Int. J. Exp. Res. Rev., Vol. 6: 62-66 (2016)

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 Original Article

Study of rhizospheric bacterial population of *Azadirachta indica* (Neem) of North 24 Parganas district of West Bengal for bioprospective consideration

K.Biswas[1], J. Basu[2], A. Ghosh[3] & P.Giri[4]*

[1]Department of Physics, Dum Dum Motijheel College, Kolkata, West Bengal, India; [2]Department of Microbiology, Dum Dum Motijheel College, Kolkata, West Bengal, India; [3]Department of Physics, Dum Dum Motijheel College, Kolkata, West Bengal, India; [4]Department of Botany, Dum Dum Motijheel College, Kolkata, West Bengal, India.

*Corresponding Author

Abstract

The rhizospheric microbial population has immense role in agriculture and crop improvement. This article deals with the preliminary information about the rhizospheric bacterial population of *Azadirachta indica* growing at the different site of North 24 parganas district of West Bengal, India. The information will serve as a data base for future exploration of bioprospective potentialities of rhizospheric bacteria prevailing in the unique ecological niche of the medicinal plants.

Key words: Rhizosphere, rhizospheric effect, inoculum, antimicrobial, cultural characteristics.

Introduction

Azadirachta indica (Meliaceae), commonly known as neem or margosa tree is an indigenous plant of our country having wide range of distribution in different agroclimatic conditions. The medicinal value of the plant is well known due to wide diversity of chemical compounds present in its different parts which exert combative and curative effect on health hazards. The root and its surrounding region or rhizosphere is a novel ecosystem which is the harbor of wide variety of microorganisms including bacteria, fungi, algae etc. The root of the host plant secretes certain growth adjuvant which provide uniqueness of the growing microbial population in the rhizosphere. The microorganisms as an inhabitant in the rhizosphere administer antimicrobial property against harmful or pathogenic microorganisms which infect root system in particular. The diversity of root associated microorganisms of selected medicinal plants has been studied by Tamilarasi et al., (2008). The study reveals that the rhizomicroorganisms have antimicrobial properties. Bacteria from rhizospheric region of medicinal plants have also reported to produce antibiotics (Pandey and Malviya, 2014).

The antagonistic effect of soil rhizospheric microorganisms on *Bipolaris sorokinianana*, the causal agent of wheat seedling blight has been established by Dalbello et al., (2003).The biocontrol efficiency of *Pseudomonas*

aeruginosa and *Bacillus licheniformis* isolated from the rhizosphere of medicinal plants have been demonstrated by different workers. Under laboratory condition the bacterial isolates were inhibitory to *Alternaria alternate*, *A. dianthicola* and *A. longipes*, causal agents of foliar diseases of some of the common medicinal plants cultivated in West Bengal.

A wide variety of growth enhancing principles released in the rhizosphere helps to grow microbes luxuriantly over there. Effects of neem aqueous extract on the growth of *Frankia* –nitrogen fixing rhizospheric bacteria have been demonstrated by Sayed et al., (2011). Flavonoids released naturally from Alfalfa seeds have been shown to enhance the growth of *Rhizobium meliloti* – a potent nitrogen fixing bacteria (Hartwiq et al., 1991). A rhizospheric signal molecule lumichrome has been shown to alter the developmental pattern of seedling (Mathesius and Michelle, 2005).The role of phosphate solubilising bacteria from soil in plant growth promotion have been worked out in detail by Rodríguez and Fraga (1999).

Keeping in view the growth promotive and protective role of rhizospheric microorganisms of medicinal plants their isolation, characterization, quantification and identification is imperative and need based one for better crop production. In this context the rhizosphere of *Azadirachta indica* is very interesting because of its appreciable medicinal values and initiatives are being taken as well for mass cultivation of this plant in different districts of West Bengal by private and Government entrepreneurs. The bacterial population of the rhizosphere of this plant has not been studied so far in detail. Moreover, such studies might have enormous possibilities to provide an important data base regarding the bacterial population of such novel ecosystem for exploration and evaluation of their bioprospective potentialities in future. Therefore, our preliminary investigation was aimed to:

i) Isolate and quantify the bacterial population from the rhizospheric soil from the plants growing at the different site of North 24 Parganas district of West Bengal.
ii) Study of the rhizospheric effect of the bacterial population.
iii) Study of gross morphology and cultural characteristics of the isolated bacterial population.

Materials and Methods

Soil Sample collection

Soil samples in replica were collected from eight different locations of North 24 Parganas of West Bengal to study diversity of microbes in rhizosphere of Neem Plant. The sites considered under present investigation are Dum Dum Cantonment, Bongaon, Basirhat, Naihati, Barrackpore, Sodepur, Taki, Hingalganj. The rhizospheric soil samples are labelled properly to demarcate the collection site .Soil samples were collected below 16 inches depth form the surface of root. Collected Soil samples are preserved in sterile plastic packets with locked mouth.

Study of rhizospheric effects of bacterial population

The study of rhizospheric effect of bacterial population was studied by using the following formula (Subbarao, 2000):

Rhizospheric effect (RE) = Number of microorganisms / gm of soil ÷ Number of microorganisms in a gm of non rhizospheric soil.

Site of collection of rhizosphere soil	Total number of isolates observed	Name of the Bacterial isolates	No. of microorganisms available / gm of soil	Gram nature of bacterial isolates	Cultural characteristics of the isolates
Dum Dum Cant.	06	RHSAN1	$33x10^6$	Gram negative	White fused colony, rapidly growing, and irregular margin, slimy.
Bongaon			$39x10^6$		
Basirhat		RHSAN2	$32x10^6$	Gram negative	Yellowish, shiny and smooth, moderate growth.
Naihati		RHSAN3	$42x10^6$	Gram negative	Whitish, dry matt, slow growing.
Barrackpore			$44x10^6$		
Sodepur		RHSAN4	$46x10^6$	Gram positive	Creamy white, translucent, shiny, moderately growing.
Taki		RHSAN5	$48x10^6$	Gram positive	White colony, slow growing with raised margin.
Hingalganj		RHSAN6	$40x10^6$	Gram negative	White colony with hallow zone of inhibition at the surrounding.

Microbiological Analysis

The samples were subjected to microbiological analysis within 24 hours after collection. To analyze microbial load from collected soil, 5gms of soil was dissolved in 50ml of water in 250 ml conical flask. The conical flask was kept in a shaker for 3 hrs to disperse the microbial load from the soil particles. To evaluate the cultivable bacterial density, 1 ml of each supernatant was tenfold serially diluted in a sterile water solution (9 ml). The serial dilution was continued until it reaches upto 10^{-6}. Then 0.1ml and 1ml aliquot respectively of each sample of 10^{-4} and 10^{-6} dilution was inoculated into the plates following pour plate method. The plates were incubated at 37^0C temperature for 18-24 hours in a BOD Incubator. The visible colonies on the plates were counted and colony characteristics were recorded. The quantification of organisms per ml of suspension was measured by using the following formula:

No. of microorganisms/ ml = No. of colony x dilution factor. The cultures were maintained by periodical sub-culturing (Fig 2).

Results and Discussion

The bacterial isolates from rhizosphere soil of *Azadirachta indica* of eight different sites of the district North 24 Parganas (West Bengal) clearly reveals that diversity of bacterial population in the rhizosphere is not much significant. The types of bacterial colony isolated from the soil samples of different sites show uniformity in their exo-morphological characters on the basis of which bacterial types have been discriminated and the identity of each type has been demarcated by using specific code RHSAN1 to RHSAN6 (RHS= Rhizospheric Soil; A= *Azadirachta*, N= North 24 Parganas).

Fig 1. Vigogurouly growing bacterial colony (Isolate number :RHSAN1).

A total six types of bacterial colony have been isolated from different soil samples as shown in Table (1) which are more or less available in all soils samples tested. Among the bacterial isolates two (2) are Gram positive and four (4) are Gram negative (Fig3).

Fig 2 : Bacterial isolates under growing condition in slant.

Though the types of bacterial isolates in the rhizosphere soil of this plant are more or less similar but the total quantity per gram of soil is different. This variation may be due to the differences in chemical composition of soil samples collected from different sites. The edaphic parameters like available carbon, nitrogen, phosphorus, electrical conductivity (EC) in addition to different specific nutritional milieu influence the bacterial population in rhizosphere soil.

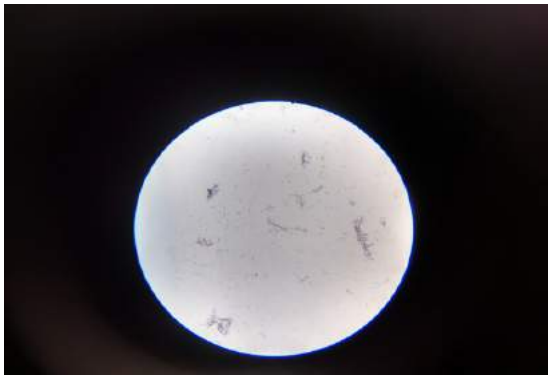

Fig 3. Gram nature of the isolate RHSAN1.

The physical parameters like pH, soil temperature, moisture, porosity etc are not less important to administer bacterial population in the rhizosphere. Detail investigation with aid of statistical technique is therefore required to find out which microclimatic parameters are positively correlated in a direct or indirect manner to influence the growth of bacterial population in the rhizosphere of this plant. Phenotypic, genotypic and agropath analysis only could point out such specific parameters for better biomass production of these isolate *in vivo*.

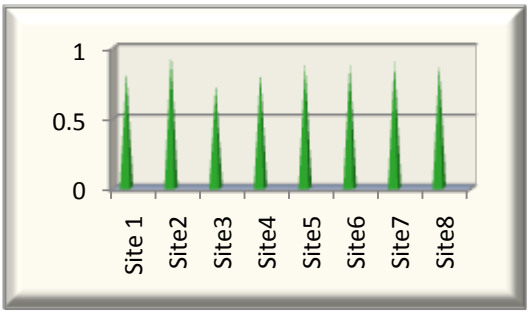

Fig 4. Rhizospheric effect of *Azadirachta indica* of different sites of North 24 Parganas district of West Bengal.

Other domains of investigation on these isolates could be carried out to test their nitrogen fixing ability, phosphate solubilising efficiency, antagonizing activity of harmful root infecting pathogens. Once such efficacy is proved the biomass of the particular isolate or isolates in combination could be amended in the soil of different crop plants concurrently after proper manipulation of rhizospheric microclimatic parameters for achievement of biomass at the satisfactory level which could be helpful for the enhancement of the productivity of desired crops. If the antagonizing activity of any isolates could be established such properties of the same could be exploited for abatement of the adverse effect of root infective pathogens of crop plants. In strict

sense therefore, the rhizosphere isolates of neem plant might have sufficient bioprospective potentiality in terms of suitable biofertilizer formulations for better crop production.

Another significant observation during the course of our investigation is the luxuriant growth of one particular colony (RHSAN1) over the others. This rapidly growing colony forms cloudy mass (Fig 1) of cells on the growth medium merely within overnight period of incubation. The colony of other isolates show moderate expansion or growth in cultural environment. Such vigorously growing bacteria in the rhizosphere may be responsible for the suppression of growth of other microorganisms in the rhizosphere by making the environment adverse for their growth. This attribute supposed to be accounted for low microbial diversity in the rhizosphere as mentioned earlier. Root exudates from the plant itself and its inhibitory constituents may also be the other attributes for low microbial diversity. The exact reasons however could be revealed through extensive studies on the analyses of the chemical constituents of the rhizosphere soil.

The figure showing the rhizospheric effect (Fig4) also corroborates our findings that the rhizosphere of neem is not suitable for the growth of common microbial inhabitants of the soil. It allows the growth of some selected bacteria which have the ability to avert the existing unfavourable microenvironment.

Acknowledgement

The authors are grateful to the Principal, Dum Dum Motijheel College, Kolkata for his constant encouragement and support. Authors are also thankful to Dr. H. Saha, IQAC Coordinator for his valuable suggestion, constant encouragement and help.

References

Dal, B.G. M., Sisterna, M. N. and Monaco, C.L. (2003). The antagonistic effect of soil rhizosphere microorganisms on Bipolaris sorokiniana, the causal agent of wheat seedling blight. *International Journal of Pest Management*. 49 (4): 313-317.

Hartwig, U.A., Joseph, C.M. and Phillips, D.A. (1991). Flavonoids released naturally from Alfalfa seeds enhance growth of *Rhizobium meliloti. Plant Physiol.* 95(3): 797-803.

Mathesius, U. and Michelle, W. (2005). Rhizosphere signals for plant–Microbe interaction: Implications for field grown plants*. In Book: Progress in Botany*. 72: 125-161.

Pandey, A. and Malviya, T. (2014). Production of Antibiotics isolated from soil bacteria from rhizospheric and nonrhizospheric region of Medicinal Plants. *Indian Journal of Applied Research.* 4 (8): 216-223.

Rodríguez, H. and Fraga, R. (1999). Phosphate solubilising bacteria and their role in plant growth promotion. *Biotechnology advances*. 17:319–339.

Sayed, W. F., Salem, W. M. and Ali, M. S. (2011). Effect of neem aqueous extract on *Casuarina equisitifolia* seed germination, growth of Frankia and some rhizospheric microorganisms. *African Journal of Microbiology Research*. 5 (14): 1874-1880.

Subbarao, N. S. (2000). Soil Microbiology. Oxford and IBH publications. pp82-87.

Tamilarasi, S., Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy, P. (2008). Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum*. *Journal of environmental Biology*. 29(1): 127-134.