and fungi varied month wise and their numbers was lowest in the summer season and increased through winter season and finally reached in height value in the February month. From these studies it is concluded that 20-30^{0}C temperature are suitable for microbial growth. The soil conditions i.e., pH from 5.5 to 7.8 , EC below 0.75 dSm^{-1} and OC above 0.75% range are suitable for better bacterial proliferation. From the regression analysis, it is concluded that soil pH, EC and OC are the good **indices** for predicting the bacteria growth efficiency and consequently this bacterial growth efficiency predict the percentage of crop yield increases on field application of bacterial inoculates in Sundarbans.

Acknowledgements

The author is grateful to Swami Sadananda Maharaj, Chairman, Sri R. K. Ashram, Nimpith and Dr. B. K. Datta, Director, Vivekananda Institute of Biotechnology, Nimpith for necessary help and continuous encouragement.

References

Ali, Sk. Jahangir (2005). Fertilizer recommendation for principal crops and cropping sequences of West Bengal. Booklet no. 1, Department of Agriculture, Govt. of W.B., 4-5.

Alexander, M. (1983). Introduction to soil microbiology. Wiley eastern limited, New Delhi, 21-59.

Basak, R.K, (2000).Soil testing and recommendation, Kalyani publishers Ludhiana, New Delhi .

Biswas, T.D. and Mukherjee, S.K. (1987). Text book of soil science. Tata McGraw-Hill publishing company limited, New Delhi, 37-49

Chhonkar, P. K., Bhadraray, S., Patra, A. K. and Purakayastha, T. J. (2002). Experiments in soil biology and biochemistry. Westville publishing house, New Delhi, 180.

Jackson, M.L. (1973) Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Mahajan, G. and Singh, K.G. (2006). Response of greenhouse tomato to irrigation and fertigation, Agricultural Water Management,84: 202-206.

Motsara, M.R., Battacharyya, P. and Srivastava, B. (1995). Biofertilizer technology, marketing and usage, Fert. Devel. and consultation organization 204. Bhanot Corner, 1-2, Pamposh Enclave, New Delhi, 110048.

Narula, N. and Gupta, K. G. (1986). Ammonia excretion by Azotobactor chroococcum in liquid culture and soil in the presence of manganese and clay minerals. Plant and Soil, 93: 205-209

Okon, Y. and Labandera Gonzalez, C. (1994). Agronomic applications of Azospirillum: an evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem., 26: 1591-1601.

Okon, Y. and Itzisohn, R. (1995). The development of Azospirillum as a commercial inoculant for improving crop yield. Biotechnol. Adv., 13: 414-424.

Rao, D.L.N. (1986). Nitrogen fixation in free living and associative symbiotic bacteria. In: Soil Microorganisms and plant growth. Subba Rao N.S. (Ed.) Oxford and IBH Pub., Co., New Delhi.

Sys, Ir. C, Van Ranst, E., Debaveye, Ir. J. and Beernaert, F. (1993). Land evaluation. Part III, crop requirements, agricultural publications (Belgium).