STUDENT STUDY PROJECTON

Isolation Of Microbial Flora In The Soil Samples Obtained From College Premises.

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CERTIFICATE

This is to certify that students of the M. Sc –MICROBIOLOGY – Second year has been successfully completed the project entitled "**Isolation Of Microbial Flora In The Soil Samples Obtained From College Premises** from the department of Microbiology ,Kakatiya government college,Hanamkonda.

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Title: Isolation of Microbial flora in the soil samples obtained from college premises.

Soil structure

Soil is the upper weathered or well ground loose parts of earth's crust and consist of loosely arranged layers of materials composed of inorganic and organic constituents, water and gaseous phases. It is the natural medium for plants and microbes. Soil provides the physical support needed for root system and is the reservoir for air, water and nutrients essential for all living organisms. Every soil has succession of layers from surface to downwards known as profile. The soil profile consists of two or more layers called horizons. Soil consists of three different horizons –A, B, &C the formation of soil horizons depends on climate, living organisms, parent rock material. The process begins with withering of rocks, release of silicate, clay minerals and their salts.

Review of literature

Soil mineral matter: soil consists of stones, gravels, sands, silts and clays. They constitute the mineral matter of soil stone, gravel and sand are known as coarse fragments ranges in size from 2mmsands and silts are the fragments of rocks as well as minerals. Coarser clay particles are composed of minerals like quartz and hydrous oxides of iron and aluminium. Three main minerals types' kaolinite, iolite and montmorillonite are mostly present.

Texture denotes the size of individual soil particles. it is an important soil characteristic determine water intakes rates, water storage in the soil, the ease of tilling of the soil, the amount of aeration and will influence the soil fertility. A triangle is used to determine the soil textural class after the percentages of sand, silt and clay are determined.

The structure of soil is dependent on stable aggregates of soil particles. A strong binding force among the soil particles forms a cluster of soil particles leads to soil aggregates. Soil organic matter including humus, polysaccharides and polyamides produced by soil

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microorganisms helps to bind soil particles together. The filamentous fungi provided mechanical support for soil aggregates. some of the fungi like *Rhizopus, Mucor, Chaetomium, Fusarium, Cladosporium, Aspergillus, Rhizoctonia and bacteria such as Aztobacter, Rhizobium, Xanthomonas and Bacillus* are known to secrete gums and provide mechanical support to bind soil particles these secreted gums consists of several components like lipopolysaccharides, fructose, arabinose, glucose, rhamnose, mannose, glucosamine, galactose, xylose and dextrin. Among all the carbohydrates dextrin containing high amount of uremic acid, and have the best soil aggregating qualities.

The water holding capacity of a soil is governed by the porosity and soil structure. Occurrence of water in soil can be three types: gravitational, capillary and hygroscopic after heavy rain. Irrigation, gravitational water passes through the soil. After the drainage of excess water, the water which is retained in the soil pores at field capacity is known as capillary water. The water absorbed by dry soil from an atmosphere of high humidity is known as hygroscopic water. Fungi are more tolerant to water stress, followed by ammonifiers such as *nitrosamines, clostridium* and *penicillium* are less tolerant.

All living organisms require oxygen. Respiration of plant root and for organic matter decomposition by microorganisms requires soil air. The most desired condition for plant growth is well-aerated soil. The condition in which oxygen exchange is very rapid between soil air atmosphericair. The soil pores are field with water and gases. Soil air contains more of CO_2 and less of O_2as compared to the atmospheric air and the difference is due to respirations of plants roots, microorganisms and other living organisms consuming O_2 realising CO_2. Oxygen content declines and CO_2 level increases with death. Changes in the atmosphere alter the size and function of micro flora as both oxygen and carbon dioxide are necessary for growth. The renewal of soil air is by diffusion.

Soil temperature is one of the most important physical property effects the crop growth. The flow of the temperature in the soil is expressed as conductivity. It increases with water content and decreases with porosity.

pH, the negative logarithm of the activity of H+ in solution indicates the acidity or alkalinity of soil. The pH values of soil vary from 3-10 and determines the mineral contents as well as microbial composition. Generally fungi are predominant in the rhizosphere under low pH conditions and the bacteria like nitrogen fixing microorganisms are favoured by neutral pH. High

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pH releases ions of potassium, Magnesium, calcium, manganese, cupper, and aluminium by weathering processes of soil where as low pH favours solubility of salts including carbonates, phosphates and sulphates.

Plant and animal residue decayed in the soil, forms soil organic matter. The great activity of microorganisms converts complex nature of plant tissue into simple organic compounds and derive their energy through the oxidation of carbohydrates, proteins and fats. The decomposed organic residues present in soil forms soil organic matter. Humus increases the fertility. Humus plays a major role in the structure of soils. Humus promotes the formation of soil aggregates.

Rhizosphere

The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, but has no distinct edge. Rather, it is an area of intense biological and chemical activity influenced by compounds exuded by the root, and by microorganisms feeding on the compounds. As plant roots grow through soil they release water soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. Microbiological activity in the rhizosphere is much greater than in soil away from plant roots. In return, the microorganisms provide nutrients for the plants. All this activity makes the rhizosphere the most dynamic environment in the soil. The underground rhizospheric activity has been largely over looked, and it is only now that many are starting to unravel the complex interactions that occur in soils. For this reason, the rhizosphere has been called the last frontier in agricultural science. The roots exude water and compounds broadly known as exudates.

Root exudates

Root exudates include amino acids, organic acids, carbohydrates, sugars, vitamins, mucilage and proteins. The exudates act as messengers that stimulate biological and physical interactions between roots and soil organisms. They modify the biochemical and physical properties of the rhizosphere and contribute to root growth and plant survival. However, the fate of the exudates in the rhizosphere and the nature of their reactions in the soil remain poorly understood. The exudates have several functions, like defending the rhizosphere and root against pathogenic microorganisms. Root cells are under continual attack from microorganisms and survive by

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secreting defence proteins and other as yet unknown antimicrobial chemicals. Research has found that exudates in the rhizosphere vary according to the stages of plant growth. For instance, there are more carboxylates and mucilage at the six leaf stage than earlier. Rhizosphere attracts and repels particular microbe species and populations. High levels of moisture and nutrients in the rhizosphere attract much greater numbers of microorganisms than elsewhere in the soil. The composition and pattern of root exudates affect microbial activity and population numbers which, in turn, affect other soil organisms that share this environment.

Rhizosphere keeps the soil moist, around the roots. Research has found that rhizosphere soil is significantly wetter than bulk soil, which protects roots from drying out. Exudates released from roots at night allow expansion of roots into the soil. When transpiration resumes with day light, the exudates begin to dry out and adhere to the soil particles in the rhizosphere. As the soil dries and its hydraulic potential decreases, exudates lose water to soil.

The exudates help roots adsorb and store ions for plant use. For instance, flavonoids in legume roots activate genes responsible for root nodulation that enable the plant roots to obtain nitrogen from the air. Exudates enable the transfer of all photo synthetically fixed carbon to the rhizosphere up to 20%. Exudates may also be responsible for encouraging vesicular arbuscular mycorrhizae that colonize roots and send out miles of thread-like hyphae into the soil, increasing the surface area and distance covered by the roots and taking up nutrients for the plant.

The rhizosphere environment generally has a lower pH, lower oxygen and higher carbon dioxide concentrations. However, exudates can make the soil in the rhizosphere more acidic or alkaline, depending on nutrients roots are taking from the soil. For example, when a plant takes up nitrogen as ammonium, it releases hydrogen ions which will make the rhizosphere more acidic. When a plant takes up nitrogen as nitrate, it releases hydroxyl ions which make the rhizosphere more alkaline. This action does not usually affect the bulk pH of the soil but is important for the small organisms that live in the rhizosphere because many soil organisms do not move far in the soil, but stabilize soil aggregates around the roots.

Sticky mucilage secreted from continuously growing root cap cells is believed to alter surrounding soil, and inhibit the growth of competing plant species. Plant roots are in continual communication with surrounding root systems and quickly recognize and prevent the presence of invading roots through chemical messengers. This process is known as allelopathy. In agriculture

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it can be beneficial when crop plants prevent weeds from growing nearby or detrimental when the weed plants prevent crops growing (Rebecca, 2005).

Rhizosphere Biology

The rhizosphere is a centre of intense biological activity due to the food supply provided by the root exudates. Bacteria, actinomycetes, fungi, protozoa, slime molds, algae, nematodes, enchytraeid worms, earth worms, millipedes, centipedes, insects, mites, snails, small animals and soil viruses compete constantly for water, food and space. Soil chemistry and pH can influence the species mix and functions of microbes in the rhizosphere. Most soil microorganisms do not interact with plant roots, possibly due to the constant and diverse secretion of antimicrobial root exudates. However, there are some microorganisms that do interact with specific plants. These interactions can be pathogenic (invade and kill roots and plants), symbiotic (benefit plant growth), harmful (reduce plant growth), saprophytic (live on dead roots and plants) or neutral (no effect on plants). Interactions that are beneficial to agriculture include mycorrhizas, legume nodulation, and production of antimicrobial compounds that inhibit the growth of pathogens.

Microorganisms convert organic forms of nutrients into inorganic forms that plants roots can take up. In legumes, microbial root nodulations enable plants to fix nitrogen from the air. Encourage plant growth. Rhizosphere microorganisms produce vitamins, antibiotics, plant hormones and communication molecules that all encourage plant growth. Stabilize soil aggregates, waste products and secretions from microorganisms (Lynch, 1994).

Plant Growth Promoting Rhizobacteria (PGPR) have gained worldwide importance and interest because of their agricultural benefits and are thus potential tools for sustainable agriculture for the future (Kloepper*et al.,* 2007). The use of PGPR offers an alternative way to replace and or supplement chemical fertilizers and pesticides. Soil bacteria living in the rhizosphere can enhance plant growth by several mechanisms like antagonism against plant pathogens, solubilisation of phosphates (De Freitas *et al.*, 1997) production of phytohormones (Frankenberger and Arshad, 1995; Arshad and Frankenberger,1998) siderophores production (Kloepper*et al.*,1980b; Raaska*et al.*,1993), antibiotic production (Schneider *et al.*, 1996), inhibition of plant ethylene synthesis (Glick *et al.,* 1998) and induction of plant systemic resistance to pathogens (Kloepper*et al*.,1999). Utilization of these beneficial microorganisms can reduce the use of pesticides and fertilizers that are potential pollutants of the environment. The

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concept of using PGPR mixture for disease control and plant growth promotion has already been well demonstrated in several crops. However, in some instances the root colonization of participating member in a consortium was severely affected. Compatibility of individual strains in mixture among themselves, and with commonly used agrochemicals is also some of the constraints in the use of multiple strain mixture of PGPR. Isolation of a PGPR strain with multiple plant growth promoting activities might help to address these problems.

Diversity of trophic and functional groups of rhizosphere micro-organisms

A variety of microbial forms can be found growing in rhizosphere micro-habitats. It is universally accepted that members of any microbial group can develop important functions in the ecosystem (Giri*et al*., 2005). The rhizosphere, which is the micro environment, directly influenced by root exudates, is a "hot spot" for antagonistic microorganisms and therefore an important reservoir for biocontrol agents (Kloepper*et al*., 1980b; Sorenson,1997; Berg *et al*., 2002, 2006). The rhizosphere (the zone directly surrounding and influenced by plant roots) contains a large majority of the soil's biota populations (> 10-fold of that in the bulk soil) and the plant-microbe interaction in the rhizosphere is one of the major factors regulating the health of plants. It is also widely acknowledged that root exudates govern which organisms reside in the rhizosphere (Lynch, 1994; Bardgett*et al*., 1999c). Therefore any change to the quality of rhizosphere exudates will potentially modify the dynamics of the soil biota composition (biodiversity) and activity and may cause changes to both deleterious and beneficial microflora and micro fauna.

Methodology

Collection of soil sample

Rhizosphere soil samples were taken from fields of 2-3 acres of land. Five such fields were selected each for Bt and NBt cultivating lands from each village. Five transects across each plot were chosen. The soil samples were collected at different points (five points) from each transects to get 125 soil samples for Bt and NBt soils separately from one village. Like this from all the three villages separate 125 Bt cotton cultivating soils and 125 Non Bt cotton cultivating soils were collected.

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Total cfu(colony forming units) of Bacteria, Fungi and Actinomycetes were determined as per the standard methods.Bacteria cultivated on nutrient agar,fungi on rose bengal agar and Actinomycetes on starch casein agar by dilution plate method.1gm of soil for each sample was taken and serially diluted to get dilutions of 10^{-1}to10^{-6}. 1ml of inoculum from 10^{-6},$10^{-4}$and 10^{-5} dilatations for bacteria, fungi and Actinomycetes were taken respectively. Triplicates for each dilution were maintained and incubated for 24-48 Hrs, 5-7 days,and 7-14 days at 30ºC, 24ºC and 24ºC temperature for bacteria, fungi and Actinomycetes respectively [24]. After incubation number of colonies per dilution was counted and determined cfupergram soil by the formula.

Viable count (cfu/gm soil)=Average no. of colonies per plate X dilution factor/Weight of the soil.

Viable count of bacteria, fungi and Actinomycetes were determined from all the soil samples and meanvalues of each variant (Bt soil and non Bt soil) were tabulated (table IV). By analysing the soil fertility index(table.V) and microbial populations, data compared in between Bt cotton and non Bt cotton soils.

Results and Discussion

Microbial populations:

Average number of each dilution was counted and cfu /gm soil calculated for fungi, bacteria and actinomycetes. InBt soils cfu/gm soil is about $16x10^{-4}$,$26X10^{-6}$,$12X10^{-5}$ for fungi, bacteria and actinomycetesrespectively. IntheNBt soils cfu/gm soil were $20X10^{-4}$,$35X10^{-6}$& $15X10^{-5}$fungi ,bacteria and actinomycetes. The cfu of fungi and bacteria and Actinomycetes is relatively low[table. 4]. There is a significant difference in the Number of microbial populations in both Bt and NBt soils, which can be explained the little high value in nutrient index of NBt soils when compared with Bt soils.

Conclusion:

Microbial populations like fungi, bacteria and actinomycetes were low and has observable difference(cfu/gm)in between Bt and NBt soils[28][figure. 2]. Continuous cultivation of Bt cotton may change the physicochemical nature of the soil, because of large accumulations or continuous exposure of microbes to Bt toxin. The test area has less nutrient index in spite of

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moderate Organic Carbon content, this may be attributed to less microbial activity [29]. Further research is required to determine the factors affecting the soil fertility.

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