INFLUENCE OF PHOSPHATE SOLUBILIZING AND MOBILIZING MICRO-ORGANISMS ON PHOSPHORUS USE EFFICIENCY IN WHEAT USING ^{32}P AS A TRACER

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INFLUENCE OF PHOSPHATE SOLUBILIZING AND MOBILIZING MICRO-ORGANISMS ON PHOSPHORUS USE EFFICIENCY IN WHEAT USING ^{32}P AS A TRACER

By

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This is to certify that the thesis entitled "**Influence of phosphate solubilizing and mobilizing micro-organisms on phosphorus use efficiency in wheat using ^{32}P as a tracer**" submitted to the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Agronomy**, embodies the results of *bona fide* research work carried out by **Mr. Bipin Bihari Panda**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

All the assistance and help received during the course of the investigation have been duly acknowledged by him.

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CONTENTS

LIST OF TABLES

LIST OF TABLES (contd....)

Chapter 1

INTRODUCTION

India is the second most populous country with 16% of the world population. It has achieved a fair amount of self-sufficiency in food grain production. But looking at higher population growth rate compared to food grain production, it is imperative that there will be a shortage of food grain in the near future. Thus India has to step up production in order to feed the burgeoning population.

Wheat, being an important *rabi* cereal crop contributes a major portion to foodgrain production. Currently, in India, the production of wheat is 68.7 million tonnes from an acreage of 25.1 million hectares with productivity at 2.74 tonnes per hectare (Anonymous, 2002). Though, India ranks second in acreage and production of wheat, the productivity is too low compared to other countries. With limited scope of increasing cultivable area under wheat, production can only be increased through vertical expansion. Wheat is an exhaustive crop and heavily depletes the soil nutrient. Further, introduction of high yielding varieties aggravates the situation. Thus, higher productivity of wheat can only be achieved by the adoption of suitable variety and improved agronomic practices with balanced and judicious use of chemical fertilizers.

Phosphorus is one of the essential plant nutrients making up about 0.2% of plant dry weight. After nitrogen, it is the most limiting nutrient for plant growth. It is the 'master key element' for many metabolic functions in living system and plays a major role in cell division and development, photosynthesis, seed formation, break down of sugars and energy transfer. It is a component of sugar phosphate, nucleotides, nucleic acids, coenzymes, phospholipids, phytic acids etc.

Phosphorus has become a major constraint in Indian agriculture because of its short supply due to meagre deposits. It is a costly input that involves import of raw materials which erodes the foreign exchange of the country and energy intensive production process. About 95% of Indian soils are low to medium in phosphorus status requiring external supply to reap good yields (Tandon, 2001). Besides, the recovery of applied P is lower i.e. 10-20% compared to 30-50% for N by the crop. The lower recovery of applied phosphorus is mainly due to slow mobility and quick fixation of phosphorus. Thus, phosphorus applied is not lost from the soil but stored in soil reserves. A substantial component of P in any soil is in the form of sparingly soluble mineral phosphates which are largely unavailable to plants. The chemical fixation process also renders most of the applied P fertilizer unavailable and has resulted in accumulation of insoluble P complexes in agricultural soils. This could lead to environmental problems with P fertilization due to run-off from agricultural lands (Portch, 2001). Release and mobilization of phosphorus from soil phosphate, therefore, are major aspects of P management. Many soil and rhizospheric micro-organisms have the ability to release phosphate from sparingly soluble mineral phosphates found in soils and are important in providing soil phosphate for plant.

Favourable response of phosphate solubilizing bacteria and fungi such as *Pseudomonas striata* and *Aspergillus awamori*, respectively, as seed inoculation has been reported by different workers. Vesicular Arbuscular Mycorrhiza (VAM) has also been noticed to have favourable effect on P uptake. But the information available on the impact of such micro-organisms on phosphorus use efficiency under varying levels of phosphorus in wheat is very scanty and sparse.

Further, using tracer technique in fertilizer studies the amount of added fertilizer taken up by the plants could be measured and the impact of micro-organisms on phosphorus use efficiency could be evaluated accurately.

Keeping above facts in view a field experiment entitled **"Influence of phosphate solubilizing and mobilizing micro-organisms on phosphorus use efficiency in wheat using ^{32}P as a tracer"** was conducted with following objectives:

1. To study the effect of phosphorus levels and applied micro-organisms on the phosphorus use efficiency.
2. To find out the optimum level of phosphorus to wheat in combination with different phosphate solubilizing and mobilizing micro-organisms.
3. To study the influence of micro-organisms on the availability of native and applied phosphorus.

Chapter 2

REVIEW OF LITERATURE

Nutrient phosphorus is an absolute necessity for successful crop production. It is the second most important nutrient next to nitrogen and termed as "Master key" element in crop production (Pierra, 1938). The involvement in a wide range of physiological processes of crop plants starting from cell division to the development of good root system and to ensure timely and uniform ripening of the crop reveals the key role of phosphorus in plant's life. It performs a number of vital functions related to growth, development, photosynthesis and utilization of carbohydrates in life processes. It is also associated with cell organization, nucleus formation and transform of heredity (Arnon, 1956).

Indian soils are generally very poor in available phosphorus which needs heavy application of phosphatic fertilizer for maintaining and increasing the grain production. Phosphatic fertilizers are expensive as it requires costly inputs and intensive processing for its manufacture. Again, a large portion of water soluble phosphatic fertilizers gets converted to insoluble form immediately after application to soil, leading to lower availability to crop. The soil micro-organisms which are associated with plant roots and its immediate environment play a key role in the uptake of P by the plant. The rhizosphere microflora can therefore, be manipulated to allow crops to grow with lesser and cheaper fertilizer. Research efforts are needed to develop low input technology for substituting or supplementing costly phosphatic fertilizer using phosphate solubilizing and mobilizing micro-organisms. The information from field experiment is inadequate so far, to indicate the extent to which these biofertilizers are responsible for increasing availability of phosphorus to crop plants under natural condition (Khalil *et al.*, 1992 and Chhonkar, 1994).

In this chapter, the relevant literature pertaining to the present investigation is reviewed under the following headings:

1. Response of crops to:
    - (i) Phosphorus
    - (ii) Phosphate solubilizing micro-organisms
    - (iii) Vesicular Arbuscular Mycorrhizae
2. Studies on microbial population in the rhizosphere
3. Phosphorus use efficiency using radio-tracer technique
4. Economics

2.1 Response of crops

2.1.1 Phosphorus

Green revolution in India brought significant change in the production potential of wheat. There was a sudden increase in phosphorus requirement after the introduction of high yielding dwarf varieties which were more responsive to phosphorus in comparison to tall wheat varieties (Khera and Datta, 1969). Thus, effective phosphorus management became an integral part of production technology.

2.1.1.1 Effect on crop growth

Effect of phosphorus on dry matter accumulation in wheat is largely determined by the initial soil phosphorus status (Mahajan and Bisen, 1980), levels of phosphorus application (Singh *et al.*, 1987) as well as interaction of phosphorus with other nutrients like nitrogen (Hooda and Agarwal, 1987) and zinc (Alam *et al.*, 1988). Phosphorus requirement of wheat is maximum from its germination to tillering stage. An experiment carried out at Varanasi to study the effect of nitrogen, phosphorus and potassium on wheat dry matter production improved only upon 40 kg P_2O_5 ha^{-1} while higher dose of 80 kg P_2O_5 ha^{-1} failed to evoke further response (Singh *et al.*, 1987). However, a positive effect of direct application of phosphorus on dry matter production in wheat has been observed by many workers (Rai *et al.*, 1982; Singh *et al.*, 1987 and Venugopal and Prasad, 1989).

Kolar and Grewal (1989) observed that phosphorus application increased the leaf area index, chlorophyll content of the leaves and interception of photosynthetically active radiation (PAR) which resulted in increased grain yield of wheat.

A spectacular increase in the rate of root growth and the number of secondary roots due to phosphorus application had been affirmed by Israel (1991) and Lu and Barbar (1995).

Sharma and Parmar (1998) reported that the increased levels of P up to 78 kg ha^{-1} to wheat significantly improved the dry matter yield at tillering stage

(30%), flowering stage (93%) and harvesting stage (148%) in a mountain Alfisol of the Western Himalayas.

2.1.1.2. Effect on yield and yield attributes

Phosphorus application influenced various yield attributes and finally the yield of wheat. Several workers have testified the influence of phosphorus application on the number of effective tillers (Rai, 1977 and Israel, 1991), number of spikes per square metre (Hagras, 1985), number of grains per ear (Meena, 1976 and Chakravarty and Gogoi, 1991), ear weight (Meena, 1976 and Patel *et al.*, 1991) and thousand grain weight (Knapp and Knapp, 1978; Reddy and Bhardwaj, 1982 and Israel, 1991).

Elliot *et al.* (1997) observed that P deficiency decreased grain yield principally by reducing the number of fertile tillers. Severe P deficiency depressed shoot growth within 15 days of sowing and ultimately reduced plant height, root weight and grain yield.

Tandon (1987) summarized the data based on 6900 trials and noted that about 35% increase in yield was attributed to phosphorus application. Bono *et al.* (1997) from series of 18 trials reported an increase in grain yield by an average of 0.82 t ha^{-1} in 5 trials and in 9 trials grain protein was increased by an average of 2.5% with the application of phosphorus.

Randhawa *et al.* (1974) found that all phosphorus treatments gave significantly more yield than no phosphorus application. The application of higher dose of phosphorus brought about a significant increase in wheat yield over the period of times (Tell and Khattari, 1989; Modak, 1992; Ravi, 1993; Pawin *et al.*, 1988; Covacevich *et al.*, 1995 and Parmar and Sharma, 1996). However, there is no unanimity among the workers regarding the phosphorus level up to which wheat responds: 40 kg P_2O_5 ha^{-1} (Singh *et al.*, 1987), 50 kg P_2O_5 ha^{-1} (Singh, 1991), 60 kg P_2O_5 ha^{-1} (Sattar *et al.*, 1991 and Singh *et al.*, 1993), 80 kg P_2O_5 ha^{-1} (Islam *et al.*, 1987) and 90 kg P_2O_5 ha^{-1} (Rai and Sinha, 1993).

2.1.1.3 Effect on nutrient content and uptake

Fertilizer application has a definite bearing on nutrient content and uptake by the crop. Early growth stages of wheat largely depend upon fertilizer phosphorus while the later stages of crop growth are supported mainly by native phosphorus (Rao, 1975).

Parmar and Sharma (1996) observed that nutrient uptake at tillering, flowering and maturity stages of wheat was significantly improved with increasing P fertilization. Similarly many workers (Pandey, 1987; Reddy, 1980; Jaggi and Minhas, 1989 and Covacavich *et al.*, 1995) reported increased P uptake due to higher level of P fertilization.

2.1.2 Phosphate solubilizing micro-organisms (PSM)

Phosphate solubilizing micro-organisms include different groups of micro-organisms such as bacteria and fungi which convert insoluble inorganic phosphatic compounds into soluble form. The existence of such micro-organisms in the rhizosphere has been reported by many investigators. Kucey *et al.* (1989) isolated many bacteria, fungi and actinomycetes species, capable of solubilizing sparingly soluble P from soil and rhizosphere samples. The species of *Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillium, Fusarium,* *Sclerotium, Apergillus* and others have been widely cited in literature to be active in bio-conversion. These micro-organisms can grow on insoluble phosphate sources such as fluorapatite, hydroxyapatite, carbonate-apatite, tricalcium phosphate, rock phosphate, bone-meal or similar insoluble phosphate compounds. Such organisms not only assimilate phosphorus but they also cause a large portion of soluble phosphate to be released in quantities in excess of their own requirement. The solubilization is not only restricted to calcium salts but iron, aluminium, magnesium, manganese and other phosphates are also acted upon. Arora and Gaur (1979) reported that phosphate solubilizing bacteria (PSB) such as *Pseudomonas striata*, *Pseudomonas rathonis, Bacillus polymyxa* and phosphate solubilizing fungi (PSF) such as *Aspergillus awamori*, *Penicillium digitatum, Aspergillus niger* and *Schwanniomyces occidentalis* were the most efficient in solubilizing tricalcium phosphate. These efficient micro-organisms have shown consistency in their capability to solubilize chemically fixed soil phosphorus and rock phosphates. In addition, PSM were found to mineralize organic phosphorus into soluble form due to enzymic activity (Gaur, 1972). A variety of such mechanisms are implicated in the solubilization of inorganic phosphorus. Inoculation of seeds and seedling with phosphate solubilizing micro-organisms can provide substantial amount of phosphorus by solubilizing the insoluble soil and added phosphorus (Gaur, 1990) and increase the crop yield in the country (Tilak and Singh, 1994).

2.1.2.1 Mechanism of phosphate solubilization

The mechanism of solubilization of inorganic phosphatic compounds by micro-organisms into forms more available to plants is not fully understood. However, several mechanisms have been implicated in the process. The production of organic acids by micro-organisms seems to be the main cause, but the other factors such as CO_2, H_2S and alkalinity production are also implicated.

Phosphate solubilization capabilities of soil microbes have been ascribed solely to the production of some organic acids, although chelating substances also have an important role (Mishra *et al.*, 1983). But these reports suffered from a major drawback as these observations were mostly based on *in vitro* experimentation and showed solubilization under high artificial condition of culture media.

The types of organic acid produced and their amounts differ with different micro-organisms. The type of organic acid produced has a significant role on the solubilization (Banik and Dey, 1983 and Bardiya and Gaur, 1972). Tri and dicarboxylic acids are more effective as compared to monobasic and aromatic acids. The organic acids responsible for solubilization of inorganic phosphate such as lactic, glycolic and citric acid (Sperber, 1958; Molla and Chowdhury, 1984 and Lee *et al.*, 1987), succinic and malic acid (Taha *et al.*, 1969 and Patil *et al.*, 1979), ∝-keto gluconic acid (Duff *et al.*, 1963 and Banik and Dey, 1982) and fumaric and phenolic acid (Pareek and Gaur, 1973) are produced by PSMs. These acids lower the pH and bring about the dissolution of bound form of phosphate.

Banik and Dey (1982) observed that the ability of PSMs to solubilize insoluble phosphates is by virtue of their capacity to chelate Ca^{2+} ion in addition to the production of H^+ ions. Some of the hydroxy acid may chelate with Ca and Fe resulting in effective solubilization and utilization of phosphate (Ostwal and Bhinde, 1972).

Artificial acidulation of culture medium with mineral acids like HCl, thus reducing the pH, did not make considerable P solubilization suggesting that P solubilization could be through the release of organic acids and other mechanisms as well (Illmer and Schinner, 1992). Further investigations revealed that pH was not significantly related to phosphate solubilization but to the total amount of acid produced in the culture medium indicating that the solubilization resulted was not mainly from H^+ ion but from the chelating effects of organic acids (Luo *et al.*, 1993 and Illmer *et al.*, 1995).

Low molecular weight organic acids were found to increase the availability of P in soil mainly through decreased absorption of P and increased solubilization of P compounds (Bolan *et al.*, 1994). Removal of Ca and P through plant uptake and the supply of protons (H^+) through the release of organic acids were to be the main reasons for enhanced dissolution of phosphate rocks in the rhizosphere (Bolan *et al.*, 1997). Di Simine *et al.* (1999) studied the solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from forest soils. They reported that the solubilization occurred by both an increase in the H^+ concentration of the medium and the production of gluconic acid. They also reported the possible involvement of the external glucose oxidative pathway in such a phenomenon.

The liberation of P from organic phosphate compounds is mainly due to the action of enzymes of esterase type. PSMs along with acid production, produced the phosphatase enzyme which cause solubilization of P on aquatic environment (Al-Ghazoli *et al.*, 1986). Besides, the other mechanisms reported for the solubilization of insoluble phosphates include CO_2 production by micro-organisms in the rhizosphere or in the liquid medium (Waksman, 1952), H_2S production in flooded soils or under anaerobic conditions (Rose, 1957; Swaby and Sperber, 1958 and Patrick *et al.*, 1973), mineral acids, siderophores and cation exchange mechanism. But it is not clear how solubilized P could reach the root. If absorbed in micro-organisms, it is of no direct benefit to the root, if liberated into the bulk soil, it is simply readsorbed. A direct benefit would only be possible if the soil immediately around the roots were saturated with chelating substances. In addition to release of plant available phosphorus, PSMs promote plant growth by producing plant growth promoting substances like IAA, gibberellins, cytokinins etc.

2.1.2.2 Factors affecting P solubilization by PSMs

The maximum solubilization of P by PSMs mainly depends upon the growth and development of these organisms. The availability of carbonaceous substrates, nutrients and proper soil conditions are important parameters of P solubilization. Since these micro-organisms are heterotrophs and solubilize insoluble phosphate by secreting organic acids, the role of carbon sources is well understood. It was reported that glucose and sucrose were the best sources for *Pseudomonas striata* and mannitol, glucose and fructose were the best for *Aspergillus awamori* but P solubilization was minimum with galactose (Gaur, 1990). The strains of *Pseudomonas striata* utilize different forms of nitrogen, except nitrites whereas best nitrogen source for *Aspergillus awamori* was ammonium nitrate followed by asparagine.

The pH of the medium is known to affect the growth and activity of micro-organisms. While elucidating the pH requirement of some PSMs, Ahmad and Jha (1968) and Bardiya (1970) reported that pH 7.0 was optimum for bacterium and pH 4.0 for fungi. Wani *et al.* (1979) observed that for maximum solubilization of tricalcium phosphate, the best pH was around 6 for PSF (*Aspergillus awamori* and *Penicillium digitatum*) and 7 to 8 for the PSB (*Pseudomonas striata* and *Bacillus polymyxa*). A temperature range between 20 and 30°C was found suitable for phosphate solubilization by strains of *Pseudomonas striata* but for *Aspergillus awamori* and *Penicillium digitatum*, 30°C was optimum.

2.1.2.3 Effect on crop growth

A positive effect of seed inoculation with PSMs on growth and yield of various crops has been reported by many workers. Gerretsen (1948) for the first time reported increased dry matter production in oat and mustard supplied with insoluble phosphate inoculated with phosphate solubilizing micro-organisms. Taha *et al.* (1969) reported an increase in dry matter yield of barley due to inoculation with *Pseudomonas striata*, *Streptomyces* species and *Bacillus megatherium*. Gaur and Gaind (1999) reported an increase in the height of plant, root and shoot biomass and seed germination due to inoculation with *Pseudomonas striata* and *Bacillus polymyxa*. Kucey (1987) reported an increase in dry matter yield of wheat as a result of inoculation with *Penicillum bilaji.*

Similarly, growth promotion of plants by P solubilizing fungi (*Aspergillus awamori*) under field conditions has also been reported by many workers (Tiwari *et al.*, 1989; Singh and Singh, 1993 and Vaishya *et al.*, 1996).

Tarafdar and Marschner (1995) reported increase in dry matter of shoot and root and root length due to inoculation with *Aspergillus fumigatus* and VAM. Gaur *et al.* (1980) reported increase in the dry matter production of wheat under field conditions due to mixed inoculum of *Aspergillus awamori* and *Pseudomonas striata.*

2.1.2.4 Effect on yield and yield attributes

Gaur (1985) reported the positive effect of *Pseudomonas striata* on yield and yield attributes of wheat. At Pura farm, Kanpur, Gaur *et al.* (1980) found the response of wheat to phosphate solubilizing bacteria which was equivalent to the application of 50 kg P_2O_5 ha^{-1} as superphosphate. Similar result was also reported by Shinde and Patil (1985).

Gaur and Gaind (1999) reported increase in yield attributing characters of wheat due to inoculation with *Pseudomonas striata* and *Bacillus polymyxa.*

Tiwari *et al.* (1993) observed that the highest mean grain yield of 4.36 t ha^{-1} was obtained with *Pseudomonas striata* along with application of phosphorus at the rate of 60 kg P_2O_5 ha^{-1} as single superphosphate.

Jain *et al.* (1997) reported that inoculation with *Pseudomonas striata* increased the wheat grain yield along with recommended NPK. Agasimani *et al.* (1994) found that application of P solubilizer causes a replacement of 25% phosphate fertilizers. Shinde and Saraf (1994) in a similar experiment observed that seed inoculation along with 30 kg P_2O_5 ha^{-1} produced similar dry matter yield as 60 kg P_2O_5 ha^{-1} with or without seed inoculation.

In an experiment under Delhi conditions the simple inoculation of seeds of wheat with either *Pseudomonas striata, Aspergillus awamori, Penicillium digitatum* or *Bacillus polymyxa* increased the grain yield significantly without any fertilizer input. However, the effects of these organisms were at par with each other (Gaur, 1990).

Increase in yield of wheat, onion, sorghum, soybean and gram due to inoculation with PSF has been reported by several workers (Gaur, 1972; Salih *et al.*, 1989; Singh and Singh, 1993; Vaishya *et al.*, 1996 and Whitelaw *et al.*, 1999).

Tiwari *et al.* (1993) reported an increase in the wheat yield by inoculation with *Penicillium bilaji, Aspergillus awamori* and *Penicillium radicum*. Similar results were also observed by Gleddie *et al.* (1991) and Whitelaw *et al.* (1999).

Gaur (1990) obtained best yield of wheat in conjunction with organic manure and phosphate solubilizing micro-organisms (*Pseudomonas striata* and *Aspergillus awamori*). He also reported saving of 30 kg P_2O_5 ha^{-1} in rice due to inoculation with *Bacillus polymyxa*.

2.1.2.5 Effect on nutrient content and uptake

Kundu and Gaur (1982) observed an increase in nutrient uptake by grain and straw due to inoculation of wheat seeds with *Pseudomonas striata.*

Doney and Lipmonn (1988) observed that isolates of phosphate solubilizing bacteria were able to improve phosphorus nutrition of wheat and thus stimulated plant growth under phosphorus deficient condition.

Manjunath and Devi (1990) showed that phosphorus uptake of groundnut increased significantly when the seeds were inoculated with *Pseudomonas striata.* While Kim *et al.* (1997) noted the P concentration of wheat biomass increased significantly when its seeds were inoculated with PSB.

Inoculation with PSF also influenced the phosphorus uptake positively (Whitelaw, 2000). Kundu and Gaur (1984) conducted an experiment at IARI and found that P uptake in rice was increased significantly due to inoculation with *Aspergillus awamori.* While, Gaur (1972) reported increased P uptake by wheat due to inoculation with *Aspergillus awamori.*

Tarafdar and Marschner (1995) reported increase in phosphorus concentration in shoot due to inoculation with *Aspergillus fumigatus.*

The increase in phosphorus uptake in a number of crops viz., wheat, gram, soybean and sorghum due to inoculation with PSF has also been reported by several workers (Manjunath *et al.*, 1981; Salih *et al.*, 1989; Singh and Singh, 1993; Vaishya *et al.*, 1996 and Whitelaw *et al.*, 1999).

2.1.3. Vesicular-Arbuscular Mycorrhizae (VAM)

The first person to use the term 'mycorrhiza' was the botanist A.B. Frank in 1985. Vesicular-Arbuscular Mycorrhizae (VAM) is by far the most widespread type of mycorrhiza. The nomenclature refers to the formation of vesicles and arbuscules. The VAM fungus penetrates into the cortical host cells, but the invading mycelium usually lives only for a short time intracellularly (Smith, 1980). Then lysis of intracellular structure (the arbuscules in VAM) occurs, but the host cell survives and can be colonized again by the fungus. VAM fungi do not form sheaths around the roots, but a network of extra material hyphae usually develops. This grows into the soil and can extend the mycelium several centimetres beyond the root surface. The total hyphae length can reach more than one metre of hyphae per centimetre of infected root (Smith, 1980 and Hayman, 1982). These VA fungi are members of family *Endogonaceae* that are placed in genera *Glomus, Sclecystis, Gigaspora* and *Acaulospora* (Gerdemann and Trappe, 1974). Since they can not be successfully sub cultured axenically, they must be considered ecologically obligate symbionts, i.e. they do not complete their life cycle unless they colonize a suitable host plant (Lewis, 1973). The family is considered to be in an order of its own, the Endogonales are tentatively placed in the class Zygomycetes in the sub division Zygomycotina (Benjamin, 1979).

2.1.3.1 Process of VAM Root Colonization

Studies on the development of VAM infection (Powell, 1976) showed that neither spore germination nor the initial direction of hyphal growth was influenced by the presence of host root. Hyphae from spore were not attracted to the roots until they approach them closely. There are four key facts in VAM colonization i.e. (1) spore germination and mycelial development, (2) a stimulation of the germ tubes when they approach the roots closely, (3) attachment of the infective hyphae to the root surface and (4) root penetration. Once the infective hyphae arrives at the root, an appresorium is usually produced on cortical cells or on root hairs and hyphal penetration occurs into or between these cells. The infection units – internal mycelium associated with a single entry point (Wilson, 1984) grow as the hyphae spread between and through cells of cortical root layers. When the first successful entry point is established, the root become more prone to further penetration. Shortly after infection a hyphae growing into single cell may show repeated dichotomic branching and a tree like structure, the arbuscule, is formed. When the internal infection is consolidated, the penetration hyphae ramify externally. The external hyphae may grow along the root surface forming more penetration point and also grow through the surrounding soil, forming an intensive tridimensional network of mycelium (Barea, 1991). Mosse and Hepper (1975) also reported that once primary infection is established, the extensive growth of external mycelium starts and leads to the development of secondary infection. Sanders and Sheikh (1983) suggested that the proportion of root system colonized by VAM fungi depends on (a) the rate of root growth, (b) rate of secondary infection development and (c) rate of longitudinal spread of fungi within cortex. Several environmental factors influence these parameters. When the mycorrhiza is well established, the fungi may form vesicle. These are oval to spherical structure containing oil droplets that can develop inter- or intra-cellularly. They may have a temporary storage function, after which they remain thin walled or become thick walled as chlamydospore-like structures. Intracellular colonization, as in the case of arbuscules, have a characteristic feature. Fine structure studies (Dexheimer *et al.*, 1979 and Scannerini and Bonfante, 1983) revealed that the arbuscules are surrounded by the intact host-cell plasmalemma. The cytological changes that occur during arbuscules formation have been well documented by Rhodes and Gerdemann (1980). Therefore, arbuscules formation represent large surface of cellular contact between host and fungus. This facilitates the interchange of metabolites between host and fungi. The function of the arbuscule is the biotrophic bi-directional transfer of nutrients, the mechanism of which requires living fungus (Cox and Tinker, 1976). In fact, the arbuscules are considered the main site of transfer of mineral nutrients from fungus (Smith and Gianinazzi-Pearson, 1988). The ratio of arbuscules per unit root length of extramatricell hyphal length might be considered for comparisons among fungal isolates with regards to functional transport capability. Technological advances that utilize automation need to be applied to this approach (Wright and Millner, 1993).

2.1.3.2 Mechanism of phosphate mobilization

Various mechanisms have been suggested by Bolan (1991) for the increase in phosphorus uptake by mycorrhizal plants. These include:

1. Physical exploration of soil,
2. Faster movement of phosphorus into mycorrhizal hyphae and
3. Modification of root environment

Approximately 95-98% of soil phosphorus occurs in unavailable form to plant roots (Bileski, 1973). Certain sparingly soluble P compounds seemed to be utilized by VAM as a source of P. This possibility was investigated by experiments in which the labile phosphate pool was labelled with ^{32}P (Sanders and Tinker, 1971 and Powell, 1975). It is clear from isotopic studies that VAM hyphae absorb phosphate from the labile pool of P in soil as do non-mycorrhizal roots. It is known that mycorrhizal plant can respond to the addition of sparingly soluble P like rock phosphate. However, this is not necessarily a P solubilization effect. There is a controversy among the various workers whether the mycorrhiza helps in both solubilization and mobilization of nutrients which are otherwise unavailable to plants (Azcon *et al.*, 1976). But it is a general agreement that mycorrhiza helps in mobilizing P and other less mobile nutrient rather than solubilization. VAM enhances P uptake in two different ways. One mode of fungal action is merely physical and is based on the increased number of sites for absorption achieved by external mycelium. The hyphae growing through soil pore space are able to affect phosphate absorption beyond the depletion zone up to 8 cm from the root (Rhodes and Gerdemann, 1975). Thus, mycorrhizal roots explore a much greater volume of soil to take up phosphate. It is plausible that VAM hyphae come in a closer contact with the P containing particles than roots to take advantage of the phosphate ions that are slowly and naturally dissociated by physico-chemical and biochemical mechanisms (Barea, 1991). The main site of phosphate transfer to host which occurs by an active mechanism across the membrane of both partners, seems to be the arbuscules (Cox *et al.*, 1980). It is now accepted that the breakdown of arbuscules can account for only 1% of the P inflow into the host cell. Phosphate released by other structure such as hyphae or vesicles might also be involved but the extensive increase of contact surface area makes the arbuscules the more probable sites for nutrient transfer between mycorrhizal symbionts.

There are some indications that the mycorrhizae is able to take up P from soil solution with low phosphate concentration, where simple roots have difficulties in tapping the ions. In other words, mycorrhiza seems to include a lower threshold concentration of effective P uptake (Bolan *et al.*, 1983). VAM hyphae have advantageous geometry and better distribution than roots to acquire P from transitory, localized and diluted source of the nutrient (Jakobsen *et al.*, 1992).

2.1.3.3 Factors affecting VAM development

The establishment of successful entry points on host roots, the internal and external development of fungus and the resulting plant response are all dependent on interaction among prevailing fungal, plant and environmental factors. The germination of spores, infection and function of VAM are affected by biotic and abiotic factors.

Certain plant species require mycorrhiza to a much greater extent than others; this phenomenon is usually referred to as mycorrhizal dependency, which is the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). In general, plants having rootlet diameter of more than 0.5 mm and lacking root hairs are highly dependent on VAM. Conversely, plant with a dense cover of long root hairs and root system that have rootlet diameters less than 0.1 mm respond to mycorrhiza only in P deficient soils (St. John, 1980).

In general, entomycorrhizal fungi are acidophilic (Marx and Krupa, 1978), but there is no correlation between VAM and soil pH (Read *et al.*, 1976). In fact, some species are better adapted for acid soils and others to neutral and alkaline soils (Abbott and Robson, 1985).

Mycorrhizal fungi are sensitive to soil moisture status (Ponder, 1983). Redhead (1975) demonstrated that the optimal water supply for plant growth is also suitable for mycorrhizal infection.

Soil temperature affects the pre-infection stages of mycorrhizal development in that the number of "entry points" increases as the temperature rises form 12 to 25°C (Smith and Bowen, 1979 and Hetrick *et al.*, 1984).

Special attention has been paid to the effect of soluble phosphate on mycorrhizal development (Mosse, 1973). The growth of VAM fungi is influenced by the concentration of nutrients in both soil and plants. Large application of phosphatic fertilizer to soil results in the reduction of arbuscular mycorrhizal infection of host plant (Abbott *et al.*, 1984; Thomas *et al.*, 1986 and Amijie *et al.*, 1989). Development of external hyphae was found to be inhibited by high soil phosphorus levels (Abbott *et al.*, 1984).

The general conclusion obtained is that P levels in the plant, rather than those in soil, control the establishment and functioning of mycorrhiza (Menge *et al.*, 1978). It has become increasingly apparent that P inhibition of VAM formation is associated with a membrane mediated decrease in root exudation (Ratnayake *et al.*, 1978). In summary, it appears that a cause effect relationship exists; the higher the P content in the plant, the lower the soluble carbohydrate content in roots and exudates and lower frequency of "entry points" (Jasper *et al.*, 1979).

2.1.3.4 Effect on crop growth

Hetrick *et al.* (1996) reported that mycorrhizal responsiveness decreased with P fertilization in cultivars that were dependent on the symbiosis but it was unaffected by P fertilization in cultivars that were negatively impacted by the mycorrhiza. The relationship between biomass production and percentage root colonization was positive for cultivars which responded favourably to the symbiosis.

The VAM inoculation (*Glomus mosseae* and *Glomus constrictum*) significantly increased the plant height, total root and shoot dry weight of wheat (Chhabra and Jalali, 1997 and Omar, 1998).

Mikhael *et al.* (1997) observed an increase in shoot dry weight of wheat by 82.8% over the control due to inoculation with VAM fungi (*Glomus* sp.). Zaghloul *et al.* (1996) reported higher root length, root and shoot dry matter of wheat with VAM inoculation than PSB inoculation. Inoculation with *Glomus mosseae* increased the dry matter production of wheat cultivars more than inoculation with *Glomus fasciculatum* or *Glomus aggregatum* (Virhcileg and Ocampo, 1991). But Panwar (1991) found that wheat inoculated with *Glomus fasciculatum* increased chlorophyll concentration and photosynthetic rate of wheat.

Tarafdar (1995) reported that inoculation of wheat with VAM significantly increased shoot and root dry weight and total root length, although shoot:root ratio was unaffected.

Pradhan and Mohan (1996) in a pot experiment observed that seed inoculation of wheat with VAM fungi i.e. *Glomus fasciculatum*, *Glomus epigaenum* and *Glomus mosseae* significantly increased plant height and total dry matter production.

In wheat, *Glomus clarum* isolate NT_4 had no effect on shoot dry weight whereas *Glomus mosseae* isolate NT_6 improved the plant height at harvest (Talukdar and Germida, 1995). Kucey and Janzen (1987) reported increased dry matter production of wheat under varied sets of growth conditions.

Tarafdar and Marschner (1995) reported that seed inoculation of wheat with phosphatase producing fungus (PSF) *Aspergillus fumigatus* or soil inoculation with VAM fungus *Glomus mosseae* increased shoot and root dry weight, root length and phosphatase activity in the rhizosphere.

2.1.3.5 Effect on yield and yield attributes

Tu-ShiHua *et al.* (1997) reported that both P addition and VAM infection increased the grain yield of wheat. Majjigudda and Sreenivasa (1996) observed that inoculation with *Glomus fasciculatum* plus 75% of recommended phosphorus improved the grain yield of wheat. Many workers observed VAM inoculation in wheat increased the yield attributing characters i.e., number of grains per ear (Chhabra and Jalali, 1997), spike weight and grain weight per spike (Zaghloul, 1996).

Singh *et al.* (1990) reported that inoculation with *Glomus fasciculatum* resulted increased grain and straw yield of wheat.

Buwalda *et al.* (1985) concluded that at all levels of applied P up to 60 kg ha^{-1}, inoculation with mycorrhizal fungi increased the yields of both wheat and barley by 17-25% after indigenous mycorrhizal fungi had been removed by fumigation, but the effects were smaller on non-fumigated plots. Panwar (1991) explained that higher grain yield of wheat due to VAM (*Glomus fasciculatum*) inoculation could be attributed to higher chlorophyll concentration, photosynthetic rate, nitrate reductase and glutamine synthetase activity.

2.1.3.6. Effect on nutrient content and uptake

VAM infection had both positive and negative effect on P uptake depending on the growth stage of wheat and translocation of nutrients. The role of VAM in enhancing the translocation of P from root and straw to grain was beneficial towards seed setting (Goh *et al.*, 1997).

Mikhael *et al.* (1997) reported inoculation with VAM fungi increased the N and P uptake of wheat by 98.3% and 154.5%, respectively, over the control.

Jakobsen and Nielsen (1983) proposed that the final P uptake by wheat in a relatively low soil P availability was counter balanced to some extent, by a well developed VAM infection.

Buwalda *et al.* (1985) reported that mycorrhizal infection increased the P concentration in the plant tissue at very early stages of growth, but the effect generally declined with time.

Kucey and Janzen (1987) found that VAM inoculation directly increased the uptake of P, Zn, Cu and Fe by field beans and of P and Zn by wheat and decreasing the nutrient availability had minimal effects on nutrient uptake by wheat because of relatively fibrous root system. Increase in total uptake of a particular nutrient resulting from inoculation with VAM are not necessarily indicative of a direct uptake of that nutrient by the VAM.

Singh *et al.* (1990) reported higher nitrogen and phosphorus content in wheat plants (HD 2285) after soil inoculation with *Glomus fasciculatum.* Zaghloul *et al.* (1996) reported VAM inoculation gave higher N, P and K concentrations in wheat compared with PSB.

Tarafdar and Maschner (1995) observed an increased P concentration and to a lesser extent K and Mg in shoot due to seed inoculation with *Aspergillus fumigatus* or soil inoculation with VAM fungus *Glomus mosseae,* but concentration of Cu and Zn were enhanced only by VAM. It was also concluded that organic P in the form of Na-phytate is efficiently used by VAM and that use of organic P can be increased by simultaneous inoculation with phosphatase producing fungi.

Liu and Zhang (1999) observed that inoculation of VAM increased the uptake of the fraction of Olsen-P, Ca_2-P, Ca_8-P, Al-P and the growth of wheat and maize, especially in soils with low phosphorus content and reduced the difference in phosphorus content between rhizosphere and bulk soil.

2.2. Studies on microbial population in the rhizosphere

Microbial population in the rhizosphere is affected by many factors and subjected to variation with addition of fertilizers and inoculation with phosphate solubilizing and mobilizing micro-organisms. Experimental data are available showing the effects of fertilizers on the microbial populations. Voynova *et al.* (1972) observed that in wheat and maize rhizospheres, fertilizers i.e. NH_4NO_3, $(NH_4)_2SO_4$, urea and P and K additions to different soils increased the number of *Pseudomonas* and decreased those of sporulated bacteria.

Jiang and Sato (1992) reported that superphosphate application improved the growth of wheat in volcanic ash soil but it did not, however, significantly change the bacterial populations with three different levels of superphosphate although there were some variations with plant age.

Majjigudda and Sreenivasa (1996) in a pot experiment, observed that when wheat cv. DWR-162 was soil inoculated with *Glomus fasciculatum*, *Gigaspora margarita*, *Acaulospora laevis* or *Clerocystis dussii* or not inoculated and given 0, 50, 75 or 100% of the recommended dose of P (75 kg ha^{-1}), the highest root colonization, spore count and population of free living nitrogen fixers and P solubilizers were recorded in the rhizosphere soil of plants inoculated with *Glomus fasciculatum*. In general, root colonization and spore count increased with up to 75% of the recommended P rate, while the population of free living nitrogen fixers and P solubilizers increased with increased P levels.

Jensen and Jakobsen (1980) reported an inverse relationship between soil P level and intensity of VAM infection and also observed a good correlation between spore counts and infection intensity. Jalaludddin and Anwar (1991) observed a gradual decrease in number of VAM spores occurred at the seedling stage and then increased again at booting to reach a maximum at harvest i.e. 1860 sproes/100 g soil from wheat fields.

Durynina and Sokolova (1991) reported that fertilizer application resulted in low (up to 20%) mycorrhization of plants and a decrease of amount of spores in wheat rhizosphere. Similar result was also obtained by P fertilization (Covacevich *et al.*, 1995). Clapperton *et al.* (1997) reported that the application of 20 kg P significantly reduced the length of root colonized and percentage of roots colonized by vesicular arbuscular mycorrhizal (VAM) fungi compared with the treatment receiving no P. Singh and Kapoor (1999) reported that a larger population of phosphate solubilizing microorganisms was maintained in the rhizosphere of wheat that was inoculated with VAM.

2.3 Phosphorus use efficiency using ^{32}P labelled fertilizers

Diversity is a unique phenomenon of nature with respect to nutrition, growth and development of species. Each plant species requires a definite amount of a particular nutrient from different sources for the completion of its life cycle. This can be estimated by radiotracer technique for the fertilizer recommendations. Here some works regarding phosphorus utilization from soil and fertilizer has been quoted.

2.3.1 Effect of phosphorus levels

Increase in uptake of fertilizer phosphorus by wheat due to application of higher dose of phosphorus has been reported by many workers (Rai *et al.*, 1984; Modak, 1992 and Hundal *et al.*, 1993).

Application of 90 kg N ha^{-1} and 30, 60, 90 and 120 kg P_2O_5 ha^{-1} increased the average grain yield but decreased the coefficient of utilization of fertilizer P from 37% with 30 kg P_2O_5 ha^{-1} to 20% with 120 kg P_2O_5 ha^{-1} (Kushnirenko and Fedorova, 1977).

Mahajan and Bisen (1980) conducted a field experiment using labelled superphosphate to study the uptake and utilization of soil and fertilizer phosphorus under different fertility gradient and phosphorus levels. They found that phosphorus derived from fertilizer (% Pdff) and fertilizer phosphorus uptake increased with increasing P levels but decreased with increased fertility status. However, per cent utilization of phosphorus decreased significantly with phosphorus levels and fertility status.

Mitkees *et al.* (1971) observed that the per cent of P derived from fertilizer was 7.0 to 15% for beans and 3.0 to 6.8% for wheat. Correlation coefficients for the relation between soil P level and percentage P derived from fertilizer were –0.91 for beans and –0.90 for wheat.

Prasad *et al.* (1981) observed that phosphorus derived from fertilizer (% Pdff) by wheat increased with increasing phosphorus rates from 17-51 kg ha^{-1} while the utilization of applied phosphorus showed a reverse trend. Similarly, the decrease in per cent utilization of added phosphorus with increase in the level of applied phosphorus have been reported by several workers (Sinha and Rai, 1976; Pandey, 1987; Naik *et al.*, 1991 and Bahl and Pasricha, 1998).

In the maize-wheat sequence, Rai *et al.* (1984) showed that direct application of phosphorus to wheat had a favourable effect on phosphorus utilization. However, a significant response of phosphorus was observed with only up to 60 kg P_2O_5 ha^{-1}. Gupta *et al.* (1985) also reported that phosphorus utilization decreased with increasing the rate of fertilizer phosphorus.

Negrila and Negrila (1995) reported that average coefficient of variation of P utilization was 19% at optimum phosphorus rates. They found that mean coefficient of phosphorus utilization was 40 to 23% when applied with 0 to 160 kg N and 0 to 160 kg P_2O_5 ha^{-1} to wheat. Such a decrease in P utilization by wheat may be attributed to (a) no luxury consumption of phosphorus with increase in phosphorus level (b) increased retention of added phosphorus in soil with increase in the level of application.

'A' value which reflects the capacity of soil to supply phosphorus tends to increase with increase in the level of phosphorus applied to wheat (Rai, 1977 and Israel, 1991). Sharpley (1987) observed that continued application of P fertilizer increased amount of available P and increased 'A' value, thus, represents total soil P availability, including native and residual (carry over) fertilizer P. 'A' value, the measure of the amount of available phosphorus, is influenced by crop species, stages of crop growth, type of fertilizer, level of P application and method of application of phosphate fertilizer. Crop species differed significantly in 'A' values, which were higher at maturity than at flowering. The values increased with increase in level of P application (Sreenivasa, 1988). Tahir *et al.* (1971) in a pot experiment determined the 'A' values of three surface soil samples of three soils using ^{32}P labelled superphosphate and wheat as a test crop. The 'A' value increased from year to year after P treatment but the proportion of fertilizer P in the plants decreased. The amount of fertilizer P fixed increased and then decreased until in the fourth year, some fixed soil P was released. Such increase in 'A' value usually results when there is yield response to applied phosphorus.

Sharma and Parmar (1998) while studying the effect of P and mulching on P use efficiency in wheat concluded that the P use efficiency (PUE) at tillering, flowering and harvesting was greatest at the lowest dose of P and decreased as the P levels were increased from 0 to 78 kg P ha^{-1}.

2.3.2 Effect of phosphate solubilizing and mobilizing micro-organisms

Observation and demonstration of phosphate solubilization *in vitro* have led to the investigation of the ability of these micro-organisms in releasing native soil phosphorus as well as increasing availability and utilization of phosphorus from added phosphate (Kapoor *et al.*, 1989).

In an experiment phosphobacterin and *Bacillus circulans* were found to significantly increase the phosphorus utilization in wheat from super phosphate and apatite (Gaur, 1990). While Sharma and Singh (1971) conducted field trials with maize crop concluded that phosphobacterin culture enhanced the efficiency of phosphatic fertilizer, particularly superphosphate. The effect was less marked with bone meal and rock phosphate.

Presence of favourable microbes like phosphate solubilizing micro-organisms in the rhizosphere is also known to positively alter the phosphorus utilization by wheat (Alagawadi and Gaur, 1988 and Israel, 1991).

Sharma and Kamath (1991) reported that soybean seed inoculated with bacterial culture of *Pseudomonas striata* or a fungal culture of *Aspergillus awamori* along with biogas slurry and single superphosphate, significantly increased the phosphorus utilization. Similarly, in groundnut, Ravi Kumar *et al.* (1995) reported that phosphorus use efficiency was highest when the seeds were inoculated with *Aspergillus awamori* along with FYM.

2.4 Economics

Rana *et al.* (1979) observed the economic dose of the phosphorus varied according to initial soil phosphorus status. He found that 57 and 47 kg P_2O_5 ha^{-1} was economic optimum dose for low and medium phosphorus status, respectively.

Rai *et al.* (1982) concluded from their finding that the economic optimum dose need not coincide with the dose which gave greater grain yield as they obtained maximum profit in wheat at 60 kg P_2O_5 ha^{-1} while grain yield increased even up to 90 kg P_2O_5 ha^{-1}.

Pandey (1987) reported 77.3 kg P_2O_5 ha^{-1} as the economic optimum dose for wheat while Israel (1991) found it to be 71.58 kg P_2O_5 ha^{-1}. However, Haris (1995) working on green manure-wheat/rapeseed/gram cropping system found that 30 kg P_2O_5 ha^{-1} applied to wheat produced by sunn-hemp green manure was the most economic dose.

Rao *et al.* (1997) reported optimum fertilizer P rates to achieve 90% of maximum yield was 44.5 to 49.5 kg P ha^{-1} for wheat.

Singh and Uttam (1992) obtained highest gross income and net returns from a cross sown wheat applied with 120 kg N + 60 kg P_2O_5 ha^{-1}.

Sharma and Parmar (1998) reported that the economic optimum dose of P in association with 8 t ha^{-1} of lantana mulch was 53 kg ha^{-1} over two years, whereas, in the absence of mulching, it was 58 kg ha^{-1}.

Chapter 3

MATERIALS AND METHODS

A field experiment was carried out during *rabi* season of 1999-2000 and 2000-2001 to study the influence of phosphate solubilizing and mobilizing micro-organisms on phosphorus use efficiency in wheat under different levels of phosphorus. The details of the experimental materials used, procedures and techniques followed during the course of investigation are being presented in this chapter.

3.1 Experimental site

The experimental site for 1999-2000 and 2000-2001 was in Main Block 3C at the farm of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi-110 012.

3.2 Climate and weather

Geographically, Delhi is situated at 28°40′N latitude and 77°10′E longitude at an altitude of about 228.61 metres above mean sea level. It has a semi arid and subtropical type of climate with extremes of weather conditions, hot dry summers and cold winters. May and June are the hottest months with mean maximum temperatures ranging from 40-46°C. December and January constitute the coldest months of the year with minimum temperature of about 5-6°C. The daily minimum and maximum temperatures increase from February onwards up to June. Temperature decreases during July to September and drops thereafter rapidly attaining minimum value in January. Sand storms and ground frost are commonly associated with summer and winter seasons, respectively.

The normal onset period of monsoon is the 4th week of June. The mean annual rainfall is about 650 mm which is mainly received during the months of July to September. About three-fourth of the total rainfall is received during these months. The mean annual pan evaporation is about 850 mm. The mean daily pan evaporation from USWB pan evaporimeter reaches a high of 19.6 mm per day in June and a low of 2.2 mm per day in January. The mean wind velocity varies from 3.5 km hr^{-1} during October to 6.4 km hr^{-1} during April. The mean relative humidity reaches the maximum value during July and August.

The meteorological data for the period of experimentation (i.e. November 1999 to April 2000 and November 2000 to April 2001) recorded at meteorological observatory of Indian Agricultural Research Institute, New Delhi are presented in Appendices I and II and depicted in Fig. 1.

3.3 Soil characteristics

The topography of the field was fairly uniform with a gentle slope. A composite representative soil sample from 0-30 cm depth of the experimental field was collected to study the availability of major nutrients and some related physico-chemical properties of the soil of the field prior to experimentation. The results of the soil analysis are presented in Table 1. It revealed that the soil was sandy-loam, low in organic carbon and available nitrogen content. The status of available phosphorus was low to medium with high amount of exchangeable potassium. The

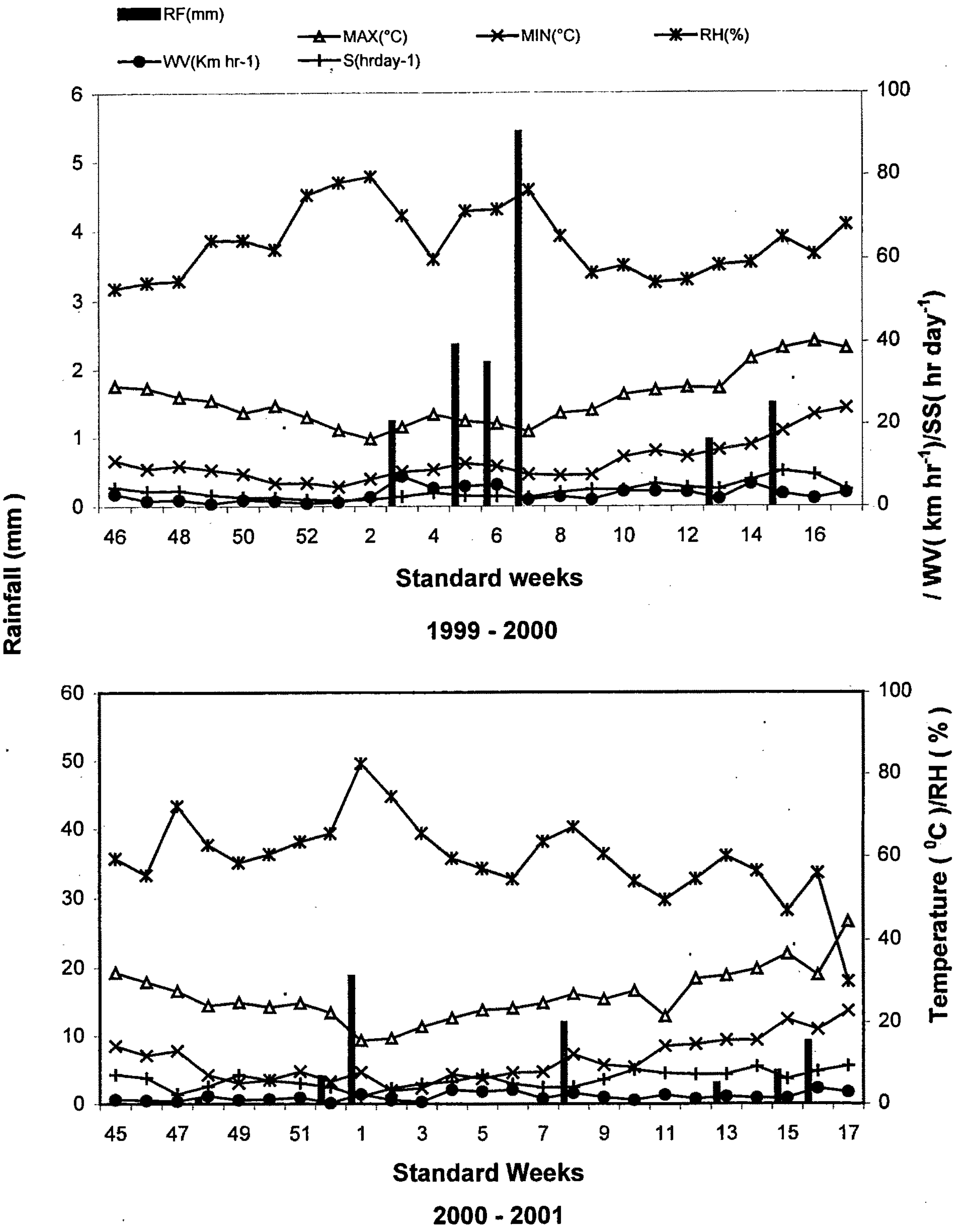


Fig. 1 Meteorological Observations during crop season

Table 1. Physical and chemical properties of soil in the experimental site

Particulars		Values		Methods used
		1999-2000	2000-2001	
I.	**Mechanical analysis**			
	Sand (%)	65.7	66.4	Hydrometer method (Bouyoucos, 1962)
	Silt (%)	14.2	14.7	
	Clay (%)	20.1	18.9	
II.	**Physical properties**			
	Bulk density (Mg m^{-3})	1.52	1.54	Piper (1950)
	Moisture content (%) at 0.33 MPa	17.82	18.11	Coleman (1944)
	Moisture content (%) at 1.5 MPa	4.60	4.79	Richards (1947)
III.	**Chemical Properties**			
	Organic carbon (%)	0.392	0.410	Walkley and Black method (Jackson, 1967)
	Available nitrogen (kg ha^{-1})	163.7	169.9	Alkaline permanganate method (Subbiah and Asija, 1956)
	Available phosphorus (kg ha^{-1})	11.20	11.58	Olsen's method (Olsen *et al.*, 1954)
	Available potassium (kg ha^{-1})	186.13	193.05	Flame photometer method (Toth and Prince, 1949)
	pH (1:2.5::soil:water)	7.10	7.20	Elico pH meter (Piper, 1950)
	Electrical conductivity at 25°C (dS m^{-1})	0.36	0.32	USDA (1967)
	CEC [c.mol(P^+) kg^{-1}]	10.10	10.00	Metson (1956)

soil reaction was in the zone of neutrality. The cation exchange capacity of the soil was low.

3.4 Cropping history of the experimental field

The cropping history of the field is represented in Table 2.

Table 2. Cropping history of the experimental field

Year	Crop season		Remarks
	Kharif	*Rabi*	
1995-1996	Maize (fodder)	Wheat	Uniform trial
1996-1997	Maize (fodder)	Wheat	Uniform trial
1997-1998	Maize (fodder)	Wheat	Uniform trial
1998-1999	Maize (fodder)	Wheat	Uniform trial
1999-2000	Maize (fodder)	Wheat	Present experiment
2000-2001	Maize (fodder)	Wheat	Present experiment

The experiment was started in the *rabi* season of 1999-2000 and repeated in 2000-2001 at different sites in the same experimental field.

3.5 Experimental details

The details of the experiment are as follows:

3.5.1 Treatments

(a) Main Plots : Phosphorus levels

P_0 : 0 kg P_2O_5 ha^{-1}

P_{20} : 20 kg P_2O_5 ha^{-1}

P_{40} : 40 kg P_2O_5 ha^{-1}

P_{60} : 60 kg P_2O_5 ha^{-1}

(b) Sub plots: Phosphate solubilizing and mobilizing micro-organisms (biofertilizers)

M_c	:	Control
M_{Ps}	:	*Pseudomonas striata* (PSB)
M_{Gf}	:	*Glomus fasciculatum* (VAM)
M_{Aa}	:	*Aspergillus awamori* (PSF)

3.5.2 Experimental design and layout

The experiment was laid out in a split plot design comprising four levels of phosphorus and four sets of biofertilizers with three replications. There were 16 treatment combinations and 48 plots in total. The plan of field layout has been depicted in Fig. 2.

Details of field layout

Spacing (R - R)	:	22.5 cm
Gross sub plot size	:	2 m x 10 m
Net sub plot size	:	1.1 m x 10 m

3.6 General features of the experimental variety (HD 2285)

The high yielding variety was selected from a cross of 249/HD 2160/HD 2186 at the Indian Agricultural Research Institute, New Delhi and was released in 1992 for commercial cultivation in Northern plain zones for late sown condition. It has a dwarfing gene Rht 1 and some rust resistant genes like Lr 23^+ (leaf rust), Sr 9b + Sr 11^+ (stem rust) and Yr 2^+ (yellow rust). It has amber, hard and medium bold grains. It matures in about 140 days and gives an average yield of about 5.5 t ha^{-1}.

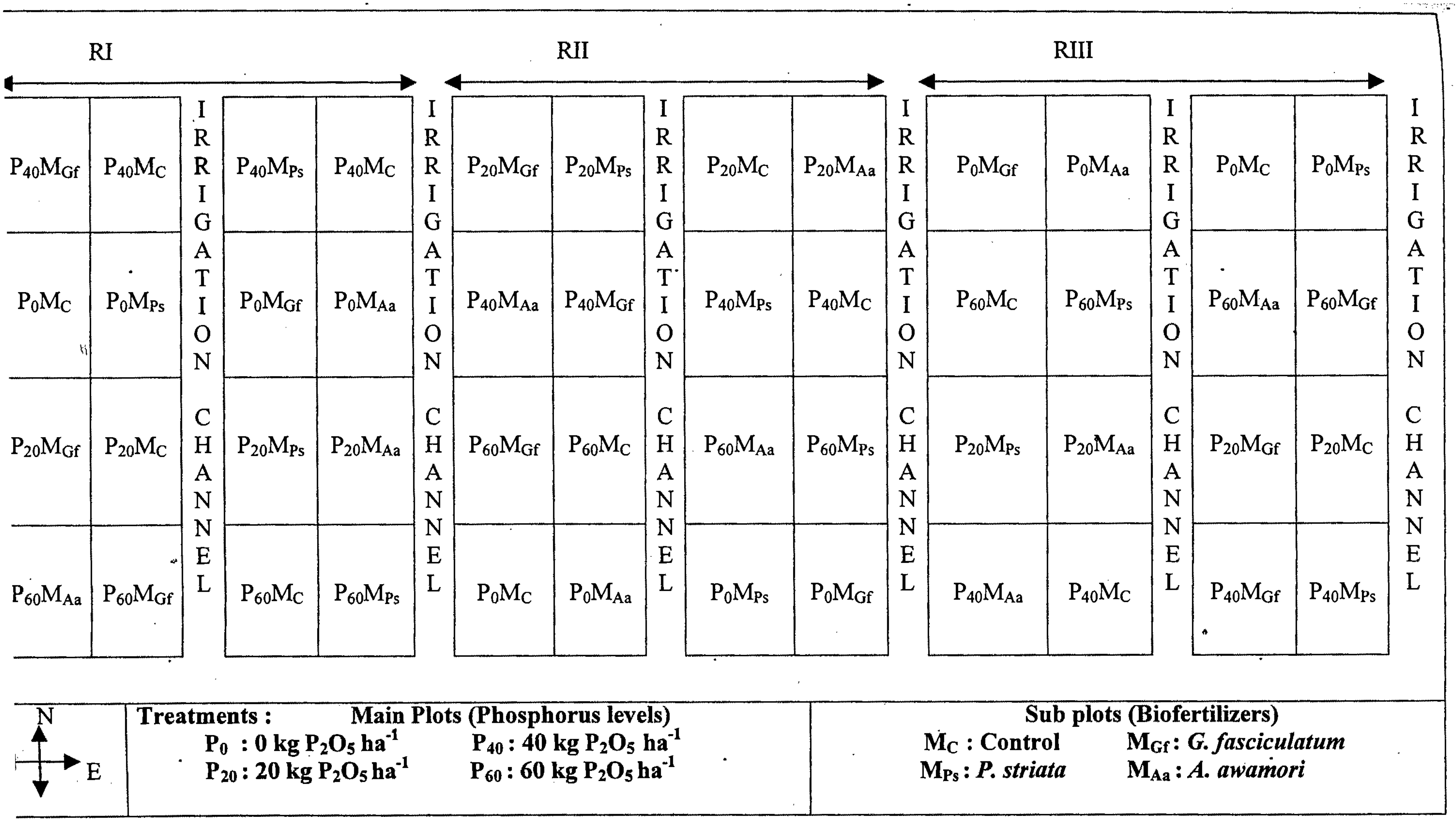


Fig 2. Layout of the experiment

3.7 Field operations

The calendar of cultural operations carried out during the investigation is given in Table 3.

3.7.1 Land preparation

A pre-sowing irrigation was given to the experimental land for field preparation. After excess water drained away and soil attained a friable tilth, the field was prepared by double harrowing with tractor followed by planking. The field was properly levelled. The plots were laid out as per plan of the field layout.

3.7.2 Fertilizer application

The crop received 120 kg nitrogen per hectare in the form of urea, half of which was applied at sowing (i.e. basal application) and remaining half was top dressed at 30 days after sowing. A recommended dose of 40 kg K_2O per hectare in the form of muriate of potash and as per the treatments the variable doses of phosphorus (0, 20, 40 and 60 kg P_2O_5 ha^{-1}) in the form of single super phosphate were applied as basal with the help of a *pora*.

3.7.3 Biofertilizer application

The carrier based inoculants of *Pseudomonas striata* and *Aspergillus awamori* were obtained from the Division of Microbiology, IARI, New Delhi. For inoculating the seeds, 10 per cent *gur* solution was prepared by boiling in water. After it cooled, the carrier based inoculants as per treatments were thoroughly mixed with it. The inoculant slurry so prepared was poured on the seeds heaped on a clean polythene sheet and was mixed with the seed uniformly. The inoculated seed was air-dried in shade and used for sowing.

Table 3. Schedule of field operations undertaken during crop season

S.No. Operations	Date	
	1999-2000	2000-2001
1. Collection of soil samples	16.11.1999	13.11.2000
2. Field ploughing and leveling	17.11.1999	14.11.2000
3. Layout of the experiment	18.11.1999	16.11.2000
4. Seed sowing and fertilizer application	21.11.1999	18.11.2000
5. Thinning	07.12.1999	03.12.2000
6. Intercultural operations		
(i) First weeding and hoeing	12.12.1999	10.12.2000
(ii) Second weeding and hoeing	05.01.2000	02.01.2001
7. Irrigations		
I	15.12.1999	13.12.2000
II	15.01.2000	15.01.2001
III	22.02.2000	12.02.2001
IV	13.03.2000	11.03.2001
8. Harvesting	08.04.2000	06.04.2001

The pure Vesicular Arbuscular Mycorrhizae (VAM) culture, *Glomus fasciculatum*, which contained 600 spores per gram of culture was obtained from the Division of Microbiology, IARI, New Delhi. The pure VAM culture was mixed with slightly moistened soil and applied below the seed with the help of metallic tube attached to the hand plough.

Pseudomonas striata and *Aspergillus awamori* were applied at the rate of 600 grams (3 packets) per hectare whereas *Glomus fasciculatum* was applied at the rate of 10 kg (10 packets) per hectare.

3.7.4 Seed sowing

Crop was sown at a row to row spacing of 22.5 cm by *kera* method at the rate of 100 kg ha^{-1}.

3.7.5 Application of labelled superphosphate

For radio chemical assay, labelled superphosphate was used. Labelled single superphosphate was obtained from Board of Radiation and Isotope Technology (BRIT), Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. It contained ^{32}P, a radioactive isotope of phosphorus tagged to give an initial activity of 0.3 millicurie g^{-1} P_2O_5. The P_2O_5 content of labelled fertilizers was 16.0% in both the years.

The labelled fertilizer was weighed in the laboratory accurately as per the treatments by adopting necessary safety precautions like use of apron, mask, surgical gloves, film badges etc. Band placement of the fertilizer was done at 3-5 cm below the seed in one meter row length of the crop.

3.7.6 Thinning

Thinning operation was carried out after 15 days of sowing to maintain the desired plant density.

3.7.7 Weeding and intercultivation

Manual weeding was done twice, 3 and 6 weeks after sowing (WAS) to keep a check on the weed growth. It was followed by shallow hoeing to provide aeration, facilitate root growth and make soil receptive to moisture and nutrients.

3.7.8 Irrigation

The schedule of irrigation indicating the number and time of irrigation is given in Table 3.

3.7.9 Harvesting and threshing

Crop was harvested manually when the leaves and stems turned yellow and became fairly dry and threshed mechanically in both the years.

3.8 Biometric observations

The second row of the plot was used for sampling purpose. The following observations were recorded at different growth stages of the crop.

3.8.1 Pre-harvest observations

3.8.1.1 Leaf area index (LAI)

Plants from 25 cm length of the sampling row were uprooted at 40, 70 and 100 DAS. The leaves of the sampled plants were separated and cleaned manually. The leaf area was measured by using leaf area meter (Model LI-COR 3100). The leaf area index was calculated by using the following formula:

$$\text{LAI} = \frac{\text{Total leaf area}}{\text{Ground area}}$$

3.8.1.2 Dry matter accumulation

Plants from 25 cm length of the sampling row were uprooted at 40, 70, 100 DAS and at harvest and above ground portions were cut for observations.

The sampled plants were first sun-dried for 2-3 days and then dried in hot air oven at 65°C for 48 hours for recording dry weight. Dry matter was expressed in gram per square metre area (i.e. g m^{-2}).

3.8.1.3 Crop growth rate (CGR)

It represents dry weight gained by a unit area of crop in a unit time and expressed as g m^{-2} day^{-1}. It was calculated by using the formula:

$$\text{CGR} = \frac{W_2 - W_1}{t_2 - t_1}$$

Where, W_1 and W_2 are plant dry weights at time t_1 and t_2, respectively.

3.8.1.4 Net assimilation rate (NAR)

It represents the rate of increase in whole plant dry weight per unit leaf area and expressed as g m^{-2} day^{-1}.

$$\text{NAR} = \frac{(\text{Log}_e L_2 - \text{Log}_e L_1)(W_2 - W_1)}{(L_2 - L_1)(t_2 - t_1)}$$

Where, L_1 and L_2 are leaf area and W_1 and W_2 are plant dry weights at time t_1 and t_2, respectively.

3.8.1.5 Root dry weight

Roots from 25 cm length of sampling row were carefully dug out at 70 DAS. After washing the roots in running water the stem portion was removed. Then these roots were sun-dried for 2-3 days and then oven dried at 65°C for 48 hours. Finally roots were weighed and dry weight per square meter was calculated based on row spacing.

3.8.1.6 Effective tillers per m^2

Number of effective tillers (ear bearing tillers) were counted from one metre row length randomly from four places in the net plot, averaged and expressed as number of effective tillers per square metre area.

3.8.2 Post harvest observations

3.8.2.1 Number of grains per ear

Ten earheads from sampled plants were randomly selected, threshed and number of grains were counted. The average was worked out to obtain the number of grains per ear.

3.8.2.2 Test weight (Thousand grains)

A representative sample of grains was taken from the produce of each plot after drying and cleaning and weight of 1000 grains were recorded.

3.8.2.3 Grain and straw yield

Crop from the net plots was harvested, sun-dried and then labelled after making bundles. The bundles were weighed to record the total biological yield. The bundles from each plots were threshed, winnowed and cleaned manually. The weight of the grain was taken separately to record grain yield. Then straw yield was calculated by deducting grain yield form the total biological yield. The yield was then converted to tonnes per hectare (i.e. t ha^{-1}).

3.8.2.4 Harvest index

Harvest index was computed by dividing the grain yield by the total biological yield and was expressed as percentage.

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.9 Microbial observations

Phosphate solubilizing micro-organisms were enumerated by serial dilution and plating method using Paikovskayas agar medium (Paikovskaya, 1948) and Rose Bengal agar medium (Martin, 1950) for bacteria and fungus, respectively. The number of VAM spores per gram of soil was enumerated by wet sieving and decanting methods (Gerdemann and Nicolsen, 1963).

Soil samples were collected from root rhizosphere at 70 DAS. After processing the soil samples 10 g of soil sample was dissolved in 90 ml of sterilized distilled water. The subsequent serial dilutions were also made using sterile water blanks. Aliquots of 0.1 ml of the appropriate dilutions were transferred to petriplates containing Pikovskaya/Rose Bengal agar medium in triplicates. The plates were incubated at 28 ± 2°C. After 4 days of incubation bacteria/fungus colonies showing clearing zones around them are counted and expressed in per gram of soil.

Fifty gram of soil sample was dissolved in 200 ml of water and kept undisturbed for few moments to settle down the heavier particles. The supernatant was passed through 30-60 mesh sieve and collected in liquid in one litre beaker. The suspension was passed through a sieve fine (100-200) enough to retain the desired spores and washed thoroughly to ensure that all colloidal material passes through the sieve. The small amount of the remaining was transferred to a petri-dish for enumerating the number of spores under dissecting microscope.

3.10 Chemical analysis of plant samples

Plants from 25 cm row length of ^{32}P applied area were cut from the ground level and kept in bundles for phosphorus analysis and radio chemical assay. The plants were oven dried and ground to fine powder to pass through 40 mesh sieve and used for analysis. The total phosphorus content was estimated by Vanadomolybdo phosphorus yellow colour method (Jackson, 1973). The phosphorus uptake by the crop was calculated by multiplying phosphorus content in plant with crop yield and expressed as kg ha^{-1}.

3.11 Assay of radioactive phosphorus

Ten millilitres of digested plant material was taken in a glass vial and counts were recorded with the help of liquid scintillation counter Model No. LKB WALLAC, 1209 RAC K BETA. Similarly a series of diluted 10 ml fertilizer solution were counted simultaneously as a fertilizer standard for computing % Pdff values.

3.11.1 Preparation of fertilizer standard

The radioactive (^{32}P) single superphosphate was obtained from BRIT, BARC, Mumbai. The P content of the radioactive sample was analysed in the laboratory and found to be 16.0 % in both the years. A fraction of labelled single superphosphate that was applied in plots was preserved for preparing labelled fertilizer standard. One gram of this fertilizer was dissolved in distilled water and volume was made up to 100 ml.

3.11.2 Specific activity

The specific activity is defined as the activity of a particular radionuclide per unit mass of its element or compound. It is commonly expressed as millicuric (MCi) per gram or counts per minute (CPM) per milligram.

$$\text{Specific activity} = \frac{^{32}P}{^{31}P + ^{32}P} = \frac{\text{CPM of the sample} - \text{CPM of the background}}{\text{P in the sample (mg)}}$$

3.11.3 Phosphorus derived from fertilizer (Pdff)

The extent to which fertilizer P is accumulated in plant can be estimated by the parameter Pdff (%) and is calculated as follows:

$$\text{Pdff (\%)} = \frac{\text{Specific activity of plant sample}}{\text{Specific activity of fertilizer standard}} \times 100$$

3.11.4 Fertilizer phosphorus in plant

The amount of fertilizer phosphorus in plants is calculated as follows:

$$\text{Fertilizer P in plant} = \frac{\text{Total P uptake by plant per unit area x Pdff (\%)}}{100}$$

3.11.5 Per cent utilization of applied phosphorus

The use of ^{32}P as a tracer provides the only accurate method of quantitative measurement of the efficiency of fertilizer use by plants. It was obtained by using the formula:

$$\text{\% Utilization of applied P} = \frac{\text{Fertilizer P uptake by plant per unit area}}{\text{Fertilizer P applied per unit area}} \times 100$$

3.11.6 'A' value

The concept of 'A' value is based on the principle that the plant, when applied with two sources of nutrients, will absorb it from each of these sources in amount directly proportional to the quantity of the nutrient available from each source (Fried and Dean, 1952; Fried, 1964). 'A' value indicates the amount of available nutrient present in soil. It is calculated as follows:

$$\text{'A' value} = \frac{\text{\% Pdfs}}{\text{\% Pdff}} \times \text{Rate of fertilizer P applied (kg ha}^{-1}\text{)}$$

Where, % Pdfs = % P derived by plant from soil

= 100 - % Pdff

and % Pdff = % P derived from fertilizer

3.11.7 Agronomic efficiency

It refers to the additional produce obtained in kg per kg of an applied nutrient. It is calculated as follows:

$$\text{Agronomic efficiency (AE)} = \frac{Y_i - Y_c}{P_{ai}}$$

Where,

Y_i = Yield from fertilized plot (kg)

Y_c = Yield from control plot (kg)

P_{ai} = Amount of fertilizer P applied (kg)

3.12 Response studies

The relationship between the grain yield and the rate of phosphorus was worked out with the help of least squares method (FAO, 1966). Optimum economic dose of phosphorus was calculated by using the formula:

$$X_0 = \frac{q/p - b}{2c}$$

Where,

X_0 = Optimum level of P_2O_5 in kg ha^{-1}
q = Cost of one kg of P_2O_5
p = Price of one tonne of grain yield

and b and c are linear and quadratic coefficients, respectively, in the regression equations.

3.12.1 Yield at optimum economic dose

The grain yield of wheat at optimum economic dose of P was calculated by fitting the values of optimum economic dose in quadratic equation given below:

$$Y = ax + bx + cx^2$$

Where,

Y = expected yield at optimum economic dose (t ha^{-1})

x = optimum economic dose of P (kg ha^{-1})

a,b and c are constants

3.13 Economics

Economics of different treatments were worked out from the costs of inputs and income from grain and straw yield. The details of cost of cultivation and sale price of the produce are given in Appendices III and IV.

The gross and net returns as well as the net returns per rupee invested were worked out as follows:

Gross returns (Rs.ha^{-1}) = Price of grain + Price of straw

Net returns (Rs.ha^{-1}) = Gross return – Total cost of cultivation

$$\text{Net returns per rupee invested} = \frac{\text{Net returns}}{\text{Total cost of cultivation}}$$

Economics of phosphatic fertilizer and microbial culture usage in terms of investment, returns and net returns per rupee invested were calculated at the prevailing prices of fertilizer, microbial culture and farm produce, the details of which were obtained from the Division of Agricultural Economics, IARI, New Delhi-110012.

3.14 Statistical analysis

The data collected in the experiment for different parameters were subjected to statistical test by following Analysis of Variance technique suggested by Cochran and Cox (1967). Wherever variance ratio ('F' value) was found significant critical difference (CD) values at 5% level of significance were computed for making comparisons between treatments.

Chapter 4

RESULTS

In this chapter, the results emanating from the experiment entitled **"Influence of phosphate solubilizing and mobilizing micro-organisms on phosphorus use efficiency in wheat using ^{32}P as a tracer"** are presented factorwise. The observations on various parameters on study have been statistically analysed and presented in tables. As no cases the interaction effect was found significant the main effects of treatments are explained in details.

4.1 Growth parameters

4.1.1 Leaf area index

The data pertaining to leaf area index (LAI) as influenced by levels of phosphorus and phosphate solubilizing and mobilizing micro-organisms are presented in Table 4. There was a significant increase in leaf area index with application of phosphorus up to 40 kg P_2O_5 ha^{-1} irrespective of growth stages. Highest leaf area index was observed at 60 kg P_2O_5 ha^{-1}, which was at par with that of 40 kg P_2O_5 ha^{-1}. The trend was similar in both the years. The leaf area index recorded due to application of 60 kg P_2O_5 ha^{-1} at 40, 70 and 100 days after sowing (DAS) were 1.79, 3.58, 3.13 and 2.20, 4.06, 3.48 in the year 1999-2000 and 2000-2001, respectively.

As regards biofertilizers in the year 1999-2000 inoculation with *Pseudomonas striata* and *Glomus fasciculatum* produced significantly higher leaf area index over control, whereas *Aspergillus awamori* could not increase

Table 4. Effect of phosphorus levels and biofertilizers on leaf area index of wheat

Treatments	1999-2000			2000-2001		
	40 DAS	70 DAS	100 DAS	40 DAS	70 DAS	100 DAS
Phosphorus levels ($kg\ P_2O_5\ ha^{-1}$)						
0	1.20	2.29	1.65	1.25	2.60	1.94
20	1.47	2.89	2.32	1.64	3.19	2.53
40	1.71	3.38	2.80	2.05	3.82	3.17
60	1.79	3.58	3.13	2.20	4.06	3.48
SEm ±	**0.056**	**0.107**	**0.101**	**0.071**	**0.123**	**0.104**
CD (P=0.05)	**0.193**	**0.371**	**0.351**	**0.245**	**0.424**	**0.360**
Biofertilizers						
Control	1.30	2.80	2.14	1.54	3.03	2.43
Pseudomonas striata	1.75	3.28	2.81	1.97	3.70	3.09
Glomus fasciculatum	1.72	3.09	2.62	1.93	3.54	2.91
Aspergillus awamori	1.42	2.97	2.34	1.70	3.40	2.69
SEm ±	**0.048**	**0.084**	**0.076**	**0.050**	**0.090**	**0.071**
CD (P=0.05)	**0.141**	**0.246**	**0.222**	**0.147**	**0.263**	**0.208**

significantly. But in the second year of study all the three biofertilizers significantly increased the leaf area index over control. Among biofertilizers, *Pseudomonas striata* recorded highest leaf area index which was at par with *Glomus fasciculatum* but significantly higher than that of *Aspergillus awamori*. *Pseudomonas striata* inoculation recorded a leaf area index of 1.75, 3.28, 2.81 and 1.97, 3.70, 3.09 at 40, 70 and 100 DAS in the year 1999-2000 and 2000-2001, respectively, which was 34.6, 17.1, 31.3 and 27.9, 22.1, 27.2 per cent higher over their respective control.

4.1.2 Dry matter production (g m^{-2})

The data on dry matter production of wheat as influenced by phosphorus levels and biofertilizers are presented in Table 5.

It is evident from Table 5 that there was increase in dry matter accumulation with addition of phosphorus up to the highest level tried. But the differences between consecutive doses were significant up to 40 kg P_2O_5 ha^{-1} in all the growth stages. Similar results were observed in both the years. The dry matter accumulation resulted due to application of 40 kg P_2O_5 ha^{-1} was 295.21, 650.16, 967.65, 1328.12 and 305.91, 679.38, 1015.14, 1390.30 g m^{-2} at 40, 70, 100 DAS and at harvest in the year 1999-2000 and 2000-2001, respectively. The increase in dry matter production due to 40 kg P_2O_5 ha^{-1} over control was in the order of 27.9, 27.9, 24.0, 13.6 and 27.2, 28.8, 24.6, 24.6 per cent at 40, 70, 100 DAS and at harvest in the year 1999-2000 and 2000-2001, respectively.

Results further indicated that inoculation with biofertilizers brought about significant increase in dry matter production in both the years at all growth stages

Table 5. Effect of phosphorus levels and biofertilizers on dry matter production of wheat (g m^{-2})

Treatments	1999-2000				2000-2001			
	40 DAS	70 DAS	100 DAS	At harvest	40 DAS	70 DAS	100 DAS	At harvest
Phosphorus levels (kg P_2O_5 ha^{-1})								
0	230.80	508.32	780.61	1071.36	240.42	527.63	814.68	1116.00
20	268.07	590.40	887.69	1217.50	277.24	609.13	920.60	1261.40
40	295.21	650.16	967.65	1328.12	305.91	679.38	1015.14	1390.30
60	308.72	679.92	1007.67	1382.40	321.58	706.38	1051.20	1440.00
SEm ±	**7.43**	**15.97**	**22.21**	**30.51**	**8.77**	**19.69**	**26.08**	**34.97**
CD (P=0.05)	**27.51**	**55.24**	**76.84**	**105.55**	**30.33**	**68.12**	**90.23**	**121.00**
Biofertilizers								
Control	248.78	547.92	831.29	1138.75	259.27	570.75	865.93	1186.20
Pseudomonas striata	298.80	658.08	974.39	1340.74	307.03	670.03	1000.72	1369.40
Glomus fasciculatum	288.13	634.56	945.36	1301.29	296.13	653.00	980.72	1343.30
Aspergillus awamori	267.09	588.24	891.88	1218.61	286.22	628.75	954.26	1307.80
SEm ±	**6.91**	**15.19**	**21.67**	**29.45**	**7.51**	**11.52**	**23.64**	**30.14**
CD (P=0.05)	**20.19**	**44.36**	**63.27**	**85.99**	**21.92**	**33.65**	**69.02**	**88.02**

except *Aspergillus awamori* in 1999-2000. The difference in dry matter accumulation between *Pseudomonas striata* and *Glomus fasciculatum* was not significant. Inoculation with *Pseudomonas striata* produced highest dry matter in both the years followed by *Glomus fasciculatum*. Inoculation with *Aspergillus awamori* produced significantly lower dry matter than *Pseudomonas striata* in 1999-2000 at all growth stages but in the year 2000-2001 the difference was significant only at 70 DAS. The dry matter yield due to inoculation with *Glomus fasciculatum*, though higher than *Aspergillus awamori* but statistically significant only at 40 and 70 DAS in the year 1999-2000. The dry matter yield due to inoculation of *Pseudomonas striata* was 298.80, 658.08, 974.39, 1340.74 and 307.03, 670.03, 1000.72, 1369.40 g m^{-2} at 40, 70, 100 DAS and at harvest in the year 1999-2000 and 2000-2001, respectively.

4.1.3 Crop growth rate (g $m^{-2}day^{-1}$)

The data recorded on crop growth rate (CGR) of wheat during different growth periods as affected by phosphorus and biofertilizers are presented in Table 6.

It is evident from the data that successive levels of phosphorus increased the crop growth rate during different growth periods. There was increase in growth rate from 0 level to 60 kg P_2O_5 ha^{-1} but the difference in growth rate between 40 and 60 kg P_2O_5 ha^{-1} was statistically at par in both the years of study at all growth stages. The crop growth rate recorded due to application of 40 kg P_2O_5 ha^{-1} was 11.83, 10.58, 9.25 g $m^{-2}day^{-1}$ in the year 1999-2000 for the period of 40-70, 70-

Table 6. Effect of phosphorus levels and biofertilizers on crop growth rate of wheat (g m^{-2} day^{-1})

Treatments	1999-2000			2000-2001		
	40-70 DAS	70-100 DAS	100 DAS-harvest	40-70 DAS	70-100 DAS	100 DAS-harvest
Phosphorus levels (kg P_2O_5 ha^{-1})						
0	9.25	9.08	7.21	9.58	9.58	7.73
20	10.74	9.91	8.46	11.07	10.39	8.74
40	11.83	10.58	9.25	12.33	11.20	9.62
60	12.37	10.90	9.60	12.83	11.48	9.96
SEm ±	**0.304**	**0.188**	**0.222**	**0.338**	**0.221**	**0.236**
CD (P=0.05)	**1.051**	**0.650**	**0.769**	**1.170**	**0.763**	**0.817**
Biofertilizers						
Control	9.97	9.44	7.88	10.38	9.84	8.21
Pseudomonas striata	11.97	10.54	9.37	12.10	11.02	9.47
Glomus fasciculatum	11.55	10.36	8.90	11.90	10.93	9.30
Aspergillus awamori	10.71	10.12	8.38	11.42	10.85	9.07
SEm ±	**0.272**	**0.183**	**0.197**	**0.317**	**0.204**	**0.186**
CD (P=0.05)	**0.795**	**0.533**	**0.574**	**0.926**	**0.597**	**0.542**

100 DAS and 100 DAS-harvest, respectively, whereas the values for the year 2000-2001 were 12.33, 11.20 and 9.62 g $m^{-2}day^{-1}$, respectively..

The data further indicates that crop growth rate increased significantly due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* throughout the growth period in both the years of investigation. Although highest growth rate was observed in case of *Pseudomonas striata*, it was statistically on par with that of *Glomus fasciculatum* inoculation. Crop growth rate achieved due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* was significantly higher over *Aspergillus awamori* during 40-70 DAS and 100 DAS-harvest in the year 1999-2000 but all the three biofertilizers were at par in the year 2000-2001 with respect to crop growth rate. The crop growth rate recorded due to inoculation with *Pseudomonas striata* was 11.97, 10.54 and 9.37 g $m^{-2}day^{-1}$ in the year 1999-2000 whereas 12.10, 11.02 and 9.47 g $m^{-2}day^{-1}$ in the year 2000-2001 during the period of 40-70, 70-100 DAS and 100 DAS-harvest, respectively.

4.1.4 Net assimilation rate (g $m^{-2}day^{-1}$)

The data recorded on net assimilation rate (NAR) of wheat as influenced by phosphorus and biofertilizers are given in Table 7.

A perusal of data from Table 7 reveals that highest NAR was observed in control plot. But application of 20 kg P_2O_5 ha^{-1} significantly reduced the NAR. Although, with addition of phosphorus, the NAR declined up to 60 kg P_2O_5 ha^{-1}, the difference between successive levels was not statistically significant. While highest NAR was observed without P fertilization, the lowest NAR was recorded

Table 7. Effect of phosphorus levels and biofertilizers on net assimilation rate of wheat (g m^{-2} day^{-1})

Treatments	1999-2000		2000-2001	
	40-70 DAS	70-100 DAS	40-70 DAS	70-100 DAS
Phosphorus levels (kg P_2O_5 ha^{-1})				
0	5.38	4.44	5.00	4.15
20	4.94	3.83	4.61	3.68
40	4.65	3.44	4.43	3.36
60	4.61	3.25	4.15	3.03
SEm ±	**0.122**	**0.119**	**0.108**	**0.100**
CD (P=0.05)	**0.421**	**0.413**	**0.374**	**0.346**
Biofertilizers				
Control	5.11	4.07	4.88	3.92
Pseudomonas striata	4.70	3.39	4.35	3.32
Glomus fasciculatum	4.81	3.60	4.46	3.41
Aspergillus awamori	4.96	3.88	4.50	3.57
SEm ±	**0.093**	**0.083**	**0.087**	**0.077**
CD (P=0.05)	**0.271**	**0.243**	**0.254**	**0.224**

due to application of 60 kg P_2O_5 ha^{-1}. The results were alike in both the years of study.

As regards biofertilizers, highest NAR was observed in control plots. Among the biofertilizer treatments, inoculation with *Pseudomonas striata* and *Glomus fasciculatum* recorded significantly lower NAR over no inoculation in both the years. Inoculation with *Aspergillus awamori* recorded significantly lower NAR in the year 2000-2001 than no inoculation but the difference was not significant in 1999-2000. There was no significant difference among the three biofertilizers with respect to NAR during 40-70 DAS. During 70-100 DAS the NAR achieved due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* was significantly lower than that of *Aspergillus awamori* inoculation in 1999-2000 but only *Pseudomonas striata* recorded lower NAR than *Aspergillus awamori* in 2000-2001. The lowest NAR i.e. 4.70, 3.39 in 1999-2000 and 4.35, 3.32 in the year 2000-2001 during 40-70 DAS and 70-100 DAS, respectively, was achieved due to inoculation with *Pseudomonas striata.*

4.1.5 Root dry weight (g m^{-2})

The data from the Table 8 indicates that the root dry weight of wheat differed significantly with the levels of phosphorus. Successive levels of phosphorus up to 40 kg P_2O_5 ha^{-1} increased the root dry weight significantly. Application of 60 kg P_2O_5 ha^{-1} improved the root dry weight marginally but could not come to the significant level. The root dry weight due to the application of 40 kg P_2O_5 ha^{-1} was 37.15 and 39.00 g m^{-2} during 1999-2000 and 2000-2001, respectively, which was 30.4 and 30.3 per cent higher over control.

Table 8. Effect of phosphorus levels and biofertilizers on root dry weight of wheat (g m^{-2})

Treatments	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})		
0	28.50	29.93
20	33.67	35.35
40	37.15	39.00
60	38.26	40.18
SEm ±	**0.961**	**1.030**
CD (P=0.05)	**3.324**	**3.563**
Biofertilizers		
Control	30.39	31.38
Pseudomonas striata	37.71	39.20
Glomus fasciculatum	36.28	38.10
Aspergillus awamori	33.21	35.80
SEm ±	**0.990**	**1.052**
CD (P=0.05)	**2.891**	**3.072**

On perusal of data from Table 8, it reveals that root dry weight of wheat produced due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* was on par but significantly higher than control in both the years. But inoculation with *Aspergillus awamori* produced significantly higher root dry weight only in second year of study. Among the micro-organisms inoculation of *Pseudomonas striata* produced significantly higher root dry weight over *Aspergillus awamori* inoculation in both the years, whereas *Glomus fasciculatum* inoculation increased the root dry weight only in first year. The root dry weight of wheat produced due to inoculation with *Pseudomonas striata* was 37.71 and 39.20 g m^{-2} in the year 1999-2000 and 2000-2001, respectively, whereas the values for *Glomus fasciculatum* inoculation were 36.28 and 38.10 g m^{-2}, respectively.

4.2 Yield attributes

4.2.1 Effective tillers

The data on number of effective tillers per square metre as influenced by phosphorus levels and biofertilizers are given in Table 9.

The data on the number of effective tillers indicates that successive levels of phosphorus increased the number of effective tillers m^{-2} up to 40 kg P_2O_5 ha^{-1}. The highest number of effective tillers were observed at 60 kg P_2O_5 ha^{-1} but it was statistically on par with that at 40 kg P_2O_5 ha^{-1}. Similar results were observed in both the years of study. The number of effective tillers due to application of 40 kg P_2O_5 ha^{-1} were 321.94 and 329.12 m^{-2} during 1999-2000 and 2000-2001, respectively.

Table 9. Effect of phosphorus levels and biofertilizers on yield attributes of wheat

Treatments	Effective tillers (m^{-2})		Grain number (ear^{-1})		Test weight (g)	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})						
0	251.28	258.56	38.96	40.32	37.98	39.06
20	292.51	297.65	43.01	44.46	40.23	41.41
40	321.94	329.12	46.14	48.15	43.01	44.30
60	332.05	344.08	49.10	50.21	44.03	45.32
SEm ±	**8.764**	**8.414**	**0.894**	**1.029**	**1.009**	**1.061**
CD (P=0.05)	**30.324**	**29.113**	**3.092**	**3.560**	**3.491**	**3.672**
Biofertilizers						
Control	271.31	276.60	43.13	44.38	39.68	40.84
Pseudomonas striata	324.57	329.89	45.47	46.90	43.00	43.86
Glomus fasciculatum	311.42	320.13	45.01	46.43	42.13	42.96
Aspergillus awamori	290.48	302.80	43.60	45.42	40.44	42.43
SEm ±	**7.932**	**6.661**	**0.622**	**0.665**	**0.812**	**0.693**
CD (P=0.05)	**23.162**	**19.451**	**1.815**	**1.943**	**2.370**	**2.024**

A critical examination of data shows that application of biofertilizers has positive impact on the number of effective tillers of wheat per square metre in both the years. Inoculation with *Pseudomonas striata* and *Glomus fasciculatum* increased the number of effective tillers m^{-2} in both the years of study, whereas inoculation with *Aspergillus awamori* produced significantly higher number of tillers over control in the year 2000-2001 only. Inoculation with *Pseudomonas striata* produced highest number of effective tillers i.e. 324.57 and 329.89 m^{-2} which was at par with *Glomus fasciculatum* inoculation i.e. 311.42 and 320.13 m^{-2} in the year 1999-2000 and 2000-2001, respectively, but significantly higher than that produced by *Aspergillus awamori* inoculation. The increase in the number of effective tillers m^{-2} due to inoculation of *Pseudomonas striata* over control was in the order of 19.6 and 20.4 per cent in the year 1999-2000 and 2000-2001, respectively.

4.2.2 Number of grains per ear

The data on number of grains per earhead are presented in Table 9.

A perusal of data reveals that application of phosphorus had positive and significant effect on this parameter. Application of 60 kg P_2O_5 ha^{-1} produced highest number of grains per earhead during both the years. However, it was found to be on par with 40 kg P_2O_5 ha^{-1}. The number of grains per earhead recorded due to application of 40 kg P_2O_5 ha^{-1} were 46.14 and 48.15 in the year 1999-2000 and 2000-2001, respectively.

Besides phosphorus, application of biofertilizers also had positive impact on number of grains per earhead. Inoculation with *Pseudomonas striata* and

Glomus fasciculatum significantly enhanced the number of grains per earhead over control but inoculation with *Aspergillus awamori* failed to prove its superiority. In the year 1999-2000 the number of grains produced per earhead due to inoculation with *Pseudomonas striata* was at par with that of *Glomus fasciculatum* but significantly higher over *Aspergillus awamori* inoculation. But the differences among the three micro-organisms were not significant. The highest number of grains ear^{-1} was recorded due to inoculation with *Pseudomonas striata* and was in the order of 45.47 and 46.90 ear^{-1} in the year 1999-2000 and 2000-2001, respectively.

4.2.3 Test weight (g)

The effects of phosphorus levels and biofertilizers on test weight are given in Table 9.

It is evident from the data that test weight was increased due to application of phosphorus. There was increase in test weight from zero level of P_2O_5 ha^{-1} to highest level tried but the difference in test weight between two consecutive levels was not significant. However, application of 40 and 60 kg P_2O_5 ha^{-1} produced significantly heavier grains over control. The test weight recorded at 40 kg P_2O_5 ha^{-1} was 43.01 g in the year 1999-2000, whereas 44.30 g in the year 2000-2001. The trends were identical in both the years.

Results further indicate that inoculation with *Pseudomonas striata* and *Glomus fasciculatum* recorded significantly higher test weight over control. The difference between *Aspergillus awamori* and no inoculation was not significant. Among biofertilizers, inoculation with *Pseudomonas striata* resulted in significantly higher test weight compared to *Aspergillus awamori* inoculation in the year 1999-2000 while there was no significant difference with respect to test weight in the year 2000-2001. The highest test weights of 43.30 and 43.86 g were observed in the years 1999-2000 and 2000-2001, respectively, due to inoculation of *Pseudomonas striata*.

4.3 Yield and Harvest index

4.3.1 Grain yield

The data on grain yield as influenced by phosphorus levels and biofertilizers are presented in Table 10.

The grain yield of wheat was increased significantly with application of phosphorus up to 40 kg P_2O_5 ha^{-1} in both the years of experimentation. Although, the highest grain yield was obtained with 60 kg P_2O_5 ha^{-1}, it was on par with that of 40 kg P_2O_5 ha^{-1}. The trend was similar in both the years. During 1999-2000 the increase in grain yield was 26.6 and 43.5% with 20 and 40 kg P_2O_5 ha^{-1} over control, respectively, whereas the corresponding figures for 2000-2001 were 28.3 and 46.6%.

Application of biofertilizers showed a positive influence on grain yield of wheat. Seed inoculation of wheat with *Pseudomonas striata* (PSB) and soil inoculation with *Glomus fasciculatum* (VAM) significantly increased the grain yield of wheat over control in both the years. But the difference in grain yield due to inoculation with PSB and VAM was not significant. Inoculation with *Aspergillus awamori* (PSF) could not increase the grain yield significantly in the year 1999-2000 but the increase was significant in 2000-2001. Inoculation with

Table 10. Effect of phosphorus levels and biofertilizers on grain yield, straw yield and harvest index of wheat

Treatments	Grain yield (t ha^{-1})		Straw yield (t ha^{-1})		Harvest index (%)	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})						
0	4.02	4.21	5.95	6.37	40.34	39.74
20	5.09	5.40	7.27	7.66	41.11	41.36
40	5.77	6.17	7.98	8.38	41.94	42.44
60	5.94	6.44	8.20	8.59	42.04	42.84
SEm ±	**0.154**	**0.170**	**0.195**	**0.197**	**0.798**	**0.928**
CD (P=0.05)	**0.533**	**0.589**	**0.674**	**0.681**	**NS**	**NS**
Biofertilizers						
Control	4.71	5.14	6.86	7.38	40.61	40.85
Pseudomonas striata	5.65	5.82	7.73	7.96	42.18	42.35
Glomus fasciculatum	5.43	5.74	7.63	7.96	41.52	41.68
Aspergillus awamori	5.04	5.52	7.18	7.72	41.13	41.49
SEm ±	**0.118**	**0.123**	**0.145**	**0.152**	**0.630**	**0.688**
CD (P=0.05)	**0.345**	**0.359**	**0.423**	**0.445**	**NS**	**NS**

PSB or VAM recorded significantly higher yield as compared to inoculation with PSF in the first year but were at par during the second year of study. The highest yield was recorded due to inoculation of PSB i.e. 5.65 and 5.82 t ha^{-1} in 1999-2000 and 2000-2001, respectively, which were 20.0 and 13.2% higher over control.

4.3.2 Straw yield

The data on effect of phosphorus levels and biofertilizers on straw yield are given in Table 10.

The data from Table 10 indicate that straw yield of wheat showed significant response to phosphorus. Straw yield significantly increased due to application of phosphorus up to 40 kg P_2O_5 ha^{-1}. But there was no significant difference between the two levels of phosphorus i.e. 40 and 60 kg P_2O_5 ha^{-1}. The increase in straw yield due to application of P at 40 kg P_2O_5 ha^{-1} was in the order of 34.1 and 31.6% over control in the year 1999-2000 and 2000-2001, respectively.

Analysis of data on straw yield reflects the superiority of inoculation with phosphate solubilizing and mobilizing micro-organisms. There was significant increase in straw yield of wheat due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* over control but it was statistically on par with *Aspergillus awamori*. However, inoculation with *Pseudomonas striata* and *Glomus fasciculatum* produced significantly higher straw yield compared to *Aspergillus awamori* in the first year but there was no significant difference among all the three micro-organisms in the second year. The increase in straw yield due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* over control was in the order of 12.7 and 11.2%, respectively, in the year 1999-2000, whereas the value for the year 2000-2001 was 7.9% for both the micro-organisms.

4.3.3 Harvest index

The data on harvest index are presented in Table 10. It is evident from the Table that phosphorus application did not show significant difference on harvest index of wheat in both the years of study.

It was further observed that none of the biofertilizer treatments had statistically significant influence on harvest index of wheat. However, *Pseudomonas striata* showed a higher harvest index value of 42.18 and 42.35% in 1999-2000 and 2000-2001, respectively, which was closely followed by *Glomus fasciculatum* i.e. 41.52 and 41.68%.

4.4 Microbial studies

Data on population of phosphate solubilizing and mobilizing micro-organisms in the wheat rhizosphere as affected by various treatments have been presented in Table 11.

4.4.1 Phosphate solubilizing micro-organisms

It is evident from the data that the population of phosphate solubilizing micro-organisms (PSMs) like *Pseudomonas striata* and *Aspergillus awamori* was favourably influenced by the levels of phosphorus application but the increase was significant only up to 40 kg P_2O_5 ha^{-1}. Phosphorus application at 60 kg P_2O_5 ha^{-1} marginally increased the population of phosphate solubilizing microbes as compared to lower level of P application, but the difference between 40 and 60 P_2O_5 ha^{-1} was not statistically significant The population of *Pseudomonas striata*

Table 11. Effect of phosphorus levels and biofertilizers on population of phosphate solubilizing and mobilizing micro-organisms in the wheat rhizosphere

Treatments	*Pseudomonas striata* (...x$10^3 g^{-1}$ of soil)		*Glomus fasciculatum* (g^{-1} of soil)		*Aspergillus awamori* (...x$10^3 g^{-1}$ of soil)	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})						
0	21.64	23.26	13.66	15.00	8.42	9.03
20	26.49	28.47	12.17	13.46	11.27	12.11
40	29.44	31.64	10.75	11.96	13.76	14.78
60	30.51	32.78	8.85	10.09	14.59	15.67
SEm ±	**0.816**	**0.882**	**0.418**	**0.466**	**0.627**	**0.647**
CD (P=0.05)	**2.823**	**3.052**	**1.445**	**1.613**	**2.171**	**2.237**
Biofertilizers						
Control	9.59	10.07	5.74	6.53	7.85	8.16
Pseudomonas striata	43.45	47.91	-	-	-	-
Glomus fasciculatum	-	-	16.97	18.72	-	-
Aspergillus awamori	-	-	-	-	16.17	17.63
SEm ±	**0.632**	**0.699**	**0.341**	**0.369**	**0.443**	**0.468**
CD (P=0.05)	**2.061**	**2.280**	**1.112**	**1.204**	**1.443**	**1.526**

at 40 kg P_2O_5 ha^{-1} was 29.44 and 31.64 x 10^3 cell counts g^{-1} of soil during 1999-2000 and 2000-2001, respectively, whereas the respective values for *Aspergillus awamori* were 13.76 and 14.78 x 10^3 cell counts g^{-1} of soil. It is further observed that inoculation with phosphate solubilizing micro-organisms significantly increased the population of respective micro-organisms. On an average, the population of *Pseudomonas striata* was increased 3.64 times, whereas the increase in the population of *Aspergillus awamori* was 1.11 times due to inoculation over control.

4.4.2 Phosphate mobilizing micro-organism

From the Table 11 it is obvious that the population of *Glomus fasciculatum* showed a negative response to application of phosphorus to wheat. With successive levels of phosphorus application, the number of spores of *Glomus fasciculatum* per gram of soil declined but the reduction was significant at or above 40 kg P_2O_5 ha^{-1} compared to control. There was a drastic reduction in the population of *Glomus fasciculatum* due to the highest level of P application as compared to that at 40 kg P_2O_5 ha^{-1}. The reduction in the population of *Glomus fasciculatum* was in the order of 35.2 and 32.7% during the years 1999-2000 and 2000-2001, respectively, at 60 kg P_2O_5 ha^{-1} over control. However, due to inoculation with *Glomus fasciculatum*, there was a significant increase in its population in the rhizosphere of wheat.

4.5 Nutrient Studies

The data pertaining to phosphorus content and uptake as influenced by phosphorus application and inoculation with phosphate solubilizing and mobilizing micro-organisms are given in Table 12.

4.5.1 Phosphorus content (%)

The data furnished in Table 12 reveal that the phosphorus content of the crop was significantly influenced by phosphorus application. Highest phosphorus content was recorded due to application of P at 60 kg P_2O_5 ha^{-1}. But significant increase was observed only up to the level of 40 kg P_2O_5 ha^{-1} during both the years.

Besides phosphorus, biofertilizers had a positive impact on the phosphorus content of wheat. It is observed that there was a significant increase in phosphorus content due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* during both the years, whereas the response was significant only in the year 2000-2001 for *Aspergillus awamori*. Inoculation with *Pseudomonas striata* and *Glomus fasciculatum* recorded significantly higher phosphorus content over *Aspergillus awamori* inoculation in the first year. However, the difference in the phosphorus content of wheat inoculated with *Glomus fasciculatum* and *Aspergillus awamori* was not significant in the second year of study. The highest phosphorus content of 0.118 and 0.117% was recorded due to inoculation with *Pseudomonas striata* during 1999-2000 and 2000-2001, respectively.

Table 12. Effect of phosphorus levels and biofertilizers on phosphorus content and uptake by wheat

Treatments	Phosphorus content (%)		Phosphorus uptake (kg ha^{-1})	
	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})				
0	0.104	0.106	10.42	11.26
20	0.109	0.112	13.47	14.68
40	0.114	0.116	15.63	16.95
60	0.116	0.118	16.49	17.78
SEm ±	**0.0012**	**0.0010**	**0.515**	**0.582**
CD (P=0.05)	**0.0042**	**0.0036**	**1.782**	**2.014**
Biofertilizers				
Control	0.103	0.107	12.06	13.57
Pseudomonas striata	0.118	0.117	15.77	16.07
Glomus fasciculatum	0.116	0.116	15.09	15.96
Aspergillus awamori	0.106	0.113	13.09	15.05
SEm ±	**0.0013**	**0.0011**	**0.409**	**0.439**
CD (P=0.05)	**0.0037**	**0.0031**	**1.193**	**1.281**

4.5.2 Phosphorus uptake (kg ha^{-1})

A perusal of data presented in Table 12 reveals that phosphorus application significantly increased the total uptake of phosphorus by wheat. The uptake of phosphorus by wheat increased progressively and significantly up to 40 kg P_2O_5 ha^{-1}. Although, there was an increase in phosphorus uptake at 60 kg P_2O_5 ha^{-1} over 40 kg P_2O_5 ha^{-1}, the increase was marginal and not significant. The results were identical in both the years.

As regards biofertilizers, all the three biofertilizers enhanced phosphorus uptake by wheat. *Pseudomonas striata* registered the maximum phosphorus uptake values of 15.77 and 16.07 kg P ha^{-1} followed by *Glomus fasciculatum* with values of 15.09 and 15.96 kg P ha^{-1} during 1999-2000 and 2000-2001, respectively, though they were on par with each other. They registered significantly higher uptake over control in both the years and *Aspergillus awamori* only in first year of study. However, inoculation with *Aspergillus awamori* significantly increased the phosphorus uptake over control in the second year. The percentage increase in phosphorus uptake due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* over control was in the order of 30.8 and 25.1% in the year 1999-2000 and 18.4 and 17.6% in the year 2000-2001, respectively.

4.6 Radio chemical studies

4.6.1 Phosphorus derived from fertilizer (Pdff)

Values for per cent phosphorus derived from fertilizer (% Pdff) by wheat when supplied with radioactive phosphorus (single superphosphate) at various levels of phosphorus along with different biofertilizers are presented in Table 13.

Table 13. Effect of phosphorus levels and biofertilizers on Pdff (%) and 'A' value (kg P ha^{-1})

Treatments	Pdff (%)		'A' value (kg P ha^{-1})	
	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})				
20	17.83	18.71	40.98	38.46
40	20.93	21.56	66.61	64.08
60	22.04	22.53	93.44	90.80
SEm ±	**0.380**	**0.423**	**1.975**	**1.779**
CD (P=0.05)	**1.495**	**1.661**	**7.762**	**6.993**
Biofertilizers				
Control	19.15	20.06	70.76	67.24
Pseudomonas striata	21.18	21.63	63.82	61.94
Glomus fasciculatum	21.05	21.21	64.32	63.69
Aspergillus awamori	19.68	20.84	69.14	64.92
SEm ±	**0.281**	**0.257**	**1.579**	**1.182**
CD (P=0.05)	**0.834**	**0.763**	**4.691**	**3.512**

The data in Table 13 indicate that there was a significant increase in per cent Pdff due to application of P at 40 kg P_2O_5 ha^{-1} over 20 kg P_2O_5 ha^{-1} The percentage increase in per cent Pdff was in the order of 17.4 and 15.2 in the year 1999-2000 and 2000-2001, respectively. Application of 60 kg P_2O_5 ha^{-1} improved the %Pdff over 40 kg P_2O_5 ha^{-1} but could not achieve the significant level. The results were identical in both the years of investigation.

The data also reveal that inoculation with phosphate solubilizing and mobilizing micro-organisms enhanced the per cent Pdff in wheat. In the first year *Pseudomonas striata* and *Glomus fasciculatum* recorded significantly higher per cent Pdff over control but *Aspergillus awamori* did not increase significantly. However, *Pseudomonas striata* and *Glomus fasciculatum* were at par with each other with respect to per cent Pdff. During 2000-2001 all the three micro-organisms recorded significantly higher per cent Pdff over control. Inoculation with *Pseudomonas striata* resulted in significantly higher per cent Pdff over *Aspergillus awamori*, but it was found at par with *Glomus fasciculatum.* The difference in per cent Pdff between *Glomus fasciculatum* and *Aspergillus awamori* was not significant. The per cent Pdff recorded due to inoculation with *Pseudomonas striata* was 21.18 and 21.63 in the year 1999-2000 and 2000-2001, respectively, whereas the corresponding values for *Glomus fasciculatum* were 21.05 and 21.21.

4.6.2 'A' value

The data pertaining to 'A' value are presented in Table 13.

A perusal of data reveals that successive increase in levels of phosphorus application increased the 'A' value significantly in both the years. The increase in 'A' value with application of 40 kg P_2O_5 ha^{-1} over 20 kg P_2O_5 ha^{-1} was 62.5 and 66.6% in the year 1999-2000 and 2000-2001, respectively. The respective values for application of P at 60 kg P_2O_5 ha^{-1} over 40 kg P_2O_5 ha^{-1} were 40.3 and 41.7%.

Inoculation with biofertilizers reduced the 'A' value in both the years of experimentation. Inoculation with *Pseudomonas striata* and *Glomus fasciculatum*, though they were statistically at par, significantly lowered the 'A' value compared to no inoculation in both the years but *Aspergillus awamori* did not decrease the 'A' value significantly. However, *Aspergillus awamori* inoculation recorded significantly higher 'A' value over both *Pseudomonas striata* and *Glomus fasciculatum* in first year, whereas no significant difference was observed among the three biofertilizers with respect to 'A' value in the second year of study. The maximum 'A' values i.e. 70.76 and 67.24 kg P ha^{-1} were recorded in the control plots, whereas *Pseudomonas striata* inoculation recorded lowest 'A' values i.e. 63.82 and 61.94 kg P ha^{-1} in the year 1999-2000 and 2000-2001, respectively.

4.6.3 Phosphorus utilization

The data on phosphorus utilization as influenced by various treatments are presented in Table 14.

From perusal of data, it is evident that the per cent phosphorus utilization followed a reverse trend with increasing levels of phosphorus during both the years of study. Wheat registered the highest per cent phosphorus utilization (27.59 and 31.33% during 1999-2000 and 2000-2001, respectively) when 20 kg P_2O_5 ha^{-1}

Table 14. Effect of phosphorus levels and biofertilizers on utilization and agronomic efficiency of phosphorus in wheat

Treatments	Utilization efficiency (%)		Agronomic efficiency (kg kg^{1})	
	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})				
20	27.59	31.33	53.38	59.75
40	18.65	20.76	43.64	49.00
60	13.77	15.19	32.05	37.13
SEm ±	**0.825**	**0.904**	**1.622**	**1.864**
CD (P=0.05)	**3.243**	**3.554**	**6.374**	**7.325**
Biofertilizers				
Control	16.19	19.23	27.31	35.11
Pseudomonas striata	23.75	24.59	58.91	59.56
Glomus fasciculatum	22.04	23.62	47.39	51.44
Aspergillus awamori	17.98	22.26	38.14	48.39
SEm ±	**0.661**	**0.684**	**1.492**	**1.805**
CD (P=0.05)	**1.963**	**2.032**	**4.431**	**5.362**

was applied to it. As the level of phosphorus goes on increasing the per cent phosphorus utilization decreased significantly. The magnitude of decrease due to application of 40 kg P_2O_5 ha^{-1} was 32.4 and 33.7% during 1999-2000 and 2000-2001, respectively, when compared with lower dose of 20 kg P_2O_5 ha^{-1}. The respective figures due to application of 60 kg P_2O_5 ha^{-1} over 40 kg P_2O_5 ha^{-1} were 26.2 and 26.8%.

Further analysis of data shows that significantly higher phosphorus utilization efficiency was recorded due to inoculation with *Pseudomonas striata* and *Glomus fasciculatum* during both the years, whereas *Aspergillus awamori* inoculation resulted in significant effect only during second year of experimentation over control. Among the biofertilizers, *Pseudomonas striata* recorded highest phosphorus utilization efficiency (23.75 and 24.59% during 1999-2000 and 2000-2001, respectively) which was at par with *Glomus fasciculatum* in both the years but recorded significantly higher efficiency compared to *Aspergillus awamori* in the second year. However, the difference between *Glomus fasciculatum* and *Aspergillus awamori* with respect to phosphorus utilization efficiency was significant during 1999-2000 only. The percentage increase in utilization efficiency due to inoculation with *Pseudomonas striata* was 46.7 and 27.9% over control during the year 1990-2000 and 2000-2001, respectively.

4.7 Phosphorus use efficiency (Agronomic efficiency)

The data relating to agronomic efficiency of phosphorus as influenced by phosphorus levels and biofertilizers are presented in Table 14.

A perusal of data reveals that agronomic efficiency of phosphorus decreased significantly with increase in phosphorus levels. The highest agronomic efficiency of phosphorus was recorded at 20 kg P_2O_5 ha^{-1} i.e. 53.38 and 59.75 kg grain per kg of P_2O_5 in the year 1999-2000 and 2000-2001, respectively. The lowest agronomic efficiency was recorded at 60 kg P_2O_5 ha^{-1}. The results were identical in both the years of study.

Further, the result shows that inoculation with different biofertilizers positively influenced the agronomic efficiency of phosphorus. All the three micro-organisms registered significantly higher phosphorus use efficiency over control in both the years. The highest agronomic efficiency was recorded due to inoculation with *Pseudomonas striata* (58.91 and 59.56 kg grain per kg P_2O_5 during 1999-2000 and 2000-2001, respectively) followed by *Glomus fasciculatum*.

4.8 Response studies

The relationship between grain yield of wheat and rates of P application were studied for both the years under different biofertilizer treatments. Response equations were fitted with the help of second degree polynomial. The linear and quadratic components of P were significant in both the years, thus the quadratic equations were fitted. The response equations evolved are presented in Table 15.

The expected yields were calculated at various levels of P by using these equations and presented in Table 16.

The optimum economic dose of P was calculated by using the formulae ½ c (q/p-b). Whereas 'p' and 'q' represent the prices of one tonne grain of wheat and one kg P_2O_5, respectively. The average price of wheat grain was taken as Rs

Table 15. Response equations of P application for different biofertilizers

Biofertilizers	Year	Response equation	'r' value	Optimum economic dose (kg P_2O_5 ha^{-1})	Yield at optimum economic dose (t ha^{-1})
Control	1999-2000	$Y = 3.30 + 0.0692 P - 0.00048 P^2$	1.000	66.50	5.78
	2000-2001	$Y = 3.59 + 0.0749 P - 0.00049 P^2$	0.997	70.96	6.41
Pseudomonas striata	1999-2000	$Y = 4.29 + 0.0841 P - 0.00083 P^2$	1.000	47.43	6.41
	2000-2001	$Y = 4.39 + 0.0842 P - 0.00078 P^2$	0.997	50.54	6.67
Glomus fasciculatum	1999-2000	$Y = 4.67 + 0.0432 P - 0.00039 P^2$	0.999	48.51	5.85
	2000-2001	$Y = 4.77 + 0.0528 P - 0.00044 P^2$	0.996	53.90	6.34
Aspergillus awamori	1999-2000	$Y = 3.79 + 0.0663 P - 0.00053 P^2$	1.000	57.49	5.85
	2000-2001	$Y = 4.09 + 0.0757 P - 0.00061 P^2$	0.999	57.65	6.45

Table 16. Observed and expected yield (t ha^{-1}) of wheat for different biofertilizers at various P levels

Levels of P (kg P_2O_5 ha^{-1})	Biofertilizers			
	1999-2000		2000-2001	
	Observed	Expected	Observed	Expected
	Control			
0	3.32	3.30	3.62	3.59
20	4.45	4.50	4.79	4.89
40	5.35	5.30	5.89	5.79
60	5.71	5.73	6.27	6.30
	Pseudomonas striata			
0	4.29	4.29	4.36	4.39
20	5.63	5.64	5.85	5.76
40	6.33	6.32	6.43	6.52
60	6.34	6.34	6.68	6.65
	Glomus fasciculatum			
0	4.68	4.67	4.79	4.77
20	5.36	5.38	5.57	5.65
40	5.80	5.78	6.25	6.18
60	5.86	5.87	6.33	6.36
	Aspergillus awamori			
0	3.79	3.79	4.08	4.09
20	4.91	4.91	5.41	5.37
40	5.59	5.59	6.11	6.15
60	5.86	5.86	6.47	6.46

5500.00 t^{-1} and the cost of P_2O_5 Rs 29.50 per kg. The optimum economic dose of P for wheat was found to be 66.50, 47.43, 48.51 and 57.49 in 1999-2000 and 70.96, 50.54, 53.90 and 57.65 kg P_2O_5 ha^{-1} in 2000-2001 with control and inoculation with *Pseudomonas striata, Glomus fasciculatum* and *Aspergillus awamori,* respectively. The lowest optimum economic dose was observed when wheat was inoculated with *Pseudomonas striata.* The yield at optimum levels of P application was also calculated by substituting the values of P in the equations. The economic optimum yields were 5.78, 6.41, 5.85 and 5.85 in 1999-2000 and 6.41, 6.67, 6.34 and 6.45 t ha^{-1} in 2000-2001 with control and inoculation with *Pseudomonas striata, Glomus fasciculatum* and *Aspergillus awamori,* respectively.

4.9 Economics

The economics of wheat production under different levels of phosphorus and biofertilizers have been worked out and presented in Table 17.

Analysis of data from Table 17 reveals that all the three economic parameters i.e., total cost of cultivation, gross returns and net returns per hectare increased with application of phosphorus up to 60 kg P_2O_5 ha^{-1} in both the years. The maximum net returns per hectare was recorded at 60 kg P_2O_5 ha^{-1} i.e. Rs 25,179.00 and Rs 28,066.00 closely followed by 40 kg P_2O_5 ha^{-1} i.e. Rs 24,666.00 and Rs 27,075.00 in the year 1999-2000 and 2000-2001, respectively. However, the net returns per rupee invested was increased with phosphorus levels only up to 40 kg P_2O_5 ha^{-1}. The highest net returns per rupee invested was recorded at 40 kg P_2O_5 ha^{-1} i.e. 2.23 and 2.44 in the year 1999-2000 and 2000-2001, respectively.

Table 17. Economics of wheat production as influenced by phosphorus levels and biofertilizers

Treatments	Gross returns (Rs. ha^{-1})		Cost of cultivation (Rs. ha^{-1})		Net returns (Rs. ha^{-1})		Net returns per rupee	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
Phosphorus levels (kg P_2O_5 ha^{-1})								
0	25083	26355	9865	9865	15218	16490	1.54	1.67
20	31615	33553	10455	10455	21160	23098	2.02	2.21
40	35711	38120	11045	11045	24666	27075	2.23	2.44
60	36814	39701	11635	11635	25179	28066	2.16	2.41
Biofertilizers								
Control	29384	31970	10685	10685	18699	21285	1.73	1.97
Pseudomonas striata	34895	36010	10715	10715	24180	25295	2.24	2.35
Glomus fasciculatum	33648	35540	10885	10885	22763	24661	2.09	2.26
Aspergillus awamori	31296	34203	10715	10715	20581	23488	1.90	2.16

Among the biofertilizer treatments, inoculation with *Pseudomonas striata* recorded highest gross returns, net returns per hectare and net returns per rupee invested, closely followed by *Glomus fasciculatum*. The total profit obtained due to inoculation with *Pseudomonas striata* was Rs 24,180.00 and Rs 25, 295.00 ha^{-1} whereas with *Glomus fasciculatum* the profit was Rs 22,763.00 and Rs 24,661.00 in the year 1999-2000 and 2000-2001, respectively. The net returns per rupee invested for inoculation with *Pseudomonas striata* and *Glomus fasciculatum* treatments were 2.24 and 2.09 in the year 1999-2000 whereas the values for the second year were 2.35 and 2.26, respectively.

Chapter 5

DISCUSSION

In this chapter an attempt has been made to establish the cause and effect relationship among various observations as described in the previous chapter. Since, grain yield is the most important agronomic criteria to evaluate treatment effect, hence discussion have been centered around observations on grain yield. Grain yield is determined by yield attributes which are affected by growth parameters. Therefore, a sincere effort has been made to establish logical and scientific relationship among yield, yield attributes and growth parameters. Studies on phosphorus uptake and efficiency have been discussed in relation with grain and straw yield. Economics of various treatments have also been discussed.

5.1 Effect of weather on crop growth

Growth and yield of crop plants are greatly influenced by prevailing weather conditions. The weekly meteorological data for both the crop seasons have been tabulated in Appendices I and II and depicted in Fig. 1.

Weather condition in terms of mean temperature, relative humidity, rainfall and sunshine hours was optimum for the growth of wheat during both the crop seasons. Mean atmospheric temperatures at the time of sowing were around 17.5 and 20°C in the year 1999-2000 and 2000-2001 respectively. At tillering stage the mean atmospheric temperature in both the years was around 16°C, whereas at grain filling stage it ranges between 20-25°C. This temperature condition was optimum for growth of wheat.

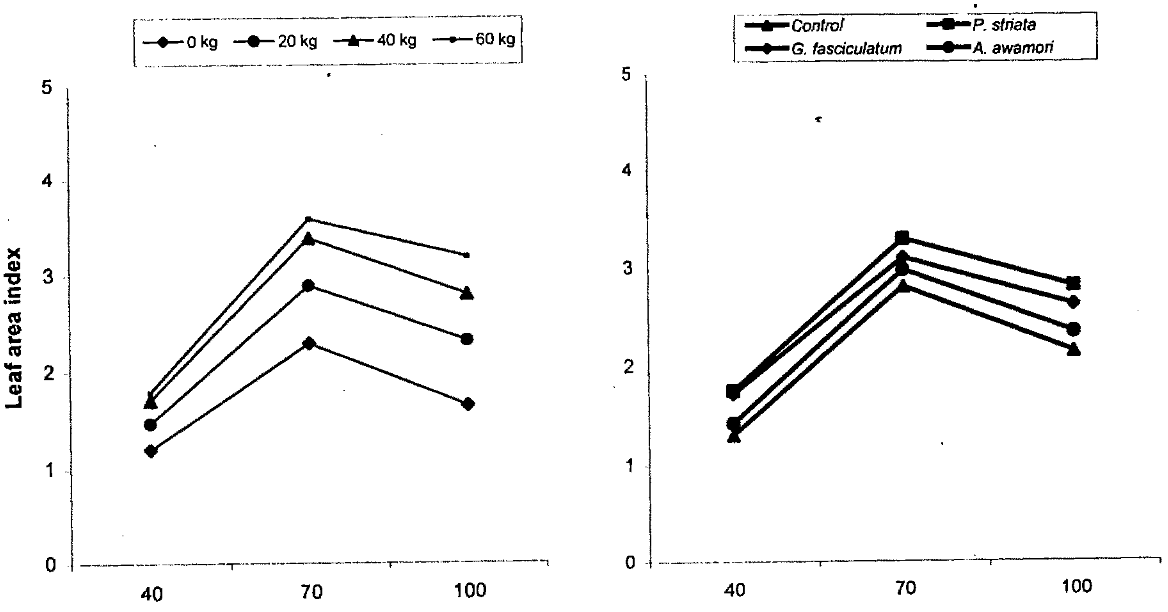
The overall crop yield was less in 1999-2000 compared to 2000-2001. This was mainly because of lodging of the crop during first year due to higher wind velocity during grain filling stage as evident from the meteorological data. Besides, a close analysis of weather data shows that though relative humidity was similar in both the years, the crop received comparatively less amount of rainfall and sunshine hours during first year. Higher rainfall in second year might have benefitted the crop whereas less sunshine hours and foggy weather conditions during first year might have limited the photosynthetic active radiation and adversely affected the crop growth resulting in lower grain yield in first year.

5.2 Effect of phosphorus fertilization

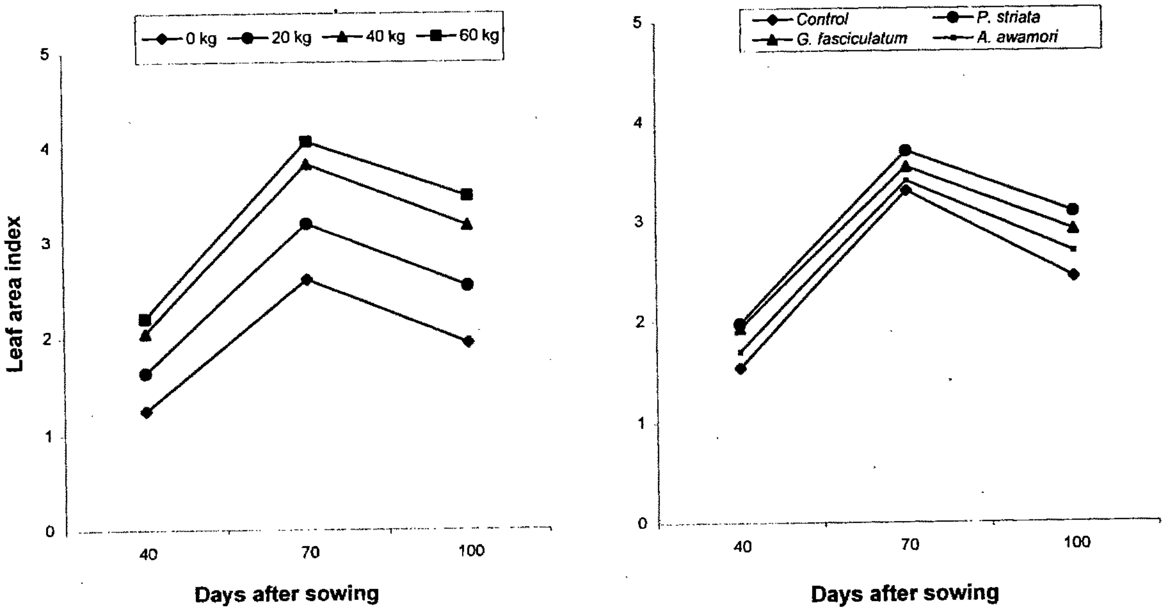
The yields of grain and straw was recorded higher with the application of 60 kg P_2O_5 ha^{-1} over 40 kg P_2O_5 ha^{-1} but the magnitude of response was failed to prove the superiority of higher dose over the lower one i.e. 60 and 40 kg P_2O_5 ha^{-1}, respectively.

The possible reason may be evidenced from the Table 12 and Fig. 9 which explains that the application of 40 kg P_2O_5 ha^{-1} enhanced the concentration and uptake of P by the wheat crop. This might have increased the immediate availability of P at initial stage of crop growth and enhanced the root:shoot ratio. This in turn provided wider absorbing surface on later growth stages and the resulting harvest index was unaffected due to higher level of P application which indicates that the proportional increase in grain yield is equal to proportional increase in total biomass yield.

1999-2000



2000-2001



Phosphorus **Biofertilizer**

Fig. 3 Leaf area index of wheat as influenced by phosperous levels and biofertilizers

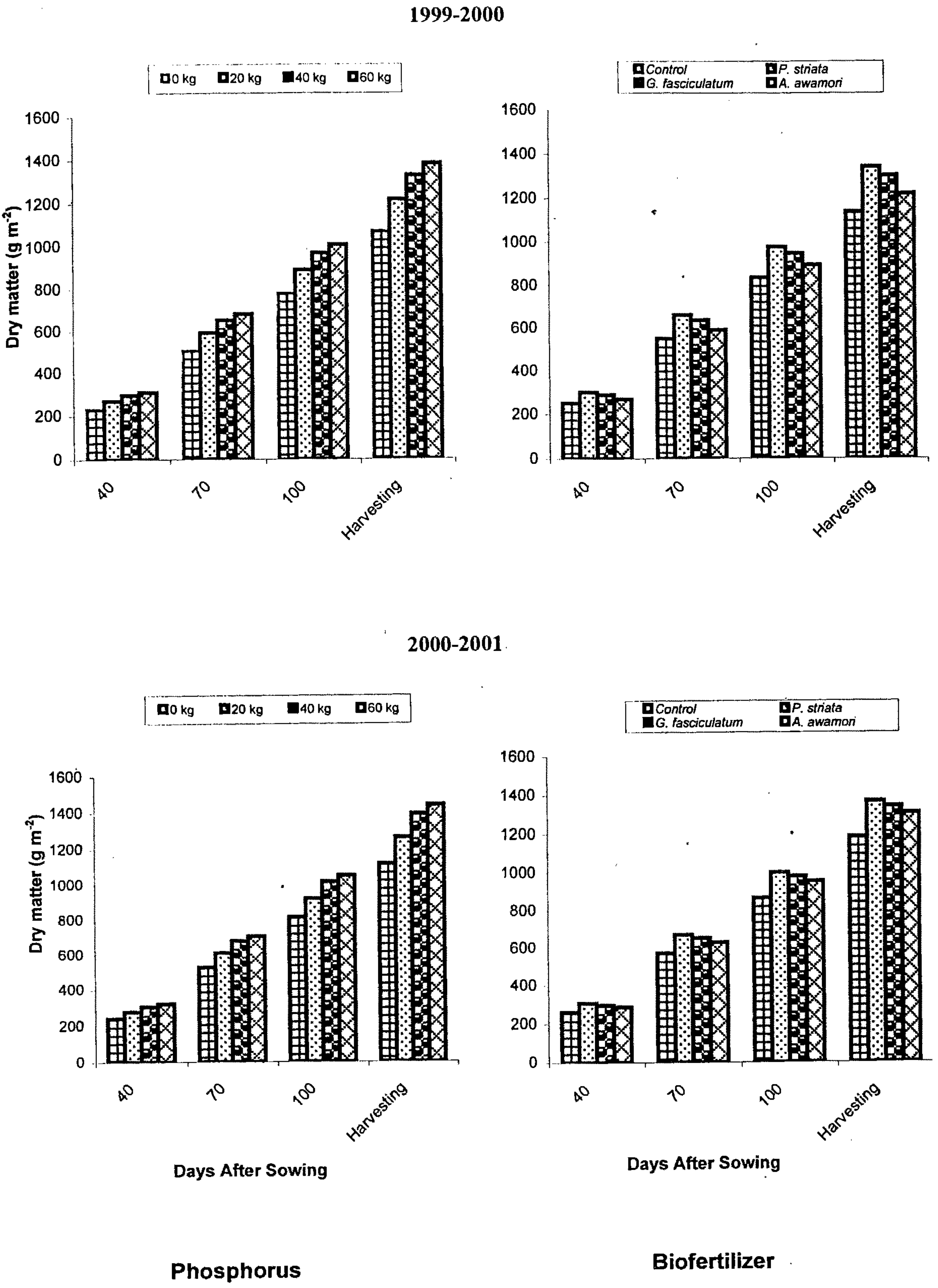


Fig. 4 Dry matter production of wheat as influenced by phosphorus levels and biofertilizers

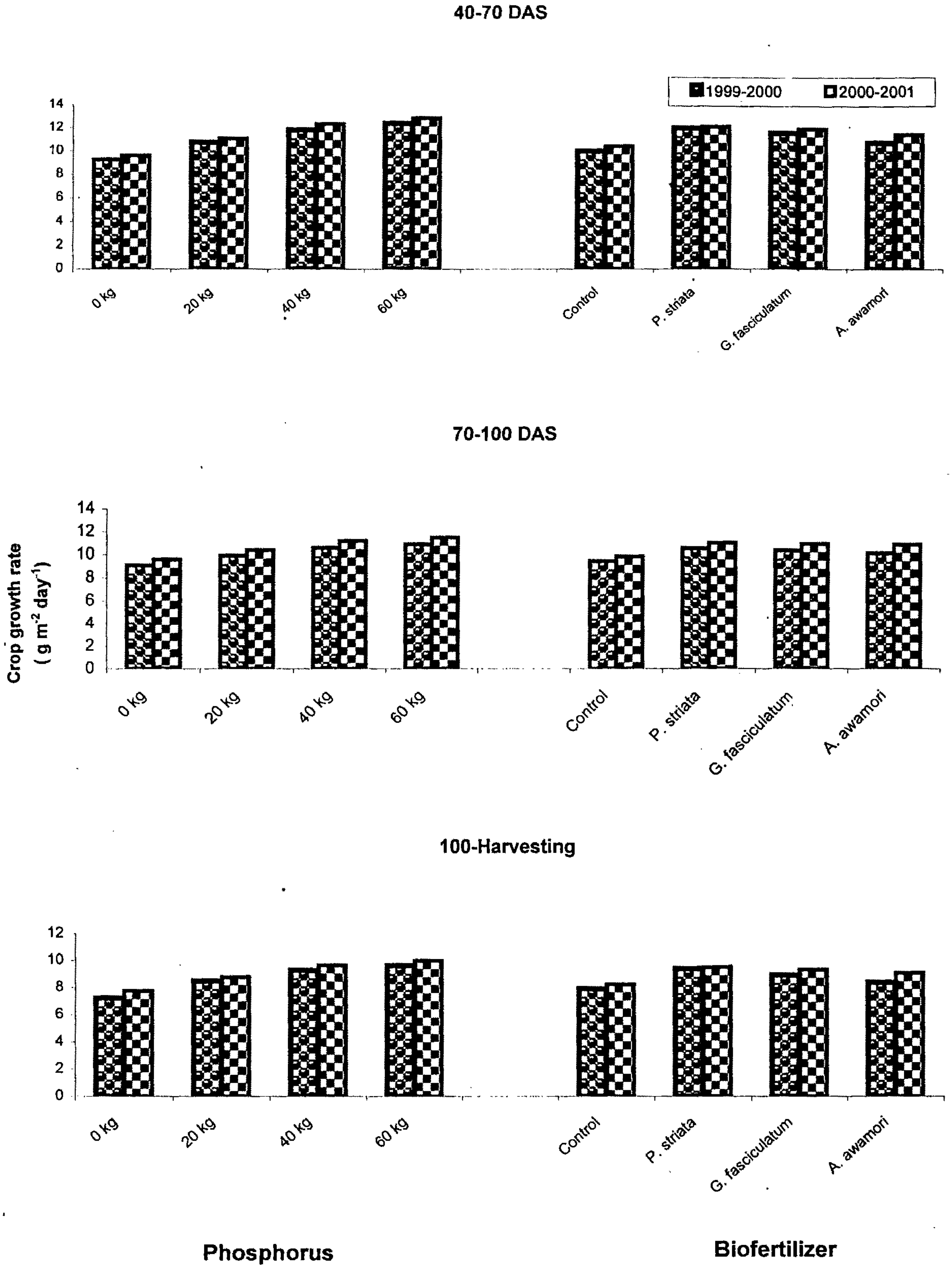


Fig. 5 Crop growth rate of wheat as influenced by phosporus levels and biofertilizers

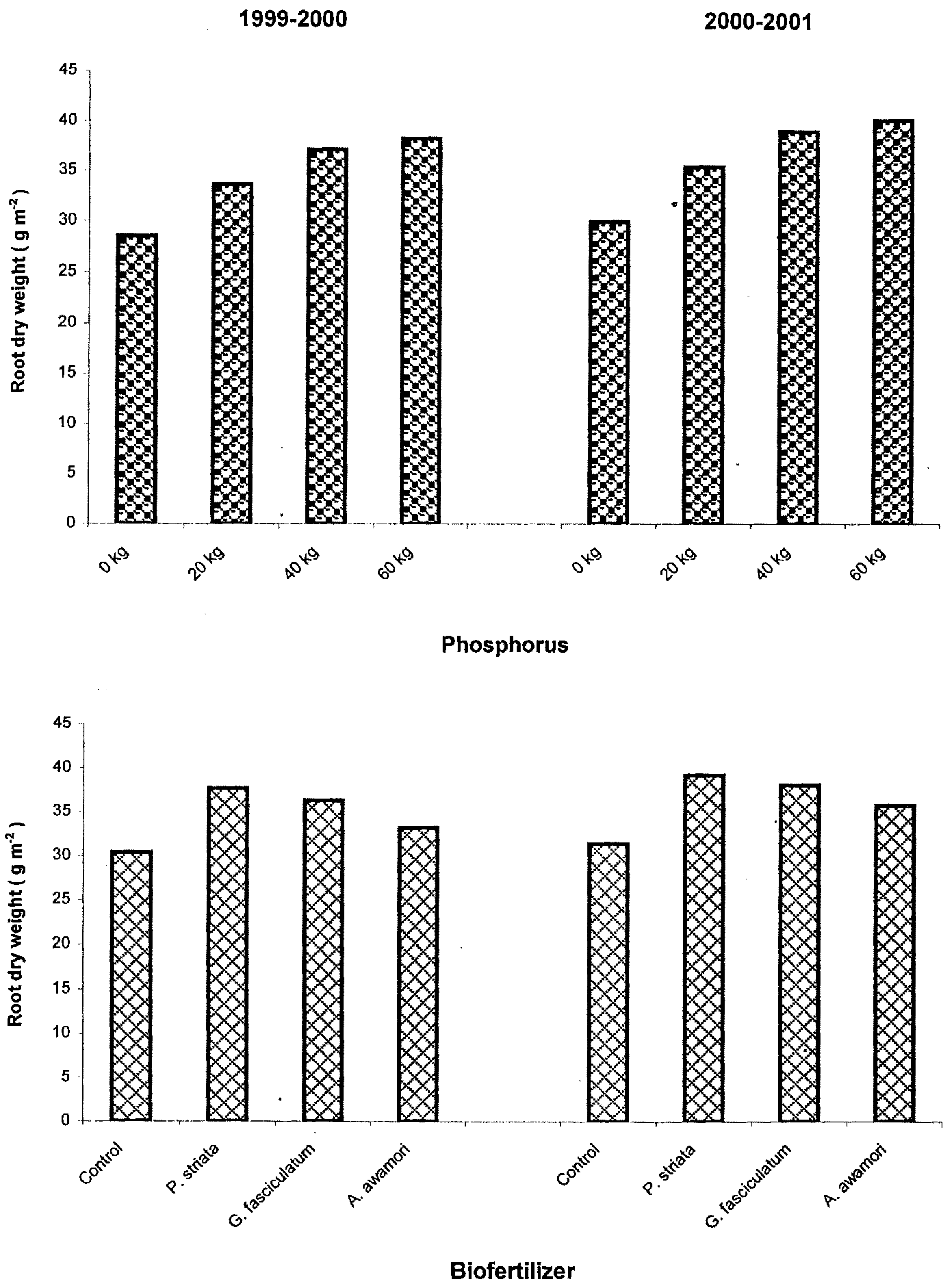


Fig. 6 Root dry weight of wheat as influenced by phosphorus levels and biofertilizers

The above finding can be further discussed in terms of yield attributes. All the yield attributing parameters such as effective tillers m^{-2}, grain number ear^{-1} and thousand grain weight were significantly increased up to 40 kg P_2O_5 ha^{-1}. Average increase in number of effective tillers m^{-2} at 40 kg P_2O_5 ha^{-1} was 27.5% over control. Low yield of wheat with no phosphorus could be attributed to lower number of effective tillers m^{-2}, grain number ear^{-1} and 1000-grain weight. It was also observed that P deficiency reduced the number of fertile tillers and sink size.

It was further illustrated in Table 5 and Fig. 4 that the dry matter of wheat (g m^{-2}) was increased significantly with application of 40 kg P_2O_5 ha^{-1} during all the growth stages. This observation could be supported by improved root growth and higher root dry weight due to 40 kg P_2O_5 ha^{-1} (Table 8 and Fig. 6).

Improved growth of wheat in terms of dry matter production can also be supported by increase in leaf area index due to P fertilization. The leaf area index increased significantly with the application of 40 kg P_2O_5 ha^{-1} (Table 4 and Fig. 3). With the successive levels of phosphorus application crop growth rate increased significantly up to 40 kg P_2O_5 ha^{-1} (Table 6 and Fig. 5). Hence it can be concluded that not only dry matter production per unit area increased but the rate of dry matter production per unit area also increased. Increased leaf area due to P application might have led to increased photosynthesis and translocation of photosynthates. In contrast to crop growth rate, net assimilation rate decreased with the application of phosphorus. Increase in accumulation of dry matter with the increase in P levels might have lagged behind the increase in leaf area expansion. Thereby decreasing the dry matter accumulation per unit leaf area.

To harvest higher economic yield a good vegetative growth is the prerequisite. Application of P led to better root growth and higher leaf area. Improved root growth might have helped in better uptake of water and nutrients. Further, higher leaf area might be responsible for interception of more solar radiation followed by higher production of photosynthates. These two factors might be synergistic for the dry matter production per unit area as indicated in Fig. 4. Since P plays an important role in translocation of photosynthates, better vegetative growth led to higher partitioning of dry matter into reproductive parts. Thereby, application of phosphorus improved number of effective tillers m^{-2}, number of grains ear^{-1} and thousand grain weight which in turn was reflected in harvesting higher grain yield.

5.3 Effect of Biofertilizers

All the three biofertilizers positively influenced the grain and straw yield compared to control, whereas the harvest index remained unaffected due to inoculation of micro-organisms. Inoculation with *Pseudomonas striata* (PSB) recorded the highest yield but it was on par with that of *Glomus fasciculatum* (VAM) and both produced significantly higher yield compared to control. The results were consistent in both the years. Unlike, *Pseudomonas striata* and *Glomus fasciculatum*, the effect of *Aspergillus awamori* (PSF) was not consistent. Although it produced higher grain and straw yield, statistically significant increase in grain yield was obtained only during second year (Table 10 and Fig. 8). This might be due to the variability in response of wheat to *Aspergillus awamori*. This could be explained on the basis of effect of weather parameters which were more

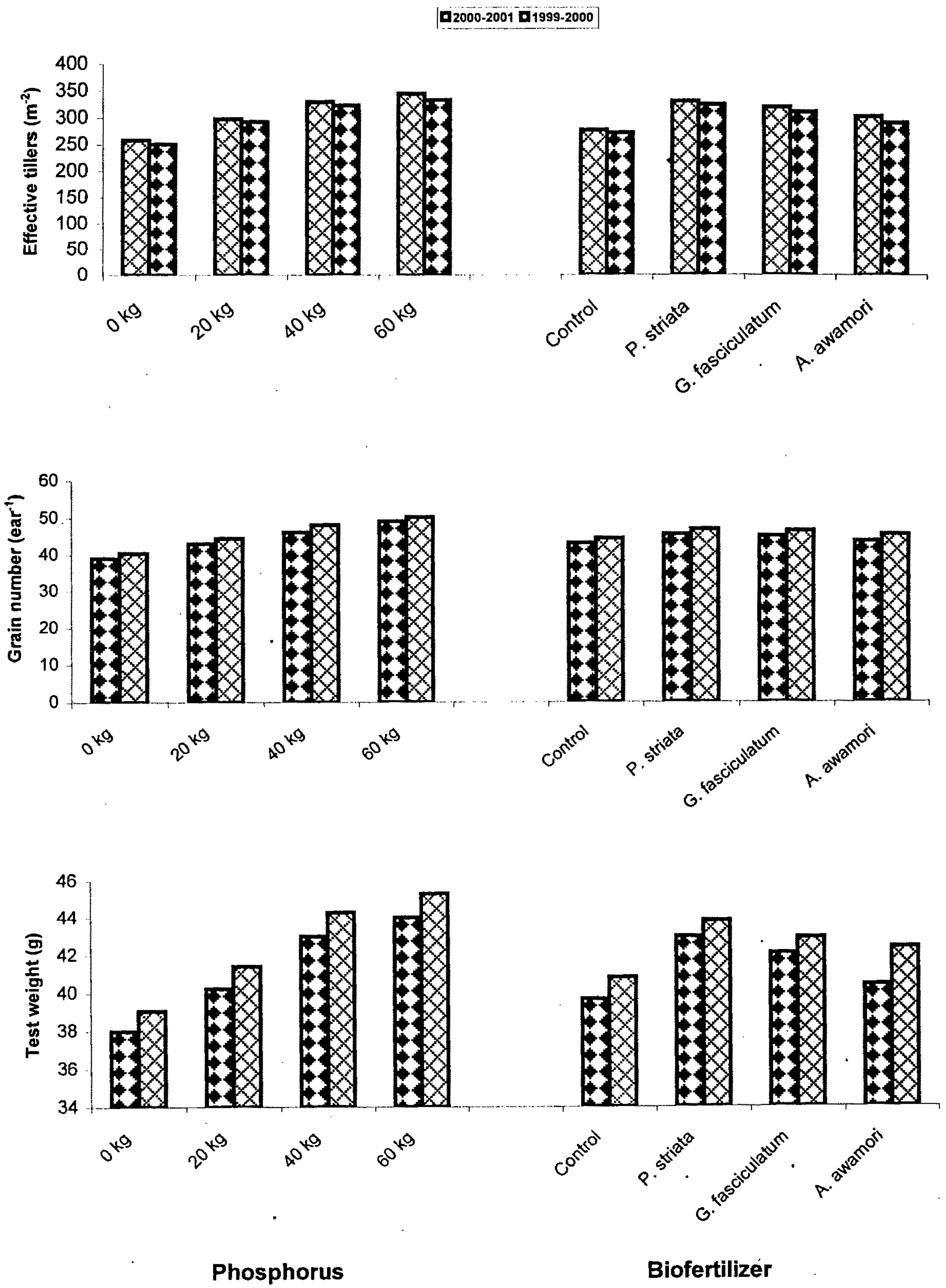


Fig.7 Yield attributes of wheat as influenced by phosphorus levels and biofertilizers

favourable in second year compared to first year and sensitiveness of *Aspergillus awamori* to adverse conditions. The lower minimum temperature at the time of sowing might have restricted the multiplication of micro-organisms in soil in second year as evident from the Table 11.

The increase in yield mainly attributed to the increase in yield parameters like number of effective tillers m^{-2}, number of grains ear^{-1} and thousand grain weight for treatments inoculated with *Pseudomonas striata* and *Glomus fasciculatum* and only number of effective tillers m^{-2} with *Aspergillus awamori* inoculated treatments (Table 9 and Fig. 7). The basic reason for increase in effective tillers m^{-2} is the enhanced root growth during crop growth which in turn enhanced the availability of phosphorus. Larger number of effective tillers provided for larger sink and increased availability of phosphorus led to increased uptake and translocation of phosphorus to reproductive part (Table 9) resulting in retention of more number of flowers and proper development of seed. This led to increase in number of grains ear^{-1} and thousand grain weight.

The difference in yield components due to inoculation with phosphate solubilizing and mobilizing micro-organisms was mainly because of difference in growth attributes viz., leaf area index, dry matter accumulation and differential rooting. These growth attributes increased progressively up to the harvesting. The highest values were observed due to inoculation with *Pseudomonas striata* which were on par with *Glomus fasciculatum* in both the years. Although inoculation with *Pseudomonas striata* and *Glomus fasciculatum* always recorded higher

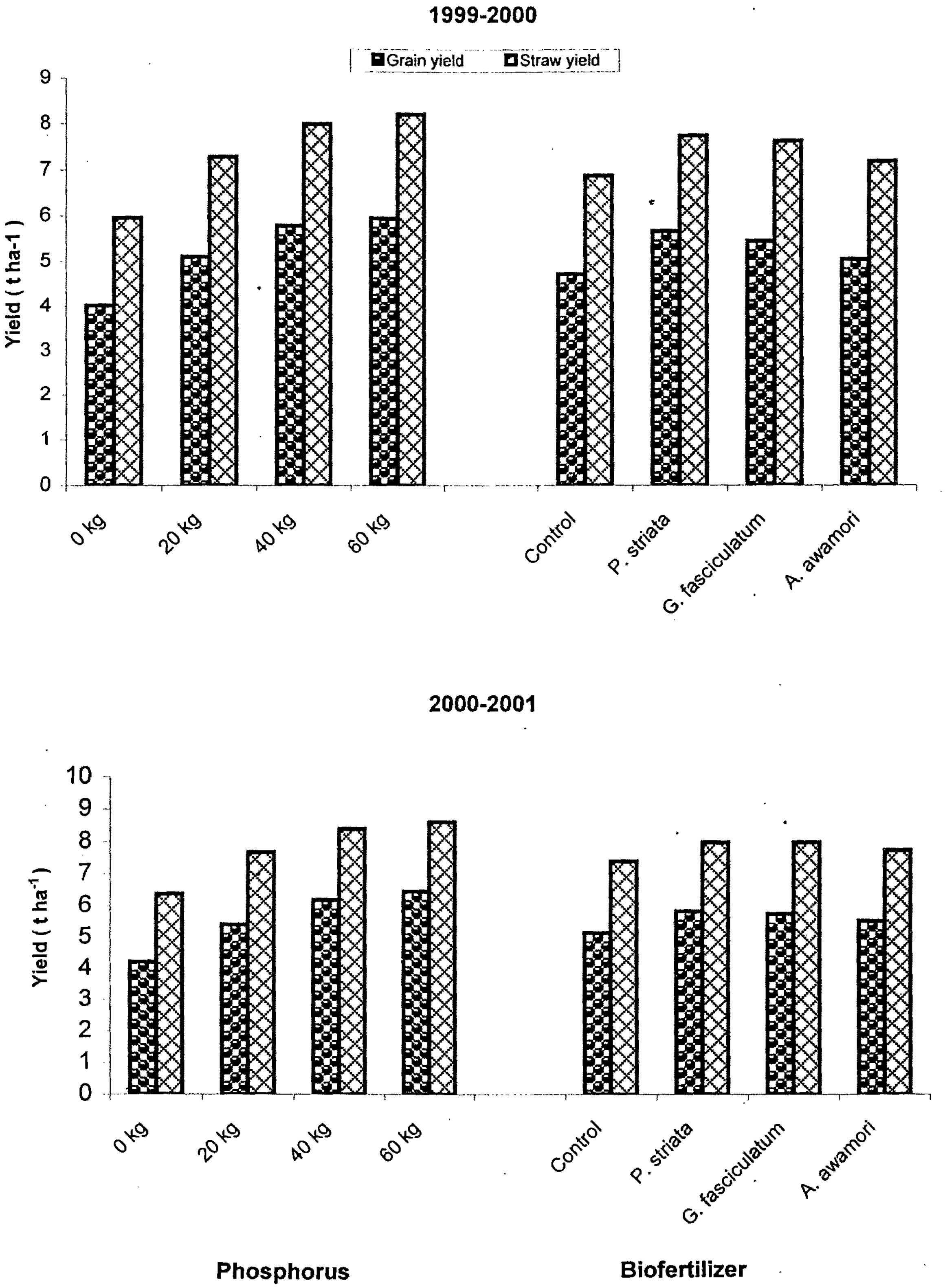


Fig.8 Grain yield and straw yield of wheat as influenced by phosperous levels and biofertilizers

values over control, their superiority over *Aspergillus awamori* was variable, whose effect was ultimately reflected on grain yield.

The positive influence on growth attributes due to inoculation with phosphate solubilizing micro-organisms viz., *Pseudomonas striata* and *Aspergillus awamori* mainly because of higher availability and uptake of phosphorus which attributed to greater mineralization of organic phosphorus. The phosphate solubilizing micro-organisms solubilize the soil phosphorus through production of organic acids, phosphatase enzyme and chelating substances. These organic acids convert the tricalcium and dicalcium phosphate to monobasic phosphate which increases the availability of phosphorus. But *Glomus fasciculatum* is a mycorrhizal fungi which might have increased the uptake of phosphorus through mobilization of phosphorus. The fungal hyphae might have helped in exploration of greater volume of soil, due to better absorbing network and higher surface area, faster movement of phosphorus through hyphae and favourably modifying the root environment (Bolan, 1991). The difference in response of wheat to *Pseudomonas striata* and *Aspergillus awamori* might be due to differential growth requirements of the microorganisms like nutrition, temperature and pH. For optimum growth, *Pseudomonas striata* requires 20-30°C temperature and 7.8 soil pH while for *Aspergillus awamori* these values are 30°C and 6.0 respectively (Wani *et al.*, 1979). During crop season the conditions like temperature and soil pH were more suitable for the growth of *Pseudomonas striata*. This can be supported from the observation on population of micro-organisms (Table 11). Besides, these micro-organisms produce plant growth promoting substances which might have helped in increasing growth and yield of wheat.

5.4 Phosphorus content and uptake

Application of phosphatic fertilizer significantly enhanced the concentration and uptake of phosphorus by wheat up to 40 kg P_2O_5 ha^{-1} (Fig. 9). The phosphorus fertilization might have increased the immediate availability at initial stage of crop growth which enhanced the root:shoot ratio and in turn provided for greater absorbing surface on later growth stages resulting in higher concentration and uptake of P.

Inoculation of wheat with PSB and VAM also significantly increased the concentration and uptake of phosphorus over no inoculation which could be possible due to higher biomass production and enhanced availability of phosphorus through solubilization effect of PSB and greater mobilization of native and applied phosphorus by VAM.

5.5 Per cent Pdff and 'A' value

The phosphorus fertilization had a positive influence on per cent phosphorus derived from fertilizer. Application of phosphatic fertilizer might have increased the amount of phosphorus in the soil solution and in immediate vicinity of root hairs which in turn resulted in higher uptake of phosphorus from applied fertilizer. The increase in per cent Pdff was not in tune with successive levels of phosphorus. This might have resulted in soil phosphorus build up. This can be supported with the observation on 'A' value which increased significantly with successive levels of phosphorus application (Table 13 and Fig. 9).

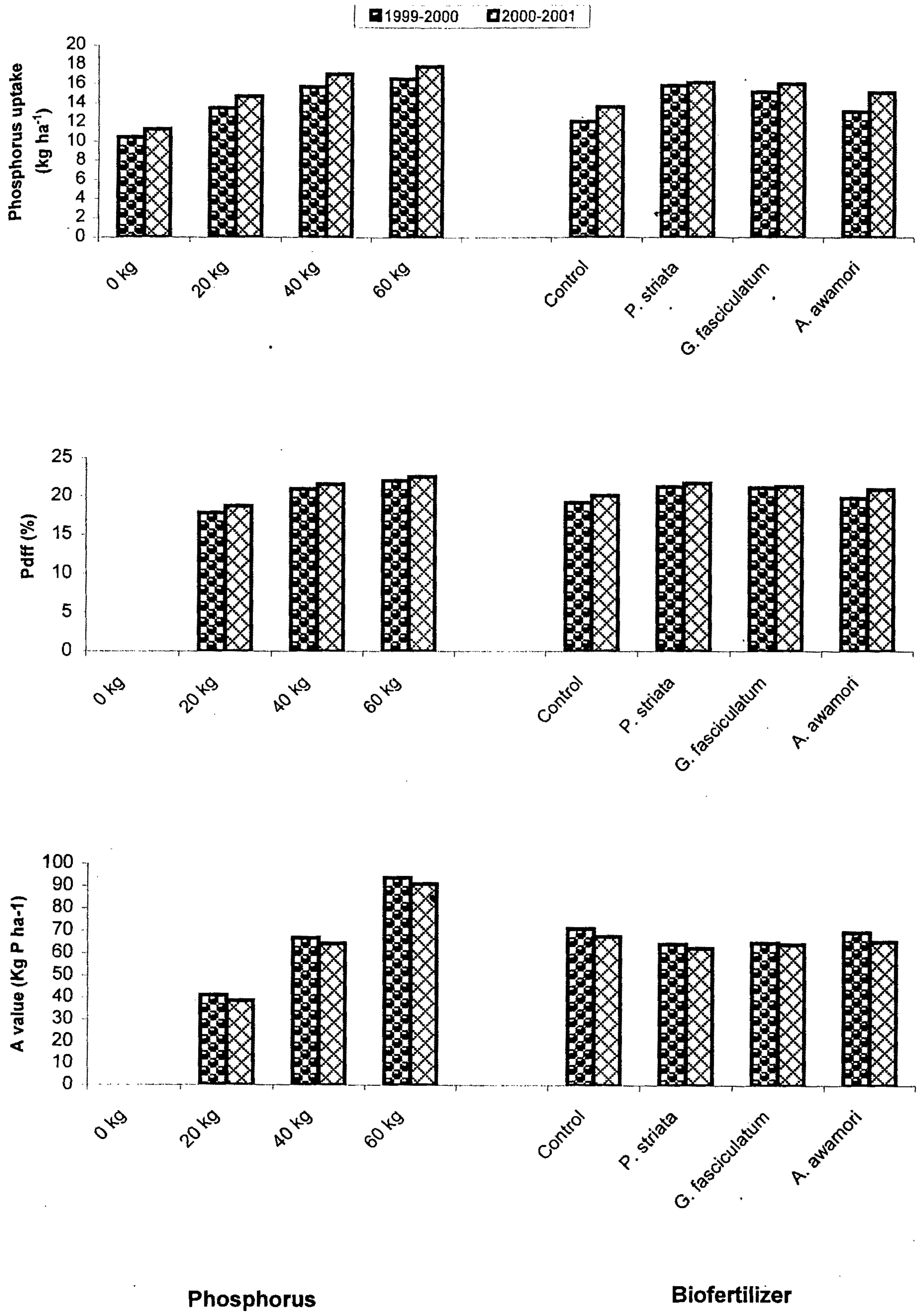


Fig. 9 P uptake, per cent Pdff and 'A' value as influenced by phosphorus level and biofertilizers

Inoculation with phosphate solubilizing and mobilizing micro-organisms had also a positive effect on per cent Pdff. Biofertilization with *Pseudomonas striata* recorded highest value of per cent Pdff which was at par with *Glomus fasciculatum*. This can be explained in the light of higher production of organic acids and chelating substances by bacteria which might have helped in maintaining the applied phosphorus in available form for a longer period of time. But in case of *Glomus fasciculatum* the hyphae might have come in direct contact with applied phosphorus and effective translocation which in turn increased the per cent Pdff. This was supported by the 'A' value (Fig. 9). The highest 'A' value was observed without any inoculation which might be due to less solubilization and mobilization of added phosphorus and consequent build up of soil phosphorus. The 'A' value was significantly lowered due to inoculation with PSB and VAM but not with *Aspergillus awamori*. But the higher uptake and per cent Pdff with PSB and VAM indicate their ability to solubilize and mobilize more native and added phosphorus.

5.6 Phosphorus utilization and Agronomic efficiency

The phosphorus utilization declined steadily and significantly with successive levels of phosphorus. The possible reason for the decline may be the lack of luxury consumption of phosphorus on one hand and greater conversion of added phosphorus into non available form and higher retention of phosphorus in soil in another.

In contrary to phosphorus levels, biofertilizer treatments had a positive effect on utilization of phosphorus. Inoculation with *Pseudomonas striata* and

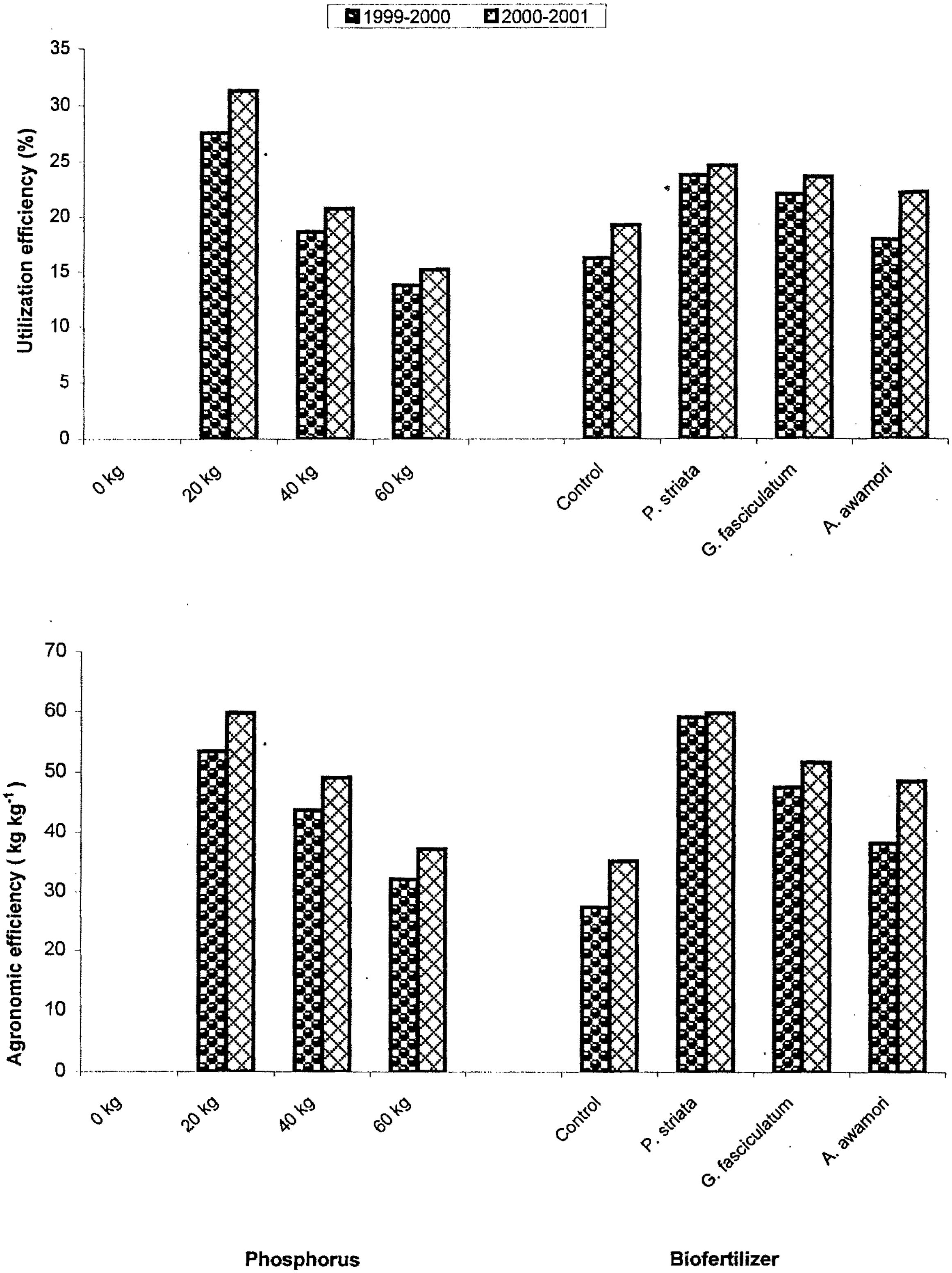


Fig. 10 Per cent utilization and agronomic efficiency of phosphrous as influenced by phosphorus level and biofertilizer

Glomus fasciculatum significantly increased the phosphorus utilization efficiency. Inoculation with PSB might have reconverted the unavailable fertilizer P to available form in the field through production of organic acids which enhanced the availability and resulted in higher uptake of added P. Whereas VAM inoculation might have helped in exploration of greater volume of soil and in turn more fertilizer P and increased the uptake of fertilizer P (Table 14 and Fig. 10).

Like, phosphorus utilization efficiency, agronomic efficiency declined steadily and significantly with successive levels of phosphorus. This was obvious as the grain yield could not increase in tune with the rate of fertilizer P application following the law of diminishing return and low P utilization efficiency.

All the three micro-organisms significantly increased the agronomic efficiency of applied phosphorus. Highest values were obtained with *Pseudomonas striata* in both the eyars. This could be explained in the light of higher per cent Pdff and utilization efficiency of phosphorus due to *Pseudomonas striata* inoculation (Table 13 and 14).

5.7 Response studies and Economics

The response of wheat to phosphorus was well fitted into quadratic equations (Table 15 and Fig. 11). On an average, the economic optimum dose of phosphorus for wheat without biofertilizer was 68.7 kg P_2O_5 ha^{-1} whereas the values with application of biofertilizer were 49.0, 51.2 and 57.6 kg P_2O_5 ha^{-1} for *Pseudomonas striata*, *Glomus fasciculatum* and *Aspergillus awamori* treatments, respectively. Hence, there can be a saving of 19.7, 17.5 and 11.1 kg P_2O_5 ha^{-1} to

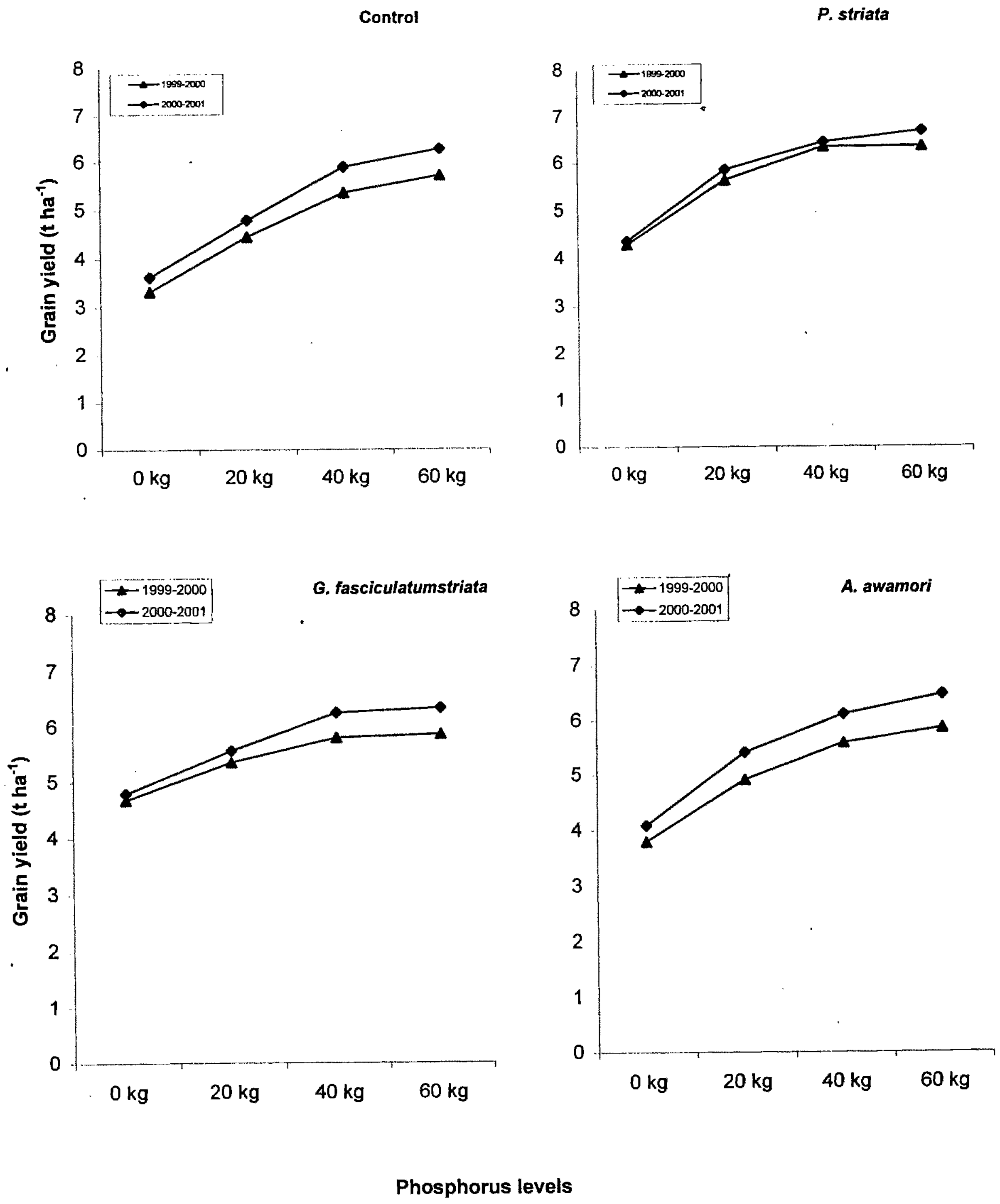


Fig. 11 Response curves showing estimated grain yield at various phosphorus levels

get the optimum economic yield due to inoculation with *Pseudomonas striata, Glomus fasciculatum* and *Aspergillus awamori*, respectively.

Application of phosphorus increased the net returns and net returns per rupee invested. Biofertilization with *Pseudomonas striata* gave highest average net returns of Rs 24,737.00 followed by *Glomus fasciculatum* (Table 17). The economic gains accrued in first year was lower compared to second year. This might be due to lower grain yield because of lodging during later stage of crop growth in the first year. But the benefits obtained from inoculation with *Pseudomonas striata* and *Glomus fasciculatum* in the form of saving of phosphorus and increase in net returns were substantial.

Chapter 6

SUMMARY AND CONCLUSION

6.1 Summary

A field experiment was conducted during *rabi* season of 1999-2000 and 2000-2001 at the farm of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi to study the "**Influence of phosphate solubilizing and mobilizing micro-organisms on phosphorus use efficiency in wheat using ^{32}P as a tracer**".

The experiment was laid out in a split plot design with four levels of phosphorus (0, 20, 40 and 60 kg P_2O_5 ha^{-1}) in main plots and four biofertilizers (control, *Pseudomonas striata*, *Glomus fasciculatum* and *Aspergillus awamori*) in sub plots replicated thrice. The soil of the experimental site was sandy loam in texture, low in organic carbon and available nitrogen, medium in available phosphorus and neutral in reaction.

A uniform dose of 120 kg N ha^{-1} and 40 kg K_2O ha^{-1} was applied in the form of urea and muriate of potash, respectively. Phosphorus was applied to different plots as per the treatments through single superphosphate. Band placement of ^{32}P labelled single superphosphate was done at 3-5 cm below the seed in one meter row length of the crop after emergence. Biofertilizer treatment was given at the time of sowing.

The experimental results of these studies presented in the previous chapter are summarized in this chapter as follows below:

1. The average wheat yield recorded in first year was comparatively lower than that of second year which was mainly due to adverse weather condition, particularly high wind velocity resulting in lodging of the crop during later stage of crop growth.
2. The leaf area index of wheat increased up to 70 days after sowing and thereafter reduced at 100 days after sowing. Phosphorus and biofertilizers positively influenced the leaf area index. Statistically highest leaf area index was recorded at 40 kg P_2O_5 ha^{-1} and with inoculation of *Pseudomonas striata*.
3. Fertilization with phosphorus and biofertilizers resulted an increasing trend of total dry matter production up to harvest stage. Application of phosphorus increased the dry matter accumulation up to 40 kg P_2O_5 ha^{-1}. Inoculation with biofertilizers brought about significant increase in dry matter production in both the years except *Aspergillus awamori* in first year. Significantly highest dry matter production was observed due to inoculation with *Pseudomonas striata* followed by *Glomus fasciculatum*.
4. The crop growth rate of wheat showed an increasing but net assimilation rate showed a decreasing trend with successive levels of phosphorus application. Among the biofertilizers highest crop growth rate was observed due to inoculation with *Pseudomonas striata* but it was statistically on par

with *Glomus fasciculatum*. In contrast, highest net assimilation rate was observed in control plots.

5. The root dry weight of wheat increased significantly with application of phosphorus and biofertilizers. A dose of 40 kg P_2O_5 ha^{-1} produced on an average 30% higher root dry weight over control. Highest root dry weight was produced due to inoculation with *Pseudomonas striata* followed by *Glomus fasciculatum.*
6. All the yield attributes i.e. effective tillers, number of grains per ear and thousand grain weight were significantly increased due to application of 40 kg P_2O_5 ha^{-1}. Application of 60 kg P_2O_5 ha^{-1} though improved the yield attributes but failed to reach the level of significance. Inoculation with *Pseudomonas striata* and *Glomus fasciculatum* significantly increased all the yield attributes over control but *Aspergillus awamori* did not increase the yield attributes except effective tillers in second year.
7. Grain yield and straw yield of wheat increased significantly up to 40 kg P_2O_5 ha^{-1} but harvest index remained unaffected by phosphorus fertilization. Though highest yields were observed at 60 kg P_2O_5 ha^{-1}, they were on par with that at 40 kg P_2O_5 ha^{-1}. Inoculation with *Pseudomonas striata* and *Glomus fasciculatum* significantly increased the grain yield and straw yield of wheat whereas effect on harvest index was not significant. *Aspergillus awamori* inoculation significantly increased the grain yield during second year only. Highest yields i.e. 5.65 and 5.82 t ha^{-1} were recorded due to inoculation with *Pseudomonas striata.*

8. Application of phosphorus and biofertilizer positively influenced the phosphorus content and uptake by wheat.
9. External supply of micro-organisms significantly enhanced the microbial population in the root zone of wheat. The population of *Pseudomonas striata* and *Aspergillus awamori* significantly increased but population of *Glomus fasciculatum* showed a negative trend with increase in phosphorus levels.
10. The per cent Pdff and 'A' value were markedly increased with application of phosphorus. Though, 'A' value was increased progressively and significantly with application of phosphorus up to highest level resulting in build up of soil phosphorus, the significant increase in per cent Pdff was observed only at 40 kg over 20 kg P_2O_5 ha^{-1}. The average increase was in order of 16.3%. Among biofertilizer treatments, inoculation with *Pseudomonas striata* and *Glomus fasciculatum* significantly increased the per cent Pdff but significantly declined the 'A' value. The highest per cent Pdff and lowest 'A' value were registered due to inoculation with *Pseudomonas striata.*
11. The phosphorus utilization efficiency and agronomic efficiency were declined significantly with application of successive levels of phosphorus, whereas inoculation with phosphate solubilizing and mobilizing micro-organisms significantly increased the same. The added phosphorus was most efficiently used by wheat due to inoculation with *Pseudomonas striata* followed by *Glomus fasciculatum.*

12. The optimum economic dose of P for wheat was found to be 68.7, 49.0, 51.2 and 57.6 kg P_2O_5 ha^{-1} for no inoculation, inoculation with *Pseudomonas striata*, *Glomus fasciculatum* and *Aspergillus awamori*, respectively. Application of phosphorus increased the net return up to 60 kg P_2O_5 ha^{-1} but highest net returns per rupee invested was observed at 40 kg P_2O_5 ha^{-1}. Among the biofertilizer treatments inoculation with *Pseudomonas striata* gave highest net returns as well as net returns per rupee invested followed by *Glomus fasciculatum*.

6.2 Conclusion

On critical appraisal of the significant research findings emerged out from the present investigation, the major conclusion can be envisaged that the application of 40 kg P_2O_5 ha^{-1} is sufficient to produce a desired yield level of wheat in association with biofertilizers. It is very clear from the optimum economic dose of phosphorus to wheat that without inoculation the optimum dose was 68.7 kg P_2O_5 ha^{-1} while in combination with *Pseudomonas striata*, *Glomus fasciculatum* and *Aspergillus awamori* the optimum economic doses were to the tune of 49.0, 51.2 and 57.6 kg P_2O_5 ha^{-1}, respectively, to fetch the same yield level which has clearly stated the beneficial effect of biofertilizers in terms of phosphorus use efficiency, production and productivity of wheat. This finding has been further confirmed from the 'A' value that the biofertilizer has played a significant role in augmenting and sustaining the soil health.

Based on the study, application of 49 kg P_2O_5 ha^{-1} and inoculation with *Pseudomonas striata* are recommended for improving phosphorus use efficiency and productivity of wheat.

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*** Original not seen**

Appendix I. Weekly meteorological data during crop season 1999-2000

Month	Standard weeks	Mean temperature (°C)		Mean relative humidity (%)	Wind velocity (km hr^{-1})	Rainfall (mm)	Sunshine (hrs day^{-1})
		Max.	Min.				
Nov.	46	29.27	10.94	52.86	2.9	0.00	4.57
	47	28.04	8.97	54.29	1.3	0.00	3.57
	48	26.53	8.69	54.71	1.5	0.00	3.74
	49	25.70	8.69	64.29	0.6	0.00	2.66
Dec.	50	22.74	7.76	64.29	1.5	0.00	2.16
	51	24.43	5.49	62.14	1.2	0.00	2.03
	52	21.53	5.57	75.14	0.7	0.00	1.51
	1	18.40	4.54	78.14	0.9	0.00	1.33
Jan.	2	16.26	6.50	79.57	2.2	0.00	1.56
	3	19.21	8.29	70.29	7.2	1.24	2.33
	4	22.26	8.74	59.86	4.3	0.00	3.20
	5	20.70	10.36	71.29	4.8	2.36	2.47
Feb.	6	19.99	9.61	79.71	5.2	2.21	2.36
	7	18.10	7.60	76.29	1.6	5.43	2.06
	8	22.57	7.36	65.29	2.3	0.00	3.31
	9	23.31	7.56	56.57	1.6	0.00	4.04
March	10	27.30	11.87	58.29	3.6	0.00	3.89
	11	28.26	13.34	54.29	3.6	0.00	5.40
	12	28.91	11.90	54.86	3.4	0.00	4.43
	13	28.71	13.60	58.43	1.8	0.96	4.16
April	14	35.93	14.80	69.00	5.4	0.00	6.33
	15	38.50	18.30	65.00	3.0	1.50	8.40
	16	40.10	22.30	61.00	1.9	0.00	7.60
	17	38.40	23.90	68.00	3.3	0.00	4.00

Appendix II. Weekly meteorological data during crop season 2000-2001

Month	Standard weeks	Mean temperature (°C)		Mean relative humidity (%)	Wind velocity (km hr^{-1})	Rainfall (mm)	Sunshine (hrs day^{-1})
		Max.	Min.				
Nov.	45	32.1	14.2	59.5	1.0	0.00	7.1
	46	29.8	11.8	55.5	0.8	0.00	6.3
	47	27.6	13.0	72.2	0.6	0.00	2.3
	48	24.1	7.0	62.8	1.9	0.80	4.2
Dec.	49	24.9	5.0	58.5	0.9	0.00	7.0
	50	23.7	5.8	60.5	1.1	0.00	5.7
	51	24.7	7.9	63.5	1.5	0.00	5.0
	52	22.3	5.4	65.5	0.2	4.00	4.1
Jan.	1	15.5	7.7	82.5	2.3	18.80	1.8
	2	16.1	3.2	74.5	0.9	0.00	3.7
	3	18.9	2.8	65.5	0.5	0.00	4.9
	4	21.0	7.2	59.5	3.4	0.00	5.4
	5	23.0	6.1	57.0	3.0	0.00	7.0
Feb.	6	23.4	7.6	54.5	3.4	0.00	4.9
	7	24.7	7.8	63.5	1.3	0.00	4.0
	8	26.9	12.1	67.0	2.6	12.0	5.1
	9	25.6	9.5	60.5	1.6	0.00	5.9
March	10	27.7	9.0	54.0	0.9	0.00	8.4
	11	21.5	14.2	49.5	2.2	0.00	7.5
	12	30.7	14.6	54.5	1.2	0.00	7.2
	13	31.4	15.6	60.0	1.8	3.00	7.2
April	14	33.0	15.6	56.5	1.4	0.00	9.1
	15	36.6	20.7	47.0	1.4	4.80	6.1
	16	31.6	18.3	56.0	3.8	9.20	8.0
	17	44.4	22.8	30.0	2.9	0.00	9.3

Appendix III. Estimation of average cost of wheat cultivation

S.No.	Particulars	Rate (Rs.)	Actual cost (Rs.)
1.	Land preparation		
	(a) Ploughing (2)	200 ha^{-1}	400
	(b) Double discing and planting (2)	200 ha^{-1}	400
2.	Pre-sowing irrigation	200 ha^{-1}	200
3.	Irrigation channel formation and layout	150 ha^{-1}	150
4.	Seed @ 100 kg ha^{-1}	13 kg^{-1}	1300
5.	Microbial culture		
	Pseudomonas striata (3 packets ha^{-1})	10 $packet^{-1}$	30*
	Glomus fasciculatum (10 packets ha^{-1})	20 $packet^{-1}$	200*
	Aspergillus awamori (3 packets ha^{-1})	10 $packet^{-1}$	30*
6.	Sowing and fertilizer application		
	(a) 8 man days	70 $man^{-1}day^{-1}$	560
	(b) 4 bullock pair	100 $unit^{-1}$	400
7.	Fertilizers		
	(a) N as urea @ 120 kg N ha^{-1}	10.50 kg^{-1} N	1260
	(b) K as MOP @ 40 kg P_2O_5 ha^{-1}	8.25 kg^{-1} K_2O	330
	(c) P as SSP @ 20 kg P_2O_5	29.50 kg^{-1} P_2O_5	590*
	@ 40 kg P_2O_5	29.50 kg^{-1} P_2O_5	1180*
	@ 60 kg P_2O_5	29.50 kg^{-1} P_2O_5	1770*
8.	Intercultural operations (2) (10 man days/operation)	70 $man^{-1}day^{-1}$	700
9.	Irrigation (4)	200 ha^{-1}	800
10.	Harvesting, threshing and winnowing (30 man days)	70 $man^{-1}day^{-1}$	2100
11.	Land rent (6 months)	200 $ha^{-1}month^{-1}$	1200

Appendix IV. Economics of different treatment combinations for the year 1999-2000

Treatments	Gross returns (Rs.ha^{-1})	Cost of cultivation (Rs.ha^{-1})	Net returns (Rs.ha^{-1})	Net returns per rupee
P_0M_c	20725	9800	10925	1.11
P_0M_{Ps}	26650	9830	16820	1.71
P_0M_{Gf}	29260	10000	19260	1.93
P_0M_{Aa}	23695	9830	13865	1.41
$P_{20}M_c$	27870	10390	17480	1.68
$P_{20}M_{Ps}$	34780	10420	24360	2.34
$P_{20}M_{Gf}$	33295	10590	22705	2.14
$P_{20}M_{Aa}$	30515	10420	20095	1.93
$P_{40}M_c$	33270	10980	22290	2.03
$P_{40}M_{Ps}$	39125	11010	28115	2.55
$P_{40}M_{Gf}$	35830	11180	24650	2.20
$P_{40}M_{Aa}$	34620	11010	23610	2.14
P_8-M_c	35670	11570	24100	2.08
$P_{80}M_{Ps}$	39025	11600	27425	2.36
$P_{80}M_{Gf}$	36205	11770	24435	2.08
$P_{80}M_{Aa}$	36355	11600	24755	2.13

Appendix V. Economics of different treatment combinations for the year 2000-2001

Treatments	Gross returns (Rs.ha^{-1})	Cost of cultivation (Rs.ha^{-1})	Net returns (Rs.ha^{-1})	Net returns per rupee
P_0M_c	22745	9800	12945	1.32
P_0M_{Ps}	27160	9830	17330	1.76
P_0M_{Gf}	29850	10000	19850	1.99
P_0M_{Aa}	25665	9830	15835	1.61
$P_{20}M_c$	29940	10390	19550	1.88
$P_{20}M_{Ps}$	36140	10420	25720	2.47
$P_{20}M_{Gf}$	34565	10590	23975	2.26
$P_{20}M_{Aa}$	33565	10420	23145	2.22
$P_{40}M_c$	36470	10980	25490	2.32
$P_{40}M_{Ps}$	39585	11010	28575	2.60
$P_{40}M_{Gf}$	38965	11180	27585	2.47
$P_{40}M_{Aa}$	37660	11010	26650	2.38
$P_{8-}M_c$	38725	11570	27155	2.35
$P_{80}M_{Ps}$	41155	11600	29555	2.55
$P_{80}M_{Gf}$	39005	11770	27235	2.31
$P_{80}M_{Aa}$	39920	11600	28320	2.44