CHAPTER 1
GENERAL INTRODUCTION

The side effects of agrochemicals on human health have renewed interest in bio-fertilizers (Aditya, et al., 2009). The present agricultural system emphasizes on high productivity for commercial purposes leading to agricultural intensification involving improved cultivars, expanding areas under single crop or cultivar devastating deforestation and above all use of agrochemicals like fertilizers, pesticides, synthetic growth regulators, etc. All these unilateral approaches of increasing production have generated several undesirable impacts viz., loss of biodiversity synonymous with destruction, deforestation and progress with pollution (Bijaya, 2001). Recent advancement in the field of bio-fertilizers creates a growing level of interest in environmental friendly sustainable agricultural practices. (Connel, 1992). Increasing and extending the role of inoculation with micro-organisms (bio-fertilizers) may reduce the need for chemical fertilizers and thereby decrease adverse environmental affects. Current developments in sustainability involve a rational exploitation of soil microbial activities (Barea et al., 1997) and the use of less expensive, though less bioavailable sources of plant nutrients, like rock phosphates (RP), which may be made available by microbiologically mediated processes (Zapata et al., 1995). Microbial populations are the key components of the soil-plant system, where they are immersed in a framework of interactions- affecting plant development (Lynch, 1990). Agriculture is a soil based industry that extracts nutrients from the soil, effective and efficient approaches that removal and returning nutrients to the soil will be required in order to maintain and increase crop productivity and for long term sustainable agriculture. Therefore, there is a need for the soil to be manipulated for increasing its fertility and for high productivity.
The bio-fertilizers include selective micro-organisms like bacteria, fungi and algae, which are capable of fixing atmospheric N or convert insoluble phosphate in the soil into forms available to plants (Sharma, 2002). Bio-fertilizers are cost effective, eco-friendly and renewable sources of plant nutrients to supplement chemical fertilizers. Bio-fertilizers play a vital role in maintaining long term soil fertility and sustainability. The use of bio-fertilizers promises to have great beneficial impact on the improvement of crop yield, quality and also upgrade eco-system, environment and soil health conserving them for future generation. Microbes that involve in the formulation of bio-fertilizers not only mobilize N and P but also secrete various growth promoting and health promoting substances. In broad sense, the term ‘biofertilizer’ may be used to include all organic resources for plant growth, which are rendered in an available form for absorption through micro-organisms or plant association or interactions.

Most of the microbial activity occurs in the soil, mainly in the surface layers and in the rhizosphere. The rhizosphere fraction is mainly influenced by the root exudates and symbiotic activities (Mestre et al., 2008), among which phosphate solubilizing micro-organisms and mycorrhizal fungi are the predominant micro-organisms. These are found inside roots, in the rhizosphere (mycorhizosphere) itself and in the bulk soil. In such soil fractions they interact with other members of the soil biota. Soil micro-organisms play a significant role in regulating eco-system processes, ranging from nutrient cycling to plant health. Studies on the interactions between plants and rhizospheric microbes are important for understanding the eco-system dynamics, in agriculture and forestry management. The mutualistic associations
between soil fungi and plant roots form a key component of soil microbial population and influence plant growth and nutrient uptake and provide a greater area for interaction with other soil microbes (Fernandez, 2008). Rhizosphere processes include reaction kinetics between root exudates, different forms of P, dissolved organic carbon, metal ions and the respective sorbed species. Equilibrium and kinetic sorption processes, besides complexation, mineralization, dissolution, precipitation, degradation and decay processes are implemented. Interaction between soil microorganisms P and exudates should be additionally accounted. Time dependent boundary conditions imply exudation of organic ligands and uptake of phosphate ions at the root surface (Klepsch, 2008).

Although use of chemical fertilizers for improving soil fertility is common approach for increasing agricultural production, a large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application and becomes unavailable to plants (Yadav and Dadarwal, 1997). Phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of the soil and ambient soil conditions (Khan et al., 2007). Soils have significant amount of phosphorus which is not immediately available to crops and only a small fraction becomes available during the cropping season. In order to maintain the amount of phosphorus available in soil for plant use, large amount of phosphorus based fertilizer is added to soil (Omar, 1998), often, the bulk also could be converted to insoluble form. Most agricultural soils contain large reserves of phosphorus, a
considerable part of which accumulates as a consequence of regular application of phosphatic fertilizer. Nearly, 95-99% is present in the insoluble form and hence cannot be utilized by the plants. Phosphorus play a vital role in plant nutrition. Phosphorus is found in soil, manure, plant and micro-organisms in various organic and inorganic forms. In soil, phosphorus is present in the form of fluoroapatite, hydroxyapatite or chloroapatite, as iron or aluminium phosphate or in combination with the clay fraction, which are unavailable to the plants. The calcium phosphates are dominant in neutral to alkaline soils, whereas iron and aluminum phosphates occur in acidic soils. From vegetation and plant decay residue, the soil also receives organic phosphates. Mobilization of such insoluble phosphates accumulated in soil is of high practical importance in intensive farming system to get optimum crop yield.

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Phosphorus is found in plants as a constituent of nucleic acids, phospholipids, the co-enzymes NAD and NADP and most important, as a constituent of ATP and other high-energy compounds. Phosphorus is found, of course, in other compounds of the plant, but these are considered most important. Higher concentrations of phosphorus are found in the meristematic regions of actively growing plants, where it is involved in the synthesis of nucleon proteins. For example, not only phosphorus is found in nucleic acid, most of the nucleoprotein molecule, but it is also involved, through ATP, in the activation of amino acids for the synthesis of protein moiety of this compound. Phospholipids, along with protein are significant constituents of cell membranes. The co-enzymes NAD and NADP are important in oxidation-reduction reactions in which hydrogen transfer takes place. Such essential plant processes as photosynthesis, respiration, nitrogen metabolism, carbohydrate
metabolism and fatty acid synthesis. To name a few, are dependent on the action of these co-enzymes. Phosphorus is associated with several vital functions and is responsible for several characteristics of plant growth, such as utilization of sugars, starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and transfer of hereditary character.

The symptoms of phosphorus deficiency can be confused with nitrogen deficiency, although the symptoms are not as pronounced as those found for nitrogen. Similar to nitrogen deficiency, phosphorus deficiency may cause premature leaf fall and purple or red anthocyanin pigmentation. Unlike plants lacking nitrogen, those lacking phosphorus may develop dead necrotic areas on the leaves, petiolis or fruits, they may have a general overall stunted appearance and the leaves may have a characteristic dark to blue-green coloration (Devlin et al., 2002).

Phosphate solubilizing micro-organisms convert insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions. Such microorganisms may not only compensate higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (Srividya, 2009).

Phosphate solubilizing soil and rhizosphere microorganisms have been distinguished by their relative abilities to dissolve calcium phosphate and apatites in pure culture and in association with plant roots. Fungi are important components of soil micro biota, typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Phosphate solubilizing microbes play
fundamental roles in biogeochemical phosphorus cycling in natural and agricultural eco-systems. Phosphate-solubilizing microbes can transform the insoluble phosphorus to soluble forms by acidification, chelation, exchange reactions and polymeric substance formation (Delvasto et al., 2006). Microorganisms substantially influence the soil productivity by solubilizing this insoluble P through their metabolic processes in soil (Ravikumar et al., 2007; Sahu et al., 2007). Phosphate solubilizing microorganisms convert insoluble Phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions. Such microorganisms may not only compensate for higher cost for manufacturing fertilizers in industry but also mobilize the fertilizers added to soil. The fact that certain soil microbes are capable of dissolving relatively insoluble phosphatic compounds (Bojinova et al., 1997) has opened the possibility for enduring microbial solubilization of phosphates in soil. Previous workers believed that the phenomenon was closely related with the ability of the microbes in producing selected organic acids and or extracellular polysaccharides (Goenadi et al., 1995; Omar, 1998; Kim et al., 1998). Solubilization of inorganic phosphate by microorganisms involve a wide range of processes involving the secretion of organic acids, lowering of pH as a result of acid production ion chelation and exchange reactions (Molla and Chowdhary, 1984) which are considered to be part of the phosphorus cycle. Several species of microorganisms isolated from soil possess the ability to solubilize soil bound inorganic phosphates. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil and ambient soil conditions. To circumvent phosphorus deficiency, phosphate solubilizing microorganisms (PSM)
play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner. The solubilization of phosphatic compounds by naturally abundant phosphate solubilizing microorganisms is very common under invitro conditions, the performance of phosphate solubilizing microorganisms in situ has been contradictory (Khan, 2007). Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment play a key role in phosphate solubilization by phosphate solubilizing microorganisms (Halder et al., 1991; Abd-Alla, 1994, Whitelaw, 2000). Fungi possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996).

These phosphate solubilizing microorganisms can, thus, be used as biofertilizers thereby improving crop yield, maintaining soil quality and soilfauna, rendering ecological balance, creating less health and environmental hazards. Moreover, the potentialities of these microorganisms can be harnessed in increasing the crop production, early maturity, shortening the crop duration, which is of particular importance. Further, agriculture, to be sustainable requires comprehensive solutions built upon by the people and the local knowledge to develop resource-conserving technology. The dimensions of sustainable agriculture for conservation of natural and biological diversity in plants is necessary to improve and sustain production of agricultural and related enterprises to keep open future options as buffer against hazardous environmental changes as the new material for much scientific and industrial innovation and a matter of morals.

The issue of sustainable development and eco-friendly measures has been gaining momentum as against the general trend of using extensive synthetic
agrochemicals for rapid increase in the productivity and disease management of crops and to feed the ever increasing human population. The conversion of many fertile lands to dust bowl were attributed to the rampant use of synthetic agrochemicals with unmanageable residual effects.

In order to find solution to enhance crop yield through an eco-friendly means by employing bio-fertilizer technology, the present work is taken up with the main objective of utilizing the natural stock resource of phosphatic bio-fertilizer from the rhizosphere. For a more scientific study, the research area has been confined to a smaller study area of studying the effect of these phosphatic fungal bio-fertilizers on the growth and yield of ginger plant. Ginger is chosen as the test crop as the crop is one of the most important commercial spice crop grown for the aromatic rhizomes, it has high medicinal value and it is economically important.

Ginger (Zingiber officinale Roscoe) is a perennial, herbaceous, monocotyledon, having underground modified rhizomatous stem and usually grown annually. The economic part of the plant is the robust branched rhizome borne horizontally near the surface of the soil. Because ginger is not found in the wild, their origins are uncertain. It is unlikely to have originated from India as ginger plants there show the most biological variability. Ginger has a long history of use in south Asia, both in dried and fresh form. The Hindu epic Mahabharata written around the fourth century B.C. describes meal where meat is stewed with ginger and other spices. Ginger is a tropical plant adapted to grow in regions of sub-tropical climate at higher elevations (Utpal et al., 2006). Ginger thrives best in well drained sandy loam rich inorganic matter and in clayey loam soil with good drainage and aeration.
supplemented with organic matter (Johny and Ravindran, 2002). The crop is sensitive to water logging, frost and salinity and tolerant to wind and drought. It thrives on a wide range of soils, but for high yield, it prefers slight, loose, friable and well-drained soil rich in humus and slightly acidic (pH 6.0 - 6.5) in reaction (Anonymous, 2002).

Ginger is carminative, pungent, stimulant, used widely for indigestion, stomachache, malaria and fevers. It is chiefly used to cure abdominal pain, anorexia, arthritis, atomic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, astic fibrosis, diarrhoea, and difficulty in breathing, dropsy, fever, flatulent, indigestion disorders of gallbladder, hyper acidity, hypercholesterolemia, hyperglycemia, morning sickness, nausea, rheumatism, sore throat, ache, stomach ache and vomiting. Ginger forms an important constituent of many pharmacopoeial Ayurvedic formulations (Misra et al., 1969, Nandakarni, 1993). Gingerol, one of the primary pungent principles of ginger helps counter liver toxicity by increasing bile secretion. Ginger has potent anti-microbial and anti-oxidant qualities as well.

The present study is propelled with the broad objective of exploring the potential phosphate solubilizing fungal strain from Rengkai, Churachandpur district and their possible application as biofertilizer for sustainable ginger production. In the light of the facts, the present study was undertaken covering the following objectives:-

1) To survey the occurrence and characterization of phosphate solubilizing fungi isolated from the rhizosphere soil of different ginger growing sites of Churachandpur District, Manipur.
2) Evaluation of the fungal species isolated from the ginger growing area for their phosphate solubilizing potential.

3) Effect of pH on the growth and phosphate solubilization potential of the fungal isolates under study.

4) Evaluation of the potential fungal isolates in relation to the growth and yield of ginger under the field condition.

5) Recommendation for the adoption of phosphatic fungal biofertilizer technology by the poor and marginal farmers for sustainable ginger cultivation.

1.1. **Study site**

The state of Manipur is located in the North Eastern part of India. Manipur has a total area of 22,327 sq. km. Manipur is a hilly state as plain covers just 10% of the total area. Manipur is endowed with wide range of climates, physiographic settings, geology and vegetative sequences. Manipur is divided into nine districts. And the biggest among them being the Churachandpur district. Manipur is situated at an elevation ranging 40m (Jiribam) to 2593m (Tamenglong) above Mean Sea Level. The average annual rainfall is recorded 967.2 mm. Most of its rains are received during summer season, which is when the south- western monsoon wind sets in.

The climate of Manipur can be broadly classified into

a) Temperate prevailing in the higher altitude of hill where temperate fruits and vegetables can be grown throughout the year,

b) Sub-tropical prevailing in the lower attitudes hills and central valley plain where winter lasts from November to February and rainy season from May to September.
The transition period of March, April and October can be described as spring and autumn though short,
c) Tropical weather prevailing in Jiri plains and foothills- during March. In this plain and foothills all the tropical crops can be raised. The temperature ranged from sub-zero to 36°C.

Manipur can be divided into 3 Agro-Climatic Zones

- Temperate and sub-alpine zone
- Sub tropical plain zone
- Mild tropical hill zone

In the Techno-economic feasibility survey conducted by National Horticulture Board, 277064 hectare (i.e. 2770.64 sq. km.) land of the state has been identified as potential horticulture area.

Churachandpur, with a geographical area of 4570 sq. km., is situated with 23°56’20.4”N & 24°36’46.8”N along the latitudes and 92°58’12”E & 93°52’58.8”E along longitudes. Churachandpur is bounded by Senapati district on the north, Bishnupur and Chandel district on the east, Mizoram and Assam state on the west and Myanmar to the south.

Major parts of the district are occupied by rugged hills constituting parts of the southern extension of Naga Hill ranges, with their elevation ranging from 350 to 1,950 m above mean sea level (AMSL). Thus making the district ideal for some horticulture crops such as Pineapple, Orange, Tomato, Ginger, Tapioca etc. Of the total geographical area of 4,570 sq. km. of the district, about 5.57%, i.e. 255 sq. km. is the valley portion. A small and narrow valley adjoining Churachandpur having an
areal extent of 200 sq. km. is present through which the Khuga River, a tributary of Manipur River is flowing towards northern direction. This valley actually forms the southern extension of Manipur valley and is locally known as Khuga valley. In addition, small valley areas are seen around Beheng, Leizangphei and Tuilaphai. The area is mainly drained by the tributaries viz. Khuga, Tuila, Tuili, Leimatak, Tuivai and Barak of Manipur River. Leimatak River and Tiupuilui River, tributaries of Irang River flow towards northerly direction, while the rest of the rivers flow in general southerly direction.

Churachandpur district enjoys sub-tropical to temperate monsoon climate varying from place to place depending on the density of rainfall and elevation. The hottest recorded lies along the foothills of Vangai Ranges, followed probably by Leijanphai area near Thanlon. They are well known for forest and cereal crops and spices. The interaction amongst these factors in the ecosystem with time and space leads to result in the formation of different kinds of soils with different properties, limitations and potentials.
Churachandpur can be divided into three Agro-ecological sub regions.

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<th>Agro ecological sub regions</th>
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<td>17c North Eastern Hills - Purvachal warm humid agro ecological sub region with deep fine red and lateritic soils of high to very high AWC(200-300 mm/m) and 330 days of LGP</td>
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<tr>
<td>15d North eastern Hills - Purvachal hot humid agro-ecological subregion with deep fine red and lateritic soils of high to very AWC(200-300 mm/m) and more than 270 to 300 days of LGP</td>
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<tr>
<td>17a North Eastern Hills - Purvachal warm perhumid agro-ecological sub-region with deep fine red and lateritic soils of high to very high AWC(200-300 mm/m) and more than 330 days of LGP</td>
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LGP : Length of Growth Period

AWC: Available Water Capacity

Churachandpur has rich variety of soils, to be precise subgroups, which are suitable for production of horticulture crops. These subgroups are Typic Dystocrept, Typic Haplaquepta, Typic Haplohumults, Typic Haplumbreplts, Typic Udorthents and Typic Palehumits.

The district has rich resources in term of land and soil fertility, rain-water, vegetation etc. and prevalence of suitable agro-climatic conditions temperate to tropical and subtropical zones for the development of horticulture in the area. Despite these natural advantages, growth of horticultural crops in the region has remained
lackluster till recently due to the wide gap between the technologies generated and their adoption by the farmers in the farms and orchards.

The National Horticulture Board has identified 38542 hectare area (i.e. 385.42 sq. km.) as potential horticulture area in its Techno Economic Feasibility Survey.

**Production of GINGER in different districts of Manipur during the year 2013-14 (in MT)**

**Production of Ginger in Churachandpur district (in MT)**

*Source: Department of Horticulture & Soil Conservation, Manipur*