2. REVIEW OF LITERATURE

2.1 THE RHIZOSPHERE AND RHIZOPLANE

The importance of the interrelationship between soil microbial communities and the root system of higher plants was emphasized by Hiltner in 1904 who coined the term 'rhizosphere', for the first time, to denote the soil region subjected to the influence of plant roots (cited by Rovira, 1965a). Since then, rhizosphere studies have been made by several authors who contributed significantly towards the understanding of this phenomenon. Among them, Starkey has been recognized as the pioneer who brought out the variation in rhizosphere effect due to difference in plant species, their age and soil factors (Starkey, 1929a, b, c, 1931, 1938). The early studies of Lockhead and associates (Lockhead, 1940; Lockhead *et al.*, 1940; Lockhead and Rouatt, 1955), Timonin (1940a, b, 1941), Katznelson (1946) and Clark (1949) and many others aroused the interest of several scientists which resulted in accumulation of tremendous amount of literature on rhizosphere phenomenon.

Rhizosphere represents a poorly defined zone of soil with a microbiological gradient in which the maximum number of the microflora occurring in the soil adjacent to the root and the number declining with distance away from the roots (Rovira, 1965a). Clark (1949) pointed out that the root surface, which he called the 'rhizoplane', is a more sensitive index of the specific qualitative effects of roots on soil microorganisms and stressed the importance of studying the rhizoplane. According to Parkinson *et al.* (1963), the root surface supports fungal population which is quite distinct from that of the plant rhizosphere and from that of the soil distant from roots. Yet another specialized micro-environment on root surface is the root surface tissues undergoing lysis by microorganisms. Bacteria may invade the empty cells of the epidermis and cortex behind the root hair zone. This specialized micro-environment has been termed the 'endorhizosphere' (Balandreau and Knowles, 1978). Newman (1985) summarized the features of the 'classical' rhizosphere as follows: (i) each root has its own rhizosphere, effectively separated from that of neighbouring roots, (ii) the rhizosphere is a region of high microbial abundance and activity, especially bacteria and (iii) soluble substances in the exudate from the root are the main substrates for the microorganisms. He reviewed the literature on rhizosphere microorganisms and their carbon substrates and

hypothesized, contrary to the classical belief, that many of the fungi in the rhizosphere were obtaining their substrate from outside the rhizosphere. The literature on various aspects of rhizosphere phenomenon has been reviewed by several other authors also (Katznelson *et al.*, 1948; Starkey, 1958; Rovira, 1965a; Parkinson, 1967; Rovira and McDougall, 1967; Rovira and Davey, 1974; Bowen and Rovira, 1976; Balandreau and Knowles, 1978; Newman, 1985; Curl and Truelove, 1986; Foster, 1986; Lynch, 1990).

2.2 FACTORS RESPONSIBLE FOR THE RHIZOSPHERE PHENOMENON

According to Starkey (1929a), the selective stimulation of the microorganisms in the root zone is due to: (i) sloughed-off root cell debris, (ii) release of soluble organic compounds by roots, (iii) higher concentration of CO_2, (iv) lower concentration of O_2 and (v) lower concentration of nutrient ions and partial desiccation of soil due to the absorption of water by roots. Rovira (1965a) and Rovira and McDougall (1967), reviewed the factors influencing the nutrition of the rhizosphere microorganisms. Though the organic compounds originating from the roots appear to be the primary determinants of the rhizosphere effect, there are additional factors which may be important in making the soil adjacent to the plant roots a favourable habitat for some microorganisms. For example, the lowering of the concentration of nutrients caused by assimilation of plants selectively favour those group of microorganisms which can compete more successfully or which has very low nutrient requirements. Other factors which may be influencing the rhizosphere microorganisms are change in soil pH and oxygen availability due to root respiration and the alteration in soil physical properties due to root penetration improving soil structure (Richards, 1987).

2.3 ORIGIN AND NATURE OF RHIZOSPHERE MICROFLORA

Many workers have studied the influence of age of plant on the rhizosphere microflora and the stage in the development of plant at which the rhizosphere effect is demonstrated (Starkey, 1929b; Rao, 1962; Mishra and Srivastava, 1969). Timonin (1940a), using bacterial counts showed that the rhizosphere population was established within three days of seed germination. Wallace and Lochhead (1951) postulated that the rhizosphere microorganisms constitute a group, morphologically, physiologically and nutritionally intermediate between the indegenous soil microflora and the epiphytic seed microflora. Rovira (1956a) found a rapid multiplication of microorganisms on the seed during the initial stages of germination and also on the root almost immediately after emergence. Natarajan (1976) found that fungi present in the rhizosphere originated from the soil rather than from the seeds.

2.4 EXTENT OF RHIZOSPHERE AND RHIZOPLANE

Foster and Rovira (1978), by bacterial counts in transmission electron micrographs of *Trifolium subterraneum* found a ten-fold drop in numbers in the soil 20 μm away from the root surface, suggesting a very narrow rhizosphere width. They defined the thickness of rhizoplane as the mean diameter of the rhizosphere microorganism, namely about 1 μm. Gilligan (1979) and Ferris (1981) proposed methods for calculating rhizosphere volume; Walker and Smith (1988), compared the suitability of the equations of the two authors for calculating rhizosphere width.

Foster (1986) reviewed the literature on the ultrastructure of the rhizoplane and rhizosphere. Electron microscopic studies have given rise to three models of root surface ultrastructure. The first, proposed by Scott *et al.* (1958) is that the root surface gel, inhabited by microorganisms, was enclosed by a fine 'cuticle'. The second, a more acceptable model was proposed by Jenny and Grossenbacher (1963) who suggested that gel had no firm boundary and become increasingly tenuous with distance from root surface. The gels become a complex mix because of the root as well as microbial secretions and Jenny and Grossenbacher (1963) termed this complex of root and microbial gels as 'mucigel'. The third model proposes formation of microfibrils from root surface making contact with soil minerals through which ions from mineral solutions are conducted to the roots (Leppard and Ramamoorthy, 1975).

2.5 METHODS OF STUDY OF RHIZOSPHERE

Knowledge of microbial population in the rhizosphere is obtained through Rossi-Cholodny-buried-slide technique (Starkey, 1938), dilution-plate technique (Lochhead, 1940; Rouatt and Katznelson, 1961), direct plating of roots (Harley and Waid, 1955), staining (Rovira, 1956a; Rovira *et al.* 1974), manometric (Katznelson and Rouatt, 1957), tracer technique (Krassilnikov, 1961), and electron microscopy (Jenny and Grossenbacher, 1963).

Of the various methods employed by investigators to study the rhizosphere, the light and electron microscopic method has been widely used in recent times to study the quantitative nature of the rhizosphere. A large number of reports are available on studies made by this method. The bacterial cover of roots as measured by light microscopy is only in the order of 7 per cent, depending on the species (Rovira *et al.*, 1974). Van Vuurde and associates (Van Vuurde *et al.* 1979; Van Vuurde and Schippers, 1979) obtained actinomycete and fungal cover ranging from 2-26 per cent and from 2-4 per cent respectively, depending

on the age of the root, with a maximum at about 9 days. Foster and coworkers (Foster and Marks, 1967; Foster and Rovira, 1978; Foster *et al.*, 1983) have studied the rhizosphere and rhizoplane using Transmission Electron Microscopy (TEM) of ultrathin sections. Reviewing the literature on ultrastructural studies of rhizoplane and rhizosphere, Foster (1986) suggested that ultrastructural studies were necessary due to the complex layering of rhizodermal cell wall, the minute size of soil microorganisms, gross underestimation of soil microorganisms derived through conventional plating techniques, easier location of organisms in the rhizosphere and for the understanding of biochemical functions of organisms. Foster and Rovira (1978) showed that there were not only more bacteria at the rhizoplane (120 x 10^9/ml compared with only 34 x 10^9/ml at 10-15 μm away), but also that the variety of types that could be recognized morphologically was also greater. Foster (1985) measured the size of more than 900 bacteria in TEMs of ultrathin sections of rhizosphere of several species and compared them with already known sizes from TEMs of bulk samples. Eighty percent of the rhizosphere bacteria were greater than 0.3 μm in diam compared with only 37 per cent in the bulk soil. Away from the rhizoplane, bacterium tend to occur in isolated discrete colonies (Foster, 1981; Foster and Martin, 1981; Foster *et al.* 1983; Foster, 1985). In the outer rhizosphere they are generally associated with substantial organic remnants. Thus the largest colonies and the largest individual bacterium were associated with cell wall remnants that still contained carbohydrate (Foster *et al.* 1983; Foster, 1985).

2.6 QUALITATIVE AND QUANTITATIVE FEATURES

Most of the qualitative and quantitative estimation of the rhizosphere effect have been done on bacteria, actinomycetes and fungi of a wide variety of crop plants (Agnihothrudu, 1953; Reddy, 1959; Ivarson and Katznelson, 1960; Rouatt and Katznelson, 1961 ; Rangaswami and Vasantharajan, 1961; 1962a, b, c; Zagallo and Bollen, 1962; Mishra and Srivastava, 1969; Parkinson and Thomas, 1969; Mall, 1975; Barber and Lynch, 1977; Gangawane and Deshpande, 1977a; Sivasithamparam *et al., 1979).*

2.6.1 Bacteria

Actively growing plant roots exert a distinct selective action on soil microorganisms, resulting in the stimulation of certain groups and in the suppression of others (Katznelson, 1965). The rhizosphere effect of an actively growing crop is most pronounced with bacteria, R:S ratio of 10 to 20 or more frequently being recorded. Observations of higher microbial population in the rhizosphere than in root free soil made by several authors have clearly

demonstrated the rhizosphere effect (Timonin, 1940a; Katznelson, 1946; Clark, 1949; Agnihothrudu, 1953; Lochhead and Rouatt, 1955; Rouatt and Katznelson, 1961; Rangaswami and Vasantharajan, 1962a; Sethunathan, 1966; Mall, 1979). The roots of most plants favour Gram-negative non-sporulating rod shaped bacteria (Lochhead, 1940; Sperber and Rovira, 1959; Vagnerova *et al.*, 1960a, b; Rouatt and Katznelson, 1961; Rangaswami and Vasantharajan, 1962b). The selective effect is more pronounced in the rhizoplane than in the rhizosphere (Vagnerova *et al.*, 1960a, b; Rouatt and Katznelson, 1961). The preponderance of motile, chromogenic, physiologically active and rapidly growing bacteria in the root zone of many plants were reported by several authors (Lochhead 1940; Katznelson, 1946; Rovira, 1956a; Katznelson and Rouatt, 1957; Rouatt and Katznelson, 1957; Zagallo and Katznelson, 1957; Katznelson and Bose, 1959; Vagnerova *et al.*, 1960a, b; Rouatt and Katznelson, 1961; Rangaswami and Vasantharajan, 1962b; Rouatt *et al.*, 1963; Zagallo and Bollen, 1969).

2.6.2 Actinomycetes

Actinomycetes which possessed important biosynthetic capabilities like production of antibiotics had not received much attention taxonomically and physiologically in relation to rhizosphere phenomenon (Katznelson, 1965). Most of the studies were aimed at finding out the presence and abundance of antagonistic types. Rouatt *et al.* (1951) found higher proportion of antibacterial actinomycetes in the rhizosphere than in root-free soil. Agnihothrudu (1955) isolated actinomycetes antagonistic to *Fusarium udum* from rhizosphere of pigeon pea, resistant to the pathogen. Rangaswami and Vasantharajan (1962c) found abundant antibacterial forms in the root zone of citrus, but no particular taxonomic group was associated with rhizosphere effect. Venkatesan (1962) observed gradual increase of R:S ratio from 2 to 7 between 15th and 150th day and found aerobic, pigmented, biochemically active, and late-sporing types of actinomycetes more commonly in the rhizosphere of rice. He also observed that nutritionally simple and soil extract-requiring types were relatively more abundant in the root free soil, while those requiring arninoacids and growth factors were more numerous in the root zone. He has also reported that Streptomycetes were the predominant group (80-90 per cent), and generally roots favoured antagonistic actinomycetes. However, Venkatesan (1962) reported that *Nocardia* species were relatively stimulated in the rhizosphere while *Micromonospora* species were favoured in the root free soil. The work of Rouatt *et al.* (1951) showed a lower percentage of incidence of antibacterial actinomycetes on old roots of oats and soybean and different patterns of antagonism with different plants. Abraham and Herr (1964) observed that developing roots of corn and soybean influenced rhizosphere

actinomycete flora qualitatively and quantitatively. Quantitative estimation of rhizosphere and rhizoplane microflora of two coriander varieties revealed greater root influence on actinomycete and bacteria than on fungi (Mall, 1977). Secilia and Bagyaraj (1987) and Mada (1990) reported increased actinomycete population in the rhizosphere of mycorrhizal plants. A brief review on actinomycetes is given separately (see section 2.10).

2.6.3 Fungi

As in the case of bacteria and actinomycetes fungi also show qualitative and quantitative differences between root free and rhizosphere soil (Peterson, 1958; Catska *et al.*, 1960; Papavizas and Davey, 1961; Gangawane and Deshpande, 1975; Roy *et al.*, 1980). Thrower (1954) studied the nutritive behaviour of rhizosphere fungi. Parkinson and Thomas (1969) reported that fungi in the rhizosphere differed both in number and species from those in non-rhizosphere soil. Katznelson (1965) is of the opinion that there is a lack of information on the rhizosphere effect on fungi in relation to nutrition and physiology.

2.7 FACTORS AFFECTING RHIZOSPHERE MICROBIAL POPULATION

As the primary factor contributing to the rhizosphere phenomenon is the plant itself, any condition that significantly affects its growth and metabolism will be reflected in quantitative and qualitative changes in the microflora of the root zone (Katznelson, 1965). In addition to the stage of plant growth (Rouatt, 1959; Vagnerova *et al.*, 1960a, b; Oblisami *et al.*, 1977), type and species of plant (Vrany, 1960; Rouatt and Katznelson, 1961), the nature, moisture content, reaction and fertility of the soil (Clark, 1947; Peterson, 1958; Ivarson and Katznelson, 1960; Grayston and Germida, 1990), environmental factors such as light and temperature (Miller and Boothroyd, 1962; Rouatt and Katznelson, 1960; Rouatt *et al.*, 1963; Fenwick, 1973), foliar sprays and soil amendments (Reddy, 1959; Venkata Ram, 1960; Horst and Herr, 1962; Vrany *et al.*, 1962; Annapurna and Rao, 1988), and the genetic constitution of the plant (Neal *et al.*, 1973) affect the rhizosphere microbial population in varying degrees.

2.7.1 Soil characters

Soil characters have profound influence on the microbial population in the rhizosphere (Peterson, 1958; Parkinson and Clarke, 1961; Baruah and Baruah 1972). Potty (1977) observed that the occurrence of microorganisms in the rhizosphere of healthy and root wilt affected coconut palms varied considerably with soil type and locality. However, Youssef and Mankarios (1968) observed that the total R/S value of fungi was not influenced by soil

types. Though it was generally accepted that populations of microorganisms in soil decline with increase in depth, the rhizosphere effect was progressively more in deeper layers (Rangaswami and Venkatesan, 1964).

Soil moisture: Early studies of Timonin (1940a) and Clark (1947) showed an increase in the density of microorganisms in the rhizosphere of flax, wheat and soybean as the soil moisture decreased. Similar results were obtained by Lochhead (1958), Peterson *et al.* (1965), Mehrothra and Kakkar (1972), and Sullia (1973b).

Taylor and Parkinson (1964) while studying the effect of soil pH on the development of root surface fungi in dwarf bean plants showed that the occurrence of *Fusarium* species on root surface was not markedly affected by different soil pH levels. *Cylindrocarpon radicicola*, on the other hand, showed increased frequency of occurrence with increasing soil pH. They also studied the effect of soil temperature on the development of fungi on the roots and reported that the number of fungi decreased on the roots with decreasing soil temperature. Ivarson and Mack (1972) also demonstrated marked influence of soil temperature on root surface microflora.

Increasing evidences of beneficial effects of rhizosphere microflora upon higher plants encouraged attempts to modify the rhizosphere population for the development of beneficial microorganisms and suppress adverse types through soil amendments and foliar sprays. Katznelson (1946) found that selective soil treatments brought about changes in the rhizosphere microflora. The rhizosphere flora of fertilised and unfertilised plants were reported to be qualitatively and quantitatively dissimilar (Papavizas and Davey, 1961; Jalaluddin, 1975; Gangawane and Deshpande, 1976; 1977b). Soil treatment with Benomyl and Captan considerably inhibited the rhizosphere mycoflora of *Allium cepa* (de Bertoldi *et al.*, 1978). Effect of pretreatment of roots of rice, maize and garlic with hormones and other substances were studied by Reddy (1968a, b), Anilkumar and Chakravarti (1970) and Bharat Rai and Agarwal (1976) respectively.

2.7.2 Environmental factors

Rouatt *et al.*, (1963) studied the effect of temperature with wheat and soybean grown at three ranges of temperature from 12.8-32.2°C. In wheat rhizosphere, bacterial counts and number of glucose fermentors, methylene blue reducers, ammonifiers and amino acid requiring bacteria were lowered with reduction of light intensity from 1000 to 300 ft. c. Srivastava (1971) recorded higher microbial population in the rhizosphere of plants exposed

to continuous light, whereas rhizosphere of plants in continuous dark harboured less microflora and those exposed to 12 hours of alternate light and dark treatments showed an intermediate condition. However, Peterson (1961) stated that shading of plants have no appreciable effect on vegetatively active fungi colonizing the primary roots of wheat and soybean seedlings.

2.7.3 Plant influence on rhizosphere microflora

The rhizosphere effect may be noted usually within a few days of planting seed, the developing microbial population being made up of organisms from both seed and soil (Wallace and Lochhead, 1951; Rouatt, 1959; Vagnerova *et al.* 1960a, b). The rhizosphere effect increases with the age of plant and generally reaches to a maximum, coincident with its greatest vegetative development. Increase in rhizosphere population with increase in plant age has been recognized by several authors (Starkey, 1929b; Timonin, 1940a; Rao, 1962; Rovira, 1965a; Kamal and Singh, 1969; Oblisami, 1973). Establishment of definite nutritional groups of bacteria at specific age of plants have been reported by Vagnerova *et al.* 1960a, b). Mishra and Srivastava (1969) found that rhizosphere population was low in the beginning, increased later and attained the peak at the time of flowering in certain legumes. Dayal and Srivastava (1973) studied the influence of varieties and age of okra on rhizosphere mycoflora.

Different plants exert varying degree of rhizosphere effect quantitatively and qualitatively. Rouatt and Katznelson (1961) reported highest rhizosphere counts with red clover followed by flax, oats, wheat, barley and corn. Fungal populations also vary considerably with age and kind of plant. Peterson (1959) showed that *Pythium* sp. predominated on barley, flax and wheat at the early stages of plant growth but declined markedly at the later stages to be replaced by *Phoma* and *Fusarium*.

Different plant species are known to harbour specific microorganisms in their rhizosphere (Peterson, 1958; Parkinson *et al.*, 1963; Mishra, 1967; Ranga Rao and Mukerji, 1971; Grayston and Germida, 1990). According to Alexander (1978) different plant species grown in the same field had widely divergent numbers of organisms in their rhizosphere. However, when the same species was cultivated in fields of different soil fertility, the composition and size of microbial population fluctuated only to a moderate extent. The impact of two soybean lines (nodulating and non-nodulating) in producing different rhizosphere pattern was demonstrated by Elkan (1962). However, absence of significant difference between rhizosphere microbial populations of different species of plants was observed by some other workers (Rangaswami and Vasantharajan, 1962a, b; Sullia, 1973b).

Root microflora of plants vary not only between different species but also between different varieties of a single species. Lochhead *et al.* (1940) showed that rhizosphere of varieties of tobacco and flax, susceptible to black root rot and wilt, had higher bacterial numbers and showed a more pronounced rhizosphere effect on the incidence of certain groups than non-susceptible varieties. The germination of species of pea-wilt fungus, *Fusarium oxysporum* f. *pisi* differed in the rhizosphere of three varieties of peas (Buxton, 1957a, b).

Occurrence of more number of microorganisms in the rhizosphere of diseased plants than in the rhizosphere of healthy plants was reported by several authors (Nair, 1964; Timonin, 1966; Gangawane and Deshpande, 1973; Goel and Mehrotra, 1974; Potty, 1977; Weste and Vitharage, 1978). Harper (1950) and Subba Rao and Bailey (1961) found more number of microorganisms in the rhizosphere of banana varieties susceptible to panama disease and tomato varieties susceptible to *Verticillium* species than in the rhizosphere of resistant varieties. Bacteria and actinomycetes antagonistic to *Fusarium udum*, the wilt pathogen, was reported by Murthy and Bagyaraj (1978) from the rhizosphere of resistant variety of *Cajanus cajan*.

Several studies have been carried out on the microbial population changes consequent to foliar treatments. Halleck and Cochrane (1950) observed that the increase or decrease of bacteria on bean rhizosphere depended upon the type of fungicide sprayed on the foliage. A sharp reduction in the rhizosphere bacterial population was observed when bean plants were sprayed with Bordeaux mixture, dithane Z-78 and malachite green. Lowering of rhizosphere microbial population of rice was observed by Sullia (1969) when sprayed with Kitazin. Foliar application of urea on rice plants increased rhizosphere fungal population nearly fifteen times while density of bacteria and actinomycetes were reduced significantly (Reddy, 1959; 1968a). Bagyaraj and Rangaswami (1972) also observed increase of rhizosphere fungal population of *Eleusine coracana* Gertn. when sprayed with N, P and K solution. However, Venkata Ram (1960) observed significant decrease in fungal population in the rhizosphere when tea plants were sprayed with urea. Effect of foliar application of various chemicals, growth regulators, fungicides and antibiotics on rhizosphere microflora was studied by several other workers (Sullia, 1968; Sethunathan, 1970; Gupta, 1971; Balasubramanian and Rangaswami, 1973; Vrany, 1974, 1978; Kannaiyan and Prasad, 1974; Van Vuurde and de Lange, 1978; Srivastava and Dayal, 1981). Rovira and McDougall (1967) suggested that modification of root exudate as a result of foliar spray and the consequent

changes in the rhizosphere may well be used to inhibit root pathogens or to stimulate the rhizosphere microflora which is beneficial to plant growth.

2.7.4 Root exudates

The importance of root exudate arises from the fact that it serves as nutrient not only for rhizosphere microorganisms (Subba Rao *et al.*, 1962; Rovira, 1965b; Balasubramanian and Rangaswami, 1971; Khalis *et al.*, 1990) but also for root pathogens (Kerr, 1956; Buxton, 1957a, b; Schroth and Hildebrand, 1964; Cook and Baker, 1983).

The first report of the evidence of root exudation was provided by Knudson in 1920 (cited from Rovira, 1965b) who observed the production of reducing sugars in sucrose solution in which peas and maize were grown aseptically. However, interest in root exudate with reference to the rhizosphere microorganisms was started in 1950s. The identification of various components of exudates, published in a series of papers (Rovira, 1956b, c, d, 1959) gave impetus to many investigators for the study of root exudates. Thereafter several reports appeared on the various aspects of root exudates (Katznelson *et al.*, 1965; Bhuvaneswari and Subba Rao, 1957; Vancura, 1964; Vancura and Hovadik, 1965; Vancura and Garcia, 1969; Martin, 1977; Meheswari and Purushothaman, 1990; Svenningsson *et al.*, 1990).

Rovira (1979) defined root exudates as "low molecular weight compounds like monosaccharides and aminoacids, which leak passively from all cells into the soil either directly or through the intercellular spaces". He differentiated root exudates from secretions which are low and high molecular weight compounds like mucilages released by metabolic processes of plant roots and microbes associated with them.

Hale *et al.* (1978) considered root exudates as part of an ecological pattern of interactions between roots and soil inhabiting organisms in the rhizosphere of plants. Release of exudates in the form of chemicals affect physical, chemical and biotic environments in the soil. Newman (1985) suggested the term 'rhizodeposition' to cover all organic materials produced or secreted by roots. The subject of root exudation has been reviewed by several authors (Woods, 1960; Schroth and Hildebrand, 1964; Rovira, 1965b; 1969; Hale *et al.*, 1978; Uren and Reisenauer, 1988; Whipps, 1990).

Role of root exudates: Root exudates are reported to be stimulatory to pathogens as well as many other microorganisms. The universality of this phenomenon is suggested to be of net benefit to plants (Baker and Cook, 1974). Stimulatory effect of root exudates on spore

germination of fungi and on germination of nematode cyst in the root region has been observed by several investigators (Kerr, 1956; Buxton, 1957a, b; Wallace, 1958; Rovira, 1965b). Brown (1973) found that wheat roots provided a suitable environment for multiplication of bacteria whose growth was otherwise restricted by bacteriostatic factors present in the soil. Indirect evidence on the role of root exudate is obtained by the observation of a modified rhizosphere microflora or restricted growth of root pathogens brought about by the foliar application of chemicals (Rovira, 1969; Baker and Cook, 1974).

The specificity shown by plant roots in their selective stimulation of certain bacteria is clearly shown in the symbiotic association between the root nodule bacterium (*Rhizobium*) and its legume host. Elkan (1961) demonstrated that a non-nodulating soybean exuded compounds which altered the morphology of *Rhizobium* and prevented nodulation of the nodulating soybean line. The qualitative and quantitative difference in the root exudates of mycorrhizal and non-mycorrhizal plants have been studied by a few authors (Graham *et al.*, 1981; Krishna and Bagyaraj, 1983; Ocampo and Azcon, 1985; Mada, 1990).

Method of exudate collection: Several types of apparatus have been developed for the root exudate collections either by maintaining the root system only in axenic condition leaving tops in the open (Blanchard and Diller, 1950; Nilsson, 1957; Reuszer, 1962; Stotzky *et al.*, 1962; Kandaswami *et al.*, 1973) or by the axenic maintenance of entire plants (German and Bowen, 1951; Waris, 1958; Estey and Smith, 1962; Lindsey, 1967). Sand culture approximate the soil, more closely, but still lacks the physical and chemical complexity of soil (Rovira, 1969). Heat sterilized soil is often regarded as phytotoxic to plants (Rovira and Bowen, 1966), while gamma ray sterilized soils are less harmful (Bowen and Rovira, 1961). The gnotobiotic cultivation of plants including axenic culture has been reviewed by Hale *et al.* (1973) and Kreutzer and Baker (1975).

Nature and amount of exudates: The nature of root exudates has been studied by many investigators. The range of compounds exuded by plant roots include substances such as carbohydrates, aminoacids, organic acids, nucleotides, vitamins, hydrocyanic acid, gaseous and volatile compounds, etc. Several reports are available on the occurrence of various kinds of carbohydrates in the root exudate of different crop plants (Rovira, 1956b; Bhuvaneswari and Subba Rao, 1957; Schroth and Snyder, 1961; Vrany *et al.*, 1962; Vancura and Hovadik, 1965; Martin, 1978). Among the carbohydrates exuded, glucose and fructose have been considered to be most abundant (Rovira, 1956b). Amino acids have been the most studied

group and various workers have reported a total of 23 aminoacids from 15 different plant species (Parkinson, 1955; Andal *et al.*, 1956; Rovira, 1956b; Sulochana, 1962a; Vancura and Hovadik, 1965). Vitamins and growth factors were reported in the root exudates of rice, bean, clover, peas, lucerne, phalaris, tomato, cotton, etc. (Bhuvaneswari and Sulochana, 1955; Rovira and Harris, 1961; Sulochana, 1962b). Organic acids were reported in the root exudates of wheat and mustard (Bhuvaneswari and Subba Rao, 1957; Vrany *et al.*, 1962). Fries and Forsman (1951) reported nucleotides in the root exudates of peas. Ljunggren and Fahreaus (1961) found several enzymes and Mitchell *et al.*, (1961) detected auxins in the root exudates of crop plants. Timonin (1941) reported hydrocyanic acids in the root exudates of flax, and Vancura and Stotzky (1976) detected gaseous and volatile compounds in plant root exudates. The amount of organic materials released into soil by the roots of growing plants were assessed by several authors (Martin, 1975; Barber and Martin, 1976; Vancura *et al.*, 1977; Martin and Kemp, 1980; Whipps and Lynch, 1983; Jansen, 1990). Reviewing the literature on root exudates, Rovira (1965b) and Hale *et al.* (1978) listed the plant species used in root exudate studies.

Factors affecting exudation: The pattern of root exudates differ depending upon plant species, age of plants and cultivar (Vancura, 1964; Balasubramanian and Rangaswami, 1969; Kraft, 1974), and plant nutrition (Bowen, 1969). The age or the stage of development has an important bearing on the quality and quantity of root exudates (Rovira, 1959; Vancura and Hovadik, 1965; Richter *et al.*, 1968; Balasubramanian and Rangaswami, 1969; Smith, 1970; Hamlen *et al.*, 1972). The alterations brought about in root exudations at different stages of plant growth with respect to pathogenesis was studied by Jalali and Suryanarayana (1970, 1971, 1972).

The influence of light and temperature on quantity of root exudates has been reported by Rovira (1959), Vancura (1967) and Whipps (1984). The presence and the type of microorganisms in the rhizosphere also influence the pattern and amount of root exudate considerably (Norman, 1955, Rovira, 1959; Martin, 1977). Foster (1986) observed that the components of root exudates vary in amount and type with distance from the root apex.

Changes in the microbial population brought about by modification of root exudates which in turn is made possible by foliar application of chemicals, have been reported by several authors (Reddy, 1959; Venkata Ram, 1960; Horst and Herr, 1962; Vrany *et al.*, 1962; Agnihotri, 1964; Vrany, 1974).

Sites of root exudation: The site of exudation on plant roots has been investigated by various workers (Pearson and Parkinson, 1961; McDougall and Rovira, 1965, 1970; Van Egeraat, 1975). According to Rovira (1973), the major zone of release of diffusible exudates is the zone of elongation.

Mechanism of root exudation: It was assumed previously that most of the organic substances released by roots were exuded or leaked passively from roots independently of metabolic processes. However, it is now believed that some metabolites are actively secreted as a result of expenditure of metabolic energy (Hale *et al.*, 1978; Richards, 1987). Rovira (1973) suggested that most of the photosynthetically assimilated materials released from the sites of elongation in wheat roots involved a 'leakiness' in that area. Ayers and Thornton (1968) have reported that injury of root was responsible for exudation from root-tips, after studying the ninhydrin reacting areas corresponding to root-tip region of wheat grown in sand.

Soil zone influenced by exudate: Rovira (1969) suggested that the distances exudates move from roots will depend on the amounts exuded, the pH of the compounds, the susceptibility of the compounds to microbial absorption and decomposition, the types and amounts of clay in the soil and moisture contents of the soil. The extent of influence of exudates away from roots in respect of growth stimulation of microorganisms was assessed by some workers (Coley-Smith, 1960; Rovira, 1969).

Role of radioisotopes in root exudate studies: The use of radioisotopes opened up new vistas in the study of root exudates and rhizosphere phenomenon. Subha Rao *et al.* (1962) incorporated $^{14}CO_2$ into tomato seedlings growing in soil and measured the radioactivity of the exudate leached from the root into the soil. They also attempted to measure the distance exudates diffuse through soil by measuring the radio-activity of soil cores taken at different distances from roots. McDougall (1968) used the technique of pulse labelling incorporating $^{14}CO_2$ into metabolic pathways and detected the radioactive exudates from roots in order to detect the site of exudation. The technique of labelling photosynthates and detecting them in root exudates have been employed by several other workers (Bowen and Rovira, 1967; Rovira, 1969; Balasubramanian and Rangaswami, 1971). Liljeroth *et al.* (1990) investigated the transport of labelled photosynthates from leaves to the rhizosphere of wheat and subsequent incorporation in the soil microbial biomass. Labelled materials have also been used in studying the effect of foliar spray on modification of root exudates and consequently on root microflora (Davey and Papavizas, 1961; Balasubramanian and Rangaswami, 1971).

2.8 INFLUENCE OF RHIZOSPHERE MICROFLORA ON PLANT GROWTH

The influence of rhizosphere microflora on plant growth has been generally considered to be favourable to plant growth (Katznelson, 1965). Alexander (1978) recorded that the increased amount of microbial carbondioxide in the root zone enhanced the supply of assimilable mineral nutrients to the plants. It is also recognised that the activity of specific physiologic group of microorganisms in the rhizosphere soil results in increased mineralization as well as high immobilisation rates. Enhanced quantity of nitrogen is made available to plants through symbiotic and asymbiotic nitrogen fixation; besides,soil microbes modify the absorption and utilization of phosphate by the root system (Bowen and Rovira, 1966; Barber, 1969). Stimulation of plant growth through greater synthesis of vitamins, aminoacids, auxins and gibberellins have also been reported (Brown, 1972; Alexander 1978). The influence of rhizosphere microflora on plant growth has been reviewed by several authors (Balandreau and Knowles, 1978; Barber, 1978; Lynch, 1982).

Physiological activity of rhizosphere microorganisms: While studying the metabolic activity of bacterial isolates from wheat rhizosphere and control soil, Zagallo and Katznelson (1957) found that bacteria isolated from rhizosphere were physiologically more active than the soil isolates in utilizing the substrates such as sucrose, glucose, acetate, succinate and alanine. Reviewing the literature on the rhizosphere effect and physiological activity of rhizosphere bacteria, Rovira (1965b) stated that most of the comparisons between rhizosphere and non-rhizosphere bacteria showed a definite selective stimulation of physiologically active organisms including Gram-ve rods, and amino acid-requiring organisms by plant roots. He also stated that roots preferentially stimulate those bacteria in soil that are capable of responding to readily available nutrients. Brown (1975) stated that differences between root and soil microflora can be explained by the presence of different energy sources and hence of special environments.

2.9 BIOLOGICAL CONTROL OF ROOT PATHOGENS

Attempts on direct application of biological control of root pathogens started in 1920s. Early attempts of biological control of soil pathogens included inoculation of forest nursery soils with antagonistic fungi in order to control damping-off of pine seedlings, and control of potato scab disease caused by *Streptomyces scabies* through soil amendments of green grass cuttings and *S. praecox*, an antagonistic actinomycete. The capability of soil inhabiting bacteria, fungi and actinomycetes in the control of take-all fungus *Gaeumannomyces*

graminis var. *tritici*. was demonstrated by Sanford and Broadfoot in 1931 and they introduced the terms "biological control" and "suppressive effect" in plant pathology (Cook and Baker, 1983). Baker and Cook (1974) defined "biological control as the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man". Ever since the publication of the book "Ecology of Soil-borne Plant Pathogens - A Prelude to Biological Control" (Baker and Snyder, 1965), tremendous interest have been shown on this topic and since then several volumes and reviews on this important topic have come up (Thirumalachar, 1968; Baker and Cook, 1974; Bruehl, 1975; Lockwood, 1977; Harley and Russel, 1979; Papavizas and Lumsden, 1980; Cook and Baker 1983; Hornby, 1983; Papavizas, 1985; Parker *et al.*, 1985; Hoitink and Fahy, 1986; Leong, 1986; Schippers *et al.*, 1987; Fravel, 1988; Mukerji and Garg, 1988a, b; Weller, 1988; Campbell, 1989). Garrett (1970) remarked that successful biological control can be achieved only as a result of the most thorough and fundamental investigations into the microbial ecology of the soil.

Mechanism of biological control: Baker and Cook (1974) suggested that biological control of plant pathogens involve: (1) antibiosis and lysis, (2) competition and (3) parasitism and predation. Antagonists include virtually all classes of organisms, namely, fungi, bacteria, actinomycetes, viruses, nematodes, protozoa, viroids and seed plants.

While antibiosis is easy to demonstrate in laboratory, there has been a great deal of argument and much speculation about its possible regulatory role in microbial ecosystem in soil (Gottlieb, 1976; Goodfellow and Williams, 1983). Reviewing the role of antibiosis in the biocontrol of plant diseases, Fravel (1988) stated that the lack of methods to evaluate the production and function of compounds mediating antibiosis has been the major impediments for acquisition of basic information on antibiosis. Several authors have put forth evidences that antibiotics function as biocontrol agents in nature (Brian, 1957; Dekker, 1963; Fravel, 1988), they can be extracted from non-sterile soil and they are produced in sterile soil inoculated with antibiotic producing microbes (Wright, 1956a, b; Rothrock and Gottlieb, 1981; Richards, 1987). Lockwood (1986), Baker (1987) and Singh and Faull (1988) discussed about the various types of microbial interactions taking place in soil and their importance in biological control of plant diseases.

Competition for nutrients had been studied earlier very little. Recently, however, with the recognition that certain strains of fluorescent Pseudomonads increase crop yield or

control biologically soil-borne plant pathogens when applied as seed inoculants (Schroth and Hancock, 1981, 1982; Suslov, 1982; Becker and Cook, 1988). This type of competition has been intensively investigated, and it appears to operate in the rhizosphere also (Schippers *et al.*, 1985, 1987).

Several reports have suggested that fluorescent *Pseudomonas* render biological control and enhance plant growth by producing fluorescent siderophores that sequester iron in the root environment, making it less available to competing deleterious microflora (Kloepper *et al.*, 1980; Scher and Baker, 1982; Sneh *et al.*, 1984). Siderophores have since been implicated in the biological control of potato seed piece decay caused by *Erwinia carotovora* (Jones) Bergy *et al.* (Xu and Gross, 1986), wilt diseases caused by *Fusarium* spp. (Sher and Baker, 1982; Sneh *et al.*, 1984), and in protection of cotton from preemergence damping-off caused by *Pythium ultimum* (Loper, 1988).

Cook and Baker (1983) considered that pathogen suppressiveness may result from the physiologically active microorganisms acting as a nutrient sink and leaving the pathogen propagules deficient in carbon, nitrogen or some other essential nutrients. Mandeel and Baker (1991) reported control of Fusarium wilt of cucumber with strains of non-pathogenic *Fusarium oxysporum*. Lockwood (1986), postulated that rapid deprivation of exudates by soil microbes resulted in disease suppression.

Mycoparasitism also is a major mechanism in the biocontrol process. Biological control of soil-borne pathogens with fungal mycoparasites has been demonstrated in a number of instances (Howell, 1982, 1987; Knudsen *et al.*, 1991). Adams (1990) reviewed the potential of mycoparasites for the biological control of plant diseases.

2.10 ACTINOMYCETES

Actinomycetes are filamentous gram positive bacteria that exhibit true branching mycelium. They are morphologically similar to filamentous fungi and can form conidia or arthrospores formed singly or in chains of various lengths. Just like bacteria, the cell walls do not contain chitin or cellulose and contain mucopeptides (Lechevalier and Lechevalier, 1981). Thus actinomycetes can be considered a transitional group between the simple bacteria and the filamentous fungi. Several volumes on Actinomycetales have come up recently (Waksman, 1959, 1961; Prauser, 1970; Sykes and Skinner, 1973; Arai, 1976; Krassilnikov, 1981a, b, c; Goodfellow *et al.*, 1988).

2.10.1 Taxonomy

The actinomycetes are classified within the order Actinomycetales. Actinomyces, a Greek derivation means ray- fungus, and actinomycetes are generally referred to as ray-fungi, particularly by Russian authors. The actinomycetes are separated into groups on the basis of morphology, physiology and chemistry of cell wall and whole cell composition, types of lipids and isoprenoid quinones (Lechevalier and Lechevalier, 1981). The vast majority of actinomycetes are oxidative and aerobic, their separation into genera carried out by utilizing morphological, physical and chemical criteria including Guanine-plus-Cytosine (G + C) content of DNA (Waksman, 1961; Lechevalier *et al.*, 1971; Cross and Goodfellow, 1973). *Streptomyces* is the important genus of this group. The number of 'species' of *Streptomyces* that have been described exceeds a thousand (Kutzner, 1981) or probably have risen to three thousand (Williams, 1986), because several different names have been given to the same species on trivial differences in morphological and cultural properties (Williams *et al.*, 1989). More than 450 species of *Streptomyces* and *Streptoverticillium* were described by collaborators of the International Streptomyces Project (ISP) under the supervision of American Society of Microbiology in order to remove the confusion regarding taxonomy and nomenclature of the genus *Streptomyces* (Shirling and Gottlieb, 1966; 1968a, b; 1969; 1972). Modern taxonomic methods like numerical taxonomy have lead to significant improvement in the classification of Streptomycetes (Kutzner, 1981; Williams, 1986; Williams *et al.*, 1989).

Role of Actinomycetes in bioctontrol of root diseases: The importance of rhizosphere microorganisms producing antibiotic substances has been mentioned by several authors (Goodfellow and Simpson, 1987; Weller, 1988; Lynch, 1990). Whaley (1965) reported the effect of nutrition in production of antibiotics by the rhizosphere actinomycetes against phytopathogenic fungi including root pathogens such as *Fusarium oxysporum, Rhizoctonia solani* and others. Dekker (1963) and Thirumalachar (1968) reviewed the role of antibiotics in the control of plant diseases.

Most of the works have been centered on the antibiotic activity of streptomycetes in the soil. There is enough circumstantial evidence suggesting that streptomycetes contribute to the control of fungal pathogens (Baker, 1968). Success in reducing disease severity by inoculation of seed or seedlings with potentially antagonistic microorganisms has been reported by a few authors (Merriman *et al.*, 1974; Singh and Mehrotra, 1980; Rothrock and Gottlieb, 1981; Sharma and Sinha, 1982).