INTRODUCTION

Nutrient needs of growing plants can be met through a number of sources. The major sources of plant nutrients are mineral fertilizers, organic manures, and recycled wastes, biological nitrogen fixation (BNF) and natural minerals and the lesser nutrients recycled through irrigation waters and precipitation, which supplement the soil nutrients reserves for nourishing crops.

Soil is a living entity which possesses a number of organism. The microbes present in the soil are responsible for the living nature of the soil. The microbial activity is enhanced due to application of organic manures like cattle manure, green manure and oil cakes, etc., to the soil. The soil microbes play a very important role in the supply of nutrients to plants by fixation, solubilization and mobilization etc., from atmosphere and soil. But the continuous use of chemical fertilizers, pesticides, insecticides, fungicides and weedicides, etc., in the soil, has eradicated all the beneficial microbes, which are very essential for plant growth, yield and quality. The microbial population and activity determine the soil fertility or soil health, which lead to crop productivity.

In recent days, the use of laboratory grown microbial inoculants or microbial cultures has attracted the world towards sustainable agriculture farming. There are two types of bioinoculants used for crop cultivation i.e., Biofertilizers and Biocontrol agents. Biofertilizers are preparations containing agriculturally useful microorganisms, which help in mobilizing plant nutrients through their biological activity. It is an eco-friendly and cost effective method. The biofertilizers are known to increase the plant growth and crop yield through increased nutrient availability, soil productivity and sustainability.
In integrated plant nutrient system, one of the major components is the carrier based microbial inoculants, commonly referred to as “Biofertilizers”. Biofertilizers generally are defined as preparation containing live or latent cells of efficient strains of N-fixing, P-solubilizing or cellulolytic microorganisms used for application to seed or soil (Motsara et al., 1995).

Biofertilizers can be divided into three main categories depending on their types of activity i.e., N$_2$ fixing biofertilizers, Phosphate solubilizing/mobilizing biofertilizers and plant growth promoting biofertilizers.

1.1 N$_2$ FIXING BIOFERTILIZERS

Nitrogen is the most effective nutrient in crop productivity. It is required in large amounts of all the nutrients by crops. Crops obtain their N from (i) soil reserves (ii) fertilizers and manures, (iii) accretion through precipitation and irrigation water and (iv) through biological nitrogen fixation (BNF) involving a number of diverse organisms

BNF is a major source of N input on the earth. The basic raw material for N is fixation, whether biological or industrial, and the air, 79% of which is N. It is abundantly clear that industrially fixed N can not meet the total N need of the world, more so in countries with an increasing population and ever growing food and fiber needs. The widely accepted strategy is economically and efficiently utilizes the available source of plant nutrients in an integrated manner. Thus the N needs of agriculture will have to be met from fertilizers, organic manures, efficient recycling and biofertilizers.

Biological nitrogen fixation offers an economically attractive and ecologically beneficial source route for augmenting nutrient supplies. Thus, Rhizobium for legumes, Blue green algae and Azolla for wet land rice, Azotobacter and Azospirillum for cereal crops can play a significant role in agriculture. The N
fixing biofertilizers makes an addition to N supplies by fixing atmospheric nitrogen for the soil-plant system.

1.2 PHOSPHATIC BIOFERTILIZERS

Phosphorous is an important nutrient, next only to nitrogen and classed along with nitrogen and potassium as a major nutrient element. Phosphatic fertilizers are in a different class in that they help in increasing the solubility/availability of nutrient P which is already present in the soil in sparingly soluble forms. These do not bring in P form outside. By doing so, they in a way deplete the soil P reserves but considering the low utilization efficiency of phosphatic fertilizers, P solubilizing biofertilizers can play an important role in improving the efficiency of P residues left in the soil. It was estimated that 140 million tones of low-grade phosphate are deposited in the soil, which cannot be directly used by the plants. The plants can use it only by the introduction of phosphorous solubilizing bacteria in to the soil. Several soil bacteria and fungi notably the species of Pseudomonas, Bacillus, Penicillium and Aspergillus secrete organic acids and lower the pH in their vicinity to bring about solubilization of bound phosphates in soil.

1.3 PLANT GROWTH-PROMOTING BIOFERTILIZERS

Pseudomonas sp. are receiving world wide attention under the broad general category known as plant growth promoting rhizobacteria (PGPR) or plant health promoting rhizobacteria (PHPR)(Schroth and Hancock, 1981, Kloepper et.al.1989). The bacteria exhibit fluorescence under u-v light and hence are also known as Fluorescent pseudomonads. Three possible mechanisms have been suggested to explain the beneficial effects of PGPR in enhancing production. They are competition for substrate and niche exclusion, production of siderospores and antibiotics. However, more than one mechanism may operate for mediating a
biological control. *Fluorescent pseudomonads* ‘mop up’ nutrients in the rhizosphere because of their versatility in grown and nutrient absorption.

These bacteria, generally, improve the plant growth through direct effects on plants by producing plant growth promoting substances (Gaskins et al. 1985, Lynch, 1982) by increasing the availability and uptake of mineral nutrients (Gaskins et al., 1985, Barber 1978, Barber et al., 1977) and by suppressing the soil-borne pathogens or other deleterious rhizosphere microorganisms (Gaskins et al., 1985, Schroth and Hancock 1982). The suppressing of the growth of soil-borne plant pathogens by the rhizosphere microorganisms is termed as biocontrol.

It is a well-known fact that biofertilizers play an important role for supplementing the essential plant nutrients for sustainable agriculture, economy and ecofriendly environment. The use of biofertilizers in crops/plants have been recommended considering the effective/sufficient dose and beneficial response on growth of plants and yield of crops.

There are different methods of application of biofertilizers in crop plants viz., (I) seed treatment (ii) seedling treatment (iii) sett treatment and (iv) soil treatment which are depending on the sowing system of crops.

Biofertilizers are known to make a number of positive contributions in agriculture. For example:

1. They supplement fertilizer supplies for meeting the nutrient needs of crops
2. They can add 20 – 200 kg N/ha (by fixation) under optimum conditions and solubilize/mobilize, 30 – 50 Kg P₂O₅/ha.
3. They suppress the incidence of pathogens and control diseases.
4. They liberate growth promoting substances and vitamins and help to maintain soil fertility.
5. They increase crop yield by 10 – 50%, N-fixers reduce depletion of soil nutrients and provide sustainability to the farming system.

6. They are cheaper, pollution free and based on renewable energy sources.

7. They improve soil physical properties, tilth and soil health in general.

The use of biological fertilizers would reduce the cost of chemical fertilizers involved in crop production. The effective utilization of these biological fertilizers for different cropping systems will not only provide economic benefits to farmers but also improve and maintain the soil fertility and sustain the soil ecosystem.

Inoculation of crop with beneficial microorganisms is gaining momentum in sustainable agriculture. In integrated plant nutrient system (IPNS), one of the major components is the carrier based microbial inoculants, commonly referred to as 'Biofertilizers'. Economically important crops are commonly included with selected strains of plant beneficial microorganisms, mass multiplied under laboratory condition and mixed with a carrier for supply to the farmers. Carrier is a medium matrix on which the inoculated microorganisms grow to a reasonably higher population for an initial period and there after decline. The nature of the carrier often determines the subsequent performance of the inoculant. The introduction of artificial microbial culture in 1890's was in the form of agar-based culture. These early cultures were essentially the agar cultures of today but they changed to carrier-based culture to overcome short shelf life of agar and liquid cultures.

The biofertilizer is manufactured using effective strains and it is first mass cultured in a liquid medium in a fermentor or containers. The broth is then mixed with a carrier, it may be peat or lignite or charcoal. Finally it is subjected to curing wherein the initial bacteria increase in number depending on the nutrient availability in the carrier. Hence the carrier plays an important role in the shelf life
of the inoculated microbial culture. In moist formulation, the carrier absorbs and retains the moisture and provides a surface for growth of the bacteria.

Wide ranges of carrier have been developed for carrying microbial inoculants in sufficient numbers. The carrier material like peat, lignite, charcoal, vermiculite, perlite, sawdust, rice husk and farmyard manure etc., are being used as carrier material by the biofertilizer manufacturers depending on the availability of quality material.

As per the ISI specifications, the carrier should be in powder form and capable of passing through 150 – 212 micron (72 – 100 mesh) IS sieve (Motsara et al., 1995). By making the carrier into powder form, it increases surface area for bacterial growth and adhesion ability for seed application. The qualities of a good carrier material are:

1. Highly absorptive
2. Non-toxic to microorganisms
3. Easy to sterilize (by autoclaving or gamma radiation)
4. Available in adequate amount and inexpensive
5. Provide good adhesion to seed.
6. Have pH buffering capacity

The carrier material determines the quality of the bioinoculants. It is found that peat is an ideal carrier material for biofertilizer. It has been stabilized as the standard and most dependable carrier worldwide (Strijdom and deschodt 1976, Burton, 1982). But in India the available peat is not as good as the Australian or American peat and it is available only in Nilagiri valley (Tamilnadu). Hence there is a high cost involved in the transportation. The availability of this peat to the commercial Biofertilizer manufactures is made very difficult because of forest conservation policy of our country.
Presently, lignite powder is being used as carrier material by most of the bioinoculant producing units in India. Often it has also been found that its availability is also made difficult, as it is being used as fuel by thermal power stations, etc. Availability of quality lignite powder is also in doubt because of adulteration by agents and improper mesh size in the pulverizing unit. Several scientists have suggested compost as carrier material for biofertilizers. But the role of good compost in maintaining microbial population has not been studied much. The existing studies exhibit that the earthworm casts is ideal material for carrying microbial culture in the agriculture point of view.

To increase the shelf life of bacteria in the carrier, the vermicompost was selected as a carrier material for the following reasons.

1. Vermicompost can be prepared by easily because of it is prepared from organic wastes, which contain all type of nutrients, vermicompost containing all type of nutrients as balanced level.

2. The vermicompost can be used directly without further processing in the production unit.

3. By using specific type of organic waste, the nutrient content of vermicompost can be standardized.

4. Vermicompost is a low cost product.

Vermicompost carrier was compared for its characters with lignite carrier, which has been used widely by biofertilizer manufacturers.

Among all the beneficial plant microorganisms, *Azospirillum* - Nitrogen Fixing Biofertilizer microorganism, *Bacillus megaterium* - Phosphate Solubilizing Biofertilizer microorganism and *Pseudomonas fluorescens* – Plant Growth Promoting biofertilizer microorganism was selected for the present study.
Azospirillum:

Azospirillum is plump gram-negative, vibrio or straight rods, 0.9-0.2μm in width, often with pointed ends. Intracellular granules of poly-β-hydroxybutyrate are present. Cells are motile with a characteristic corkscrew-like or vibratory motion in liquid media by means of single polar flagella may also be formed in addition to the polar flagellum. Lateral flagellum by some species when cells are cultured on solid media at 30° C. The colonies of some strains form a light pink or dark pink pigment on potato agar. Optimum growth temperature is 34-37°C. Some strains grow well in pH 7; others prefer more acidic conditions. They are nitrogen fixers, exhibiting N$_2$ dependent growth under microaerobic conditions. They grow well under an air atmosphere in the presence of a basic source of fixed nitrogen, such as an ammonium salt. They possess mainly a respiratory type of metabolism with O$_2$ and with some strains, and NO$_3$ as terminal electron acceptors. Weak fermentative ability may also occur. Under severe oxygen limitation some strains may dissimilate NO$_3$ to N$_2$O and N$_2$. They are oxidase positive. They are chemoorganotrophic; however, some strains are facultative hydrogen autotrophs. They grow well on the salts of organic acids, such as malate, succinate, lactate, and pyruvate; certain carbohydrates may also serve as carbon sources. Some strains require Biotin. Species occur in free living in soil or in association with the roots of cereal crops, grasses and tuber plants. Root nodules are not induced (Bergey’s manual of Determinative bacteriology-ninth edition).

Bacillus:

Cells are rod shaped and straight, 0.5 – 2.5 X 1.2 – 10 μm long and often are arranged in pairs or chains, with rounded or squired ends. Cells stain gram positive and are motile by peritrichous flagella. Endospores are oval or sometimes round or cylindrical and are very resistant to many adverse conditions. There is not more than one spore per cell, and sporulation is not repressed by
exposure to air. Aerobic or facultative anaerobic, with wide diversity of physiological ability with respect to heat, pH, and salinity. Chemoorganotrophs, with a fermentative or respiratory metabolism. Usually catalase positive. Found in a wide range of habitats; a few species are pathogenic to vertebrates or invertebrates (Bergey’s manual of Determinative bacteriology-ninth edition).

**Pseudomonas:**

Straight or slightly curved rods, but not helical, 0.5 – 1.0 X 1.5 – 5.0 μm. Many species accumulate poly-β-hydroxybutyrate as carbon reserve material, which appears as sudanophilic inclusions. They do not produce prosthecae and are not surrounded by sheaths. No resting stages are known. Cells stain gram negative. Motility occurs by one or several polar flagella; they are rarely non-motile. In some species lateral flagella of shorter wavelength may also be formed. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor, allowing growth to occur anaerobically. Xanthomonadins are not produced. Most, if not all, species fail to grow under acidic conditions (pH 4.5). Most species do not require organic growth factors. Oxidase is positive or negative. Catalase positive and chemolithotrophs, able to use H₂ or CO as energy sources. Widely distributed in nature. Some species are pathogenic for human, animals, or plants (Bergey’s manual of Determinative bacteriology-ninth edition).

To study the performance of these three types of carrier based inoculants, rice crop was selected, as it is very suitable to know the effect of biofertilizer microorganisms. Rice is the staple food in south India and is extensively grown in more than one season. Farmers raised this crop with farmyard manure or green manure in ancient days but have resorted to cultivate this crop with mainly inorganic fertilizers since 1950’s. The awakening that soil fertility is depleted due to continuous use of inorganic fertilizers and the steadily increasing cost of fertilizers made rice growers to think of alternatives or means by which the use of
inorganic fertilizers can be reduced. The low cost and ecofriendly input like blue green algal biofertilizer and *Azospirillum* biofertilizer are now widely used by farmers to supplement the nitrogen requirement of rice.

Phosphorous is second only to nitrogen, as mineral nutrient required by both plants and microorganisms. Phosphorous in soil is immobilized or becomes less soluble either by adsorption, chemical precipitation or both. Hence the plants can use this fixed form of phosphate only by introduction of phosphorous solubilizing bacteria (PSB) into the soil. Phosphatic fertilizers are applied in high amount, next to nitrogenous fertilizers and rice responds more for phosphatic fertilizers.

Probably plants require a definite level and ratio of the plant growth hormones to maintain their normal growth and development. It is also well established that several rhizosphere bacteria, especially root colonizing *Pseudomonas* sp. produce vitamins and growth hormones (auxin, cytokinines) which often lead to certain morphological changes in the plants (Brown 1972, Prikryl et al., 1985 and Derylo et al., 1993).

It is, therefore, expected that the plants may respond in a different way to the invasion of hormone producing rhizosphere bacteria at different stages of their growth and development. Apart from inducing the plant growth, *Pseudomonas fluorescens* affect the disease development like sheath blight of rice.

In the present investigation, an attempt has been made to study the influence of vermicompost in improving shelf life of inoculated bacterial cultures and the performance of vermicompost based bio inoculants on growth, yield components and biochemical properties of rice. The main objectives of the present work are as follows:
1. To develop a best carrier material for Nitrogen fixing bacteria – *Azospirillum* sp, Phosphate solubilizing bacterium - *Bacillus megaterium* and Plant Growth Regulating Rhizobacterium - *Pseudomonas fluorescens* inoculants for good performance in the field conditions.

2. To investigate the effect of vermicompost based *Azospirillum sp., Bacillus megaterium* and *Pseudomonas fluorescens* inoculants on yield and quality of low land rice.

3. To study the role of *Azospirillum sp., Bacillus megaterium* and *Pseudomonas fluorescens* on Biochemical changes during crop period.

4. To study the role of conjunctive use of *Azospirillum sp., Bacillus megaterium* and *Pseudomonas fluorescens* with inorganic source of nitrogen and Phosphorous on yield and quality of low land rice.