5. DISCUSSION

5.1 RHIZOSPHERE MICROBIAL POPULATION

The quantitative estimation of bacteria, fungi and actinomycetes from the rhizosphere and control soil obtained in the present study is in conformation with the respective microbial populations generally reported in literature. The plate count studies have revealed a pronounced rhizosphere effect for bacteria with substantial population in the rhizosphere of all the three plant species. However, the bacterial population was less than 10^9/g of rhizosphere soil, the count considered as common occurrence by Katznelson (1965) and Alexander (1978). The higher population count and R:S ratio for bacteria compared to that of actinomycetes and fungi were as expected and the results agreed with the findings of several authors (Katznelson *et al.*, 1948; Clark, 1949; Agnihothrudu, 1953; Starkey, 1958; Catska *et al.*, 1960; Katznelson, 1960; Rouatt *et al.*, 1960; Rangaswami and Vasantharajan, 1962b, c; Rovira, 1965a; Bopaiah and Shekara Shetty, 1991). Alexander (1978) considered that actinomycetes and fungi were not significantly benefited by their proximity to roots and the R:S ratio rarely exceeded 2 or 3:1. However, some investigators have reported relatively high R:S ratios for actinomycetes studying with different plants (Balagopal and Oblisami, 1972; Oblisami and Jayasheela, 1975; Mall, 1977). In the present study also, actinomycete populations in the rhizosphere soil, especially in sunflower rhizosphere soil, showed significantly higher population count and an R:S value of about 90.83, far exceeding the usual values. Rangaswami and Vasantharajan (1962a) studied the quantitative incidence of microorganisms in relation to root and shoot growth of *Citrus* spp. and reported that bacteria were about 4 to 90 times more abundant in the rhizosphere than in soil, actinomycetes were 2 to 6 times and fungi 3 to 6 times abundant. Alexander (1978) suggested that contrary to their effect upon bacteria, roots did not alter the total counts of fungi appreciably; on the other hand specific fungal genera were stimulated depending upon plant species and age and the kind of soil.

5.2 MICROBIAL POPULATION IN THE CONTROL SOIL

Compared to the rhizosphere soil, the microbial population enumerated in the control soil in this study was relatively small. However, the total count obtained for bacteria,

actinomycete and fungi could be considered as a sufficiently moderate value by soil-dilution plate-count method, inspite of the low pH, and organic content of the soil. Generally the plate-counts gave an estimate of several hundred thousand up to 200 million bacteria per gram of dry soil, the abundance being a reflection of several environmental factors (Alexander, 1978). Kuznetzov and Arjunarao (1972) reported actinomycete population ranging between 26 and 38×10^6/g in different soils from fallow and cultivated plots of the field laboratory of the Botany Department of University of Madras. Rangaswami and Oblisami (1967) and Rangaswami *et al.* (1967) reported that among the representative soils of South India, Pollibetta (Karnataka State) laterite soil contained a bacterial population of about 40.10×10^6/g dry soil in a layer of 0-7.5 cm deep soil. Similarly, the same soil contained a high population of actinomycetes (10.81×10^6/g dry soil) and fungi (1.09×10^6/g dry soil). Although the same type of soil (laterite) was used in the present study, the bacterial, actinomycete and fungal populations were less than those of Pollibetta laterite soil. This may be probably due to the collection of soil samples from the uncultivated area also of the Botanic garden.

5.3 PLANT TYPE AND QUANTITY OF RHIZOSPHERE MICROFLORA

The variation between the rhizosphere microbial population of the three crop plants was significant because of the fact that the plants were growing in the same soil. This variation can be accounted for the differential stimulation on the rhizosphere microflora by the roots of the three crop plants. The results are in agreement with the findings of Vruggink (1976) that actinomycetes are influenced by crop species. Wide variation in actinomycete populations from the rhizosphere of medicinal plants was reported by Oblisami and associates also (Jayasheela and Oblisami, 1975a, b; Oblisami and Jayasheela, 1975).

It is recognized that different plant species often establish somewhat different subterranean flora, both quantitatively and qualitatively (Agnihothrudu, 1953; Rouatt and Katznelson, 1961; Mishra and Srivastava, 1969). The occurrence of qualitative and quantitative variation among the rhizosphere microbial population of different plant species is considered mostly due to substances exuded from roots, and partly due to autolysis of moribund and dead root cells (Alexander, 1978; Richards, 1987). It has also been established that genotype of the host plant also influence the rhizosphere microflora presumably through its control of quantity and quality of root exudates (Elkan, 1962; Neal *et al.*, 1973; Cook and Baker, 1983).

5.4 MORPHOLOGICAL CHARACTERISTICS OF ACTINOMYCETES

5.4.1 Colour of aerial mycelium

Pigmentation, being a readily recognizable character, has gained considerable importance in the taxonomy of actinomycetes, streptomycetes in particular (Shirling and Gottlieb, 1966). In the present study, all the isolates from the control soil and nearly 86 per cent of the isolates from the rhizosphere of sunflower and bittergourd were variously coloured. Similar observations were reported by other investigators also. Krassilnikov (1981a) observed that above 40 per cent of the strains isolated from the soil had coloured spores and when the colour of the mycelium was also taken into account, the total number of pigmented strains will reach up to 70-80 per cent of the total strains. Among the coloured actinomycetes, gray types were predominant, both in the control and rhizosphere soils. The red and yellow coloured isolates were the second and third largest groups respectively. This type of predominance in colour variation was also reported among the species of *Streptomyces* and *Streptoverticillium* described by the collaborators of International Streptomyces Project (Shirling and Gottlieb, 1968a, b, 1969, 1972). Rangaswami *et al.* (1967) grouped actinomycetes isolated from seven soil samples from South India based on colony colour. They found considerable variation among the coloured types; there were more of white mycelial forms in the black and alluvial soils and mauve forms in the red and laterite soils. Similarly in the present study also a number of isolates showed white coloured mycelium in all the four groups. Nearly half of the isolates of okra rhizosphere soil were white in colour. Several authors gave priorities for the colour of spore and substrate mycelium in the preparation of diagnostic keys for the classification of taxa included in the International Streptomyces Project (Kuster, 1972; Nonamura, 1974; Szabo *et al.* 1975). Krassilnikov (1981a) reviewed the chemical composition, properties and importance of pigments in actinomycete taxonomy. According to him, pigmentation plays an important role in the grouping of ray fungi.

Studies on pigmentation showed that non-coloured (white) actinomycete isolates also could produce coloured soluble pigments, melanoids and coloured substrate mycelia. Among the total actinomycete isolates, approximately, 18 per cent were non- coloured. Of these isolates 40 per cent produced reverse side pigment, about 33 per cent soluble pigment and 27 per cent melanoid pigments. This characteristic feature can also be considered in the characterisation of actinomycetes, particularly in species identification.

5.4.2 Pigment production

In the present study, production of the extra-cellular pigments by the isolates was studied only in relation to its use in species identification. Soluble and melanoid pigment producers were less than non-producers except in those from sunflower rhizosphere. Rangaswami *et al.* (1967) also found less number of pigment producers than non-producers from among the various actinomycetes isolated from the soils of South India. Pigment production was an important criterion adopted by Krassilnikov (1981a) in the classification of actinomycetes. However, the procedure adopted was different from that of ISP recommendations, which is adopted in the present study. Kutzner (1981) considered formation of melanin pigment by streptomycetes as a reliable physiological character.

5.4.3 Morphological character of sporophores and spores

Methods of reproduction are different in different genera of actinomycetes. The higher forms have specialized spore-forming organs - the sporophores and sporangia. In the present study, among the *Streptomyces* and *Streptoverticillium* isolates, *Rectiflexibiles* and *Retinaculiaperti* were the predominant type of sporophores. But Oblisami and Jayasheela (1975) have observed *Spirales* as the dominant type followed by *Rectiflexibiles* among the streptomycetes isolated from the rhizosphere soil of *Solanum khasianum*. Among the ISP described *Streptomyces*, more than half of the species possessed spiral sporophores, and the remaining *Rectiflexibiles* and *Retinaculiaperti* types of sporophores.

Spore surface morphology, formed by the overgrowth of outer layer of membrane, has been recognized as one of the major criteria in actinomycete taxonomical studies. Krassilnikov (1981a) found that this character is sufficiently stable to be used to differentiate actinomycete cultures. It is reported that the shape and type of outgrowths of spore membranes do not change in different nutritional media and under different conditions of culturing. In the present study, the observation of more than 75 per cent of the total actinomycete isolates with smooth spore surface and the remaining with spiny, warty and hairy surface is, in general, agreement with the earlier reports. Among the *Streptomyces* species described by ISP also, approximately 75 per cent of the species are smooth-walled and the remaining are of spiny, hairy and warty (Nonamura, 1974). In the present study, the only hairy- spored species, identified as *S. lucensis* (C8) was isolated from control soil. Among the ISP described species, most of the smooth spores were from *Rectiflexibiles* and

Spirales, whereas in this study, smooth spores are mostly from *Rectiflexibiles* and *Retinaculiaperti* sporophores.

5.5 UTILIZATION OF CARBON SOURCES

5.5.1 Sugars

Actinomycetes are capable of utilizing a fairly wide range of carbohydrates as the carbon source. The ability to utilize various sugars has been considered as a major distinguishing character for the identification of actinomycetes, especially in the case of *Streptomyces* (Shirling and Gottlieb, 1966). Krassilnikov (1981a) observed large variation with regard to the efficiency of sugar utilization among the members of 'ray fungi'. Waksman (1959) has reported that the utilization of sucrose, cellulose and inulin can be regarded as a differentiating characteristic of actinomycetes.

In the present study, sugar utilization is used as one of the important physiological characters for species differentiation. When the actinomycetes of the different sources studied are considered as four different groups, the isolates from the bittergourd rhizosphere is found to be the most efficient utilizers followed by the isolates from okra and sunflower rhizosphere and the isolates from the control soil. The significant difference in carbon utilization between the three rhizosphere isolates is of importance. The result indicates that the actinomycetes differ not only in their taxonomic group but also nutritionally depending upon the source plants. The least efficiency of actinomycetes from control soil in the utilization of simple sugars is an indication that such carbohydrates are not naturally found in the soil away from the plant roots. Kutzner (1981) suggested that the melanin production and the sugar utilization can be regarded as the two important characters of the actinomycetes for identification compared to other physiological tests.

The efficiency of carbon utilization by the actinomycetes in the various tests can be rated clearly, based on the diameter of the colony or the width of the halo zone. The results obtained in the study are graded as 'poor', 'moderate', 'efficient', and 'unutilized', in contrast to several earlier reports as 'utilized' and 'unutilized' for the carbon utilization (Rangaswami *et al.* 1967). The results show that simple sugars are more readily utilized by most of the actinomycetes and generally monosaccharides are more favoured than other types of sugars. Compared to the percentage and mean diameter of colony growth of different sugar utilizers among the rhizosphere isolates, there is a perceptible and significant ($p = 0.05$) reduction in the percentage and mean diameter of colony growth of different sugar utilizers

among control soil isolates. This indicates an increased physiological ability of rhizosphere isolates compared to the control soil isolates. In this study, arabinose, xylose, glucose, mannose and maltose are the most commonly and efficiently used sugars. Among the described-species of *Streptomyces*, fructose is the most highly utilized sugar followed by xylose and arabinose (Nonamura, 1974). Rangaswami *et al.* (1967) found that more than 80 per cent of the actinomycetes isolated from the soil samples from South India utilized glucose, galactose and maltose. Though galactose was not included in the present study, the other two sugars were utilized by 89-100 per cent of the isolates from all the sources. They also reported that carbon sources like xylose, fructose, lactose, sucrose, rhamnose, raffinose and mannitol were less readily utilized by most of the actinomycete isolates from South India. However, in the present study, only inositol, mannitol and rhamnose are found to be relatively less utilized by actinomycete isolates from rhizosphere and control soil than the other sugars.

5.5.2 Starch hydrolysis

Actinomycetes play an important role in the decomposition of starch. While the addition of simple sugars in soil results in rapid proliferation of bacteria, actinomycetes are benefited by the addition of starch, and fungi by cellulose (Alexander, 1978). In the present study also, it was found that majority of the isolates were capable of using starch. However, starch utilizers were found to be more in the rhizosphere than those in the control soil. The percentage of utilizers ranged from 67 (control soil) to 92 (sunflower rhizosphere soil). The increased percentage of starch hydrolisers among the rhizosphere soil thus indicates that the isolates from rhizosphere soils are physiologically more active. Abraham and Herr (1964) also found that actinomycetes isolated from the rhizosphere of corn and soybean had significantly greater starch-hydrolysing activity than did their respective non-rhizosphere group of isolates. Rangaswami *et al.* (1967), however, found that 95 per cent of actinomycetes isolated from the soils of South India hydrolyse starch.

5.5.3 Cellulose utilization

Based on the liquefaction of Carboxy methyl cellulose (CMC), the majority of the actinomycetes of all the four groups were found to be cellulose utilizers. The isolates from control soil were equally efficient in hydrolysing cellulose as the rhizosphere isolates. The actinomycetes are generally known to be active in the breakdown of cellulose, starch and other polysaccharides in soil (Cochrane, 1961). The products of the metabolism of cellulolytic organisms can also provide carbonaceous substrate for the rhizosphere microorganisms

(Alexander, 1978). The occurrence of relatively high percentage of cellulolytic actinomycetes in the rhizosphere soils studied is of great importance because, the sloughed-off tissues from the plant roots may also be a source of carbon for them. A substantial quantity of root tissues are generally sloughed off as the age of plant increases. Probably more cellulolytic actinomycetes may be attracted to the rhizosphere during this stage of plant growth. The activity of actinomycetes in cellulose break down in soils is already established (Waksman, 1959; Goodfellow and Williams, 1983). However, Rangaswami *et al.* (1967) reported that among the isolates from representative soils from South India, only 1-10 per cent of the actinomycetes utilized cellulose.

5.5.4 Nitrate reduction

The percentage of actinomycetes capable of reducing nitrate is found to be relatively less in the present study. The control soil showed the lowest percentage. Although, rhizosphere isolates showed more activity on NO_3 reduction, no significant difference was found between the four sources. This finding is also in agreement with the report of Abraham and Herr (1964) who did not find any difference in the ability to reduce nitrate between rhizosphere and non-rhizosphere actinomycetes of corn and soyabean. Alexander (1978) suggested that microorganisms concerned with denitrification is typically larger in the immediate vicinity of plant roots. Krassilnikov (1981a) considered that the great majority of actinomycetes can not assimilate nitrate as the source of nitrogen. Rangaswami *et al.* (1967) also found that only a few of the actinomycete isolates from South Indian soils reduced nitrate.

5.6 TEMPERATURE TOLERANCE

It is already known that mesophilic microbes constitute the bulk of soil micro-organisms. The observation of the abundance of mesophiles and the absence of psychrophiles among the actinomycetes isolated in the present study is thus as expected. Alexander (1978) reported that although the optimum temperature of mesophiles was in the vicinity of 25 to 35°C, they could grow from about 15 to 45°C. Similarly, the thermophiles could also show growth at temperatures below 40°C. Such observations were also made in this study. For example, *S. cacoi* (A5), considered as a mesophile, had also shown growth fairly well at temperature below 30°C and above 40°C, and *S. thermophilus* (A9) considered as a thermophile, had also shown good growth at 30°C. Porter (1971) also reported that many actinomycetes isolated at elevated temperature were thermotolerant rather than truely

thermophilic; also thermophilic actinomycetes were isolated on media employed to isolate mesophiles.

5.7 MAJOR TAXONOMIC GROUPS

The identification of *Streptomyces* species as the major genus among the actinomycete isolates from the rhizosphere of all the three plants and from the control soil was in agreement with the earlier reports. Among the soil actinomycetes *Streptomyces* species were identified as the most predominant genus by Waksman (1959), Alexander (1978) and Lechevalier and Lechevalier (1981).

In the present work, *Streptomyces* spp. constituted about 80 per cent of the rhizosphere actinomycete population, while all the actinomycetes from control soil were identified as *Streptomyces* spp. Rouatt *et al.* (1951), Agnihothrudu (1955), Rhem (1961) and, Mall (1977) have also similarly reported the occurrence of *Streptomyces* as the predominant genus among the rhizosphere actinomycetes. In the study on the actinomycetes from major soil types of South India, Rangaswamy *et al.* (1967) found that *Streptomyces* spp. accounted for 87.14 to 97.34 per cent, *Nocardia* spp. 1.33 to 7.14 per cent and *Micromonospora* 1.25 to 5 per cent. The richness of *Streptomyces* microflora of South Indian soil was also reported by Kuznetsov and Arjunarao (1972) who identified 95 per cent of the 383 actinomycete strains isolated from field soils of Botany Department of University of Madras as *Actinomyces* (*Streptomyces*). Among isolation of 5000 actinomycete cultures made by Lechevalier and Lechevalier (1967) from 16 soil samples elsewhere, 95 per cent were *Streptomyces* spp. while *Nocardia* spp. were about 2 per cent and *Micromonospora* spp. less than 1.5 per cent.

The prevalence of other genera in the rhizosphere soil may be due to the presence of factors in the rhizosphere promoting growth of other genera besides *Streptomyces*. Species of other genera may be requiring more specific nutrients for growth, which might be available in the rhizosphere soil as root exudates. While reviewing the biology of actinomycetes, Lechevalier and Lechevalier (1967) noted that non-streptomycetes possess more complex nutritional requirements and grow more slowly than streptomycetes. Alexander (1978) considered *Nocardia* spp. to be the second most abundant in rhizosphere soil. However, in the present study *Micromonospora* spp. were the second predominant species, followed by species of *Streptoverticillium, Thermomonospora, Nocardia, Actinobifida* and *Thermoactinomyces*.

In two out of three cases, the similarity between the species composition of isolates (identified *Streptomyces* spp.) from the rhizosphere soils were found to be lesser than the similarity between the species composition of isolates from rhizosphere soils and control soil (Table 20). The low SI between plant types indicates that there is a very close and unique relationship between the plant and the *Streptomyces* species in their respective rhizosphere and that the unfavoured species are eliminated during competition for nutrients. Species difference even among the genus of *Streptomyces* from the rhizosphere soil of different varieties of a plant has been reported. Working with rhizosphere actinomycetes of two varieties of coriander, Mall (1977) has observed different species composition of *Streptomyces* in the rhizosphere soils and in the control soil. It is known that plant root exudates have selective effect on microorganisms (Rovira, 1969; Hale *et al.*, 1978; Cook and Baker, 1983), which may be the cause of selective harbouring of microorganisms in the rhizosphere.

5.8 ANTAGONISTIC ACTIVITY OF ACTINOMYCETES

Since microbial antagonism has tremendous ecological significance in soil, especially in rhizosphere soil, an attempt has been made to study the antagonism of rhizosphere actinomycetes from the three crop plants and the control soil against some common root pathogens. The screening of actinomycetes for the antagonistic activity is carried out to find out the potential of the isolates to prevent/control infection by common root pathogens and also to find out the ecological distribution of antagonists in the rhizosphere.

In the present study, a marked difference is observed in the number of antagonistic actinomycetes among the rhizosphere and the soil populations. The presence of the least number of antagonistic actinomycetes in the rhizosphere soil of bittergourd indicates that the rhizosphere soil has specific influence on the development of such antagonists. The difference in the number of antagonists among the rhizosphere soils of three different crop plants is in agreement with the findings of Mohamed (1985) who reported the occurrence of more antagonistic actinomycetes in the rhizosphere of wheat than in the rhizosphere of soybean. Kutzner (1981) considered that habitats rich in streptomycetes harbour more antibiotic producers. Thus rhizosphere soil is such an ecological niche and naturally more number of antagonistic actinomycetes could be expected to occur in rhizosphere soil. Baker and Cook (1974) stated that antibiotic potential resides in every soil microorganism. Plant roots can specifically favour and attract microorganisms of various types including antibiotic

producers present in the particular soil for aggregation in rhizosphere. The existance of a direct relationship between disease resistance of plants and the occurrence of antagonistic microorganisms in the rhizosphere is also reported (Bird ., 1982).

Occurrence of actinomycetes antagonistic to plant pathogenic fungi, in the rhizosphere of *Solanum khasianum* was reported by Oblisami and Jayasheela (1975). Rangaswami and Vasantharajan (1962c) reported more number of antagonistic actinomycetes in the rhizosphere of *Citrus* than in soil. They suggested that perhaps the actinomycetes with antagonistic properties are attracted to the rhizosphere because of the richness of the nutrients available in that region. Similar results have also been obtained by Agnihothrudu (1955) while studying the actinomycete population in the rhizosphere of pigeon pea. Weller (1988) is of the opinion that antagonistic species have an edge over other species as far as their competitive ability to survive and multiply in the rhizosphere soil is concerned, or in other words, they possess sufficient rhizosphere competence traits.

The percentage of antagonistic actinomycetes obtained from the control soil in the present study (38.9%) is much higher than the highest percentage of antagonists reported for a representative laterite soil of South India (Rangaswami and Oblisami, 1967). The present result is in agreement with the results obtained by Reddi and Rao (1971) and Kuznetsov and Arjunarao (1972) who reported that 40 per cent and 57.7 per cent of the actinomycete populations isolated respectively were with antagonistic properties. Nandi (1968) also reported that antibiotics producing actinomycetes were extremely rich in garden soils. Surveying the antagonistic actinomycetes from the soils of Agra region, Sharma and Sinha (1980) also reported that out of 118 strains of actinomycetes isolated, more than 50 per cent were antagonistic against fungal pathogens.

The difference in the antagonistic effect observed by the cross-streak and cylinder-plate methods showed that the degree of antagonistic effect varied based on the methods used. The inhibitory effect of antagonistic actinomycetes detected by cross-streak method may be due to the secretion of antibiotic substances and probably by the nutritional competition between the two microbes in culture (Johnson and Curl, 1972). Hsu and Lockwood (1969) also have suggested that the appearance of the zone of inhibition in cross-streak assay could also be due to nutrient deprivation. The failure of some of the antagonists to show inhibitory effect by cylinder-plate method may be due to the very little or no secretion of the antibiotic substance into the culture medium. In a study, Rothrock and

Gottlieb (1981) detected antibiotic activity of culture filtrates of only five *Streptomyces* spp. among the 10 streptomycetes antagonistic to the plant pathogens viz. *R. solani* and *Phytophthora megasperma* var. *sojae*. Similar results had been obtained by other workers also (Vittal Rao and Rao, 1966). Comparing the two assay methods employed in this study, the cross-streak method appeared to be a better method than the cylinder-plate method for screening of antibiotic producing organisms. In achieving biological control of root pathogens, it is suggested that all types of antagonists are important because a biologically suppressive soil probably cannot be explained in terms of a single antagonist (Baker and Cook, 1974).

There are several reports of actinomycetes identified as potential agents for biocontrol of plant pathogens (Rangaswami and Vasantharajan, 1962c; Broadbent *et al.*, 1971; Balagopalan and Oblisami, 1974; Merriman *et al.* 1974; Singh and Reddi, 1979; Chattopadhyay and Nandi, 1982; Rothrock and Gottlieb, 1984; Filonow and Lockwood, 1985). The suitability of antagonistic microorganisms that can thrive well in rhizosphere soil for use as biocontrol agents has been stressed by Elad (1986) and Weller (1988) recently, because rhizosphere provides the front-line defence for roots against attack by pathogens.

The ability of several species of actinomycetes from the rhizosphere soil of okra and sunflower to inhibit the growth of root pathogenic fungi tested *in vitro* indicates the potential of rhizosphere actinomycetes to prevent root diseases. Similar results have been obtained by Mall (1973) who reported the occurrence of streptomycetes antagonistic to soil-borne plant pathogens from the rhizosphere of different plants of resistant varieties. Mohammed (1985) also obtained *Streptomyces* spp. antagonistic to mycelial growth of *S. rolfsii, R. solani* and *Alternaria solani* from the rhizosphere of wheat and soybean. However, in the present study, suppression of *S. rolfsii* is confined to the inhibition of germination of sclerotia only. Complete loss of germinability of sclerotia of *S. rolfsii* was also reported by Henis and Chet (1968). Hadar and Gorodecki (1991) reported suppression of germination of sclerotia by application of compost. This is probably caused by the antagonistic microbial population present in the compost.

Besides the species of *Streptomyces*, certain species of *Thermomonospora, Micromonospora, Nocardia* and *Streptoverticillium* were also antagonistic. Kuznetsov and Arjunarao (1972) have also obtained antifungal actinomycetes belonging to species of *Actinosporangium, Amorphosporangium* and *Chainia* in addition to *Actinomyces*

(*Streptomyces*), from the experimental farm at Madurovayal under the Botany Department of University of Madras. Similarly, species of *Streptomyces*, *Actinoplanes*, *Amorphosporangium* and *Micromonospora* were also reported to be antagonistic to fungal root pathogens and those were shown to have potential as biocontrol agents by some other workers (Broadbent *et al.* 1971; Merriman *et al.*, 1974; Rothrock and Gottlieb, 1984; Filonow and Lockwood, 1985).

R. solani has been considered as a serious soil-borne pathogen with high competitive saprophytic ability (Garrett, 1970). It has been reported to cause damping-off (Sohi and Sokhi, 1974) and root rot (Ranga Rao and Mukerji, 1972), in okra, damping-off in sunflower (Subramanian *et al.*, 1975; Anilkumar and Sastry, 1978) and root rot of bittergourd (Jhooty and Grover, 1971) in India. In the present study, the results of *in vivo* tests of antagonism indicated that in a *R. solani* infested soil, severity of the pathogen attack on the plant roots could be considerably reduced by seed treatment with antagonistic *Streptomyces*.

Treatment of seeds with the antagonists may result in the establishment of the antagonist in the spermosphere utilizing seed exudates and later in the rhizosphere utilizing root exudates. Such development of antagonists on seeds or in the rhizosphere of seedlings can considerably reduce or prevent host infection. Although, complete control of the pathogen was not shown by any of the three antagonists, namely *S. vastus, S. endus* and *S. luteogriseus, the root infection by R. solani* was considerably reduced by the antagonists. This shows that the antagonistic organism probably serves as a protective mantle over the host roots against the fungal infection so as to achieve a partial control of *Rhizoctonia* infection. The establishment and domination of antagonists in the infection court is an essential prerequisite for achieving biological control of root pathogens (Hornby, 1990). The relatively low survival of seedlings of the three test plants when the pathogen- treated seeds were sown in antagonist-treated soil, indicates that the establishment of pathogen on seed surface and in the rhizosphere prior to the establishment of antagonists is the reason for the antagonist to become ineffective in reducing the fungal infection. The pathogen might have gained an advantage over the actinomycete to establish and occupy the spermosphere and rhizosphere utilizing the seed and root exudates and thereby infecting the plant.

In a similar study by Rothrock and Gottlieb (1981), root rot of pea was found to be reduced when sterile soil was simultaneously infested with an antagonist *S. cellulose* and the pathogen. They also got complete control of disease when *S. hygroscopicus*, an

antagonist, was added seven days prior to infesting the soil with *R. solani*. *S. herbaricolor* was also found to be antagonistic by them; but the same species isolated from the rhizosphere of Okra (A10) and control soil (C1), in the present study, was not found to be antagonistic.

Suppression of damping-off on pepper caused by *R. solani* through soil treatment of antagonistic *S. nobilis* and *S. ochraceiscleroticus* has been reported recently (Turhan and Turhan, 1989). Antagonistic isolates of several other species of *Streptomyces* are also capable of suppressing diseases caused by *R. solani* (Ghaffer, 1988); Roy, 1989).

Root and collar rot of okra caused by *R. bataticola* (Goel and Mehrotra, 1974) and wilt disease of sunflower caused by *F. oxysporum* (Ghodojkar *et al.*, 1976) are reported from India. The results obtained in the present experiments using seedlings of okra and sunflower further confirmed the efficiency of *S. vastus* in reducing root infections caused by *R. bataticola* and *F. oxysporum*. The antagonistic effect of these three species of *Streptomycetes* against the above pathogens is further evidenced by the high survival of the test seedlings when transplanted in soil treated first with the pathogen, followed by the antagonist, compared to the survival of the seedlings in pathogen-alone-treated soil.

5.9 ROOT EXUDATES

Root exudate is already known to be an important component of rhizosphere as the exudate serves as a source of energy for vast number of microorganisms in the rhizosphere. Hence, an attempt was made in this study to find out the amino acids and sugars present in the root exudates of the three crop plants grown in axenic condition for three weeks. None of the amino acids and sugars was found to be commonly present in the exudates of all the three plants and the number of identified amino acids and sugars varied in each type of exudate. Fructose, the only sugar found in the exudate of okra was also found in the sunflower root exudate. It is known that the amount, range and balance of compounds in root exudates differ for different plant species (Rovira, 1969). Vancura and Hovadik (1965) studied the composition of root exudates of seven species of cultivated plants belonging to four families and found that the qualitative differences in the composition of root exudates increased with phylogenetic differences between plants. The difference in the composition of amino acids and sugars detected in the root exudates of okra, bittergourd and sunflower belonging to three different families viz. Malvaceae, Cucurbitaceae and Compositae respectively, is also in agreement with these earlier studies.

Frenzel (1957) reported 11 amino acids from the root exudates of sunflower. However, only two amino acids viz., serine and phenylalanine reported by Frenzel were found to occur among the five amino acids identified in the sunflower exudate in the present study. Differences in the composition of exudates are expected when the experiments are carried out in unidentical environments (Rovira, 1965b, 1969; Vancura, 1967) and also the composition varies depending upon age and plant variety (Balasubramanian and Rangaswami, 1969; Smith, 1970; Kraft, 1974). No other reports are available on studies made on the composition of sugars in the exudate of sunflower and the composition of the exudates of okra and bittergourd. However, all the sugars detected during the present study are also reported to be present in the root exudates from other crop plants (Rovira, 1965b, 1969; Hale *et al.*, 1978).

Plant root exudates, particularly the sugars and amino acids, are reported to have selective effect on microorganism in the soil (Rovira, 1969; Hale *et al.* 1978; Baker and Cook, 1983; Khalis *et al.*, 1990). In the present study, eventhough a definite correlation between the presence of a particular sugar in the root exudate of a plant and the efficiency in utilizing the sugar by the actinomycetes isolated from the rhizosphere of the respective plant is not observed, it appears that the actinomycetes are generally favoured by the presence of those sugars which are detected in the exudate. Except rhamnose, such sugars, are found to be comparatively the better utilized carbon compounds by actinomycetes from the respective rhizosphere soil. Thus, fructose, the only compound identified in the exudate of okra was found to be a good carbon source for more than 93 per cent of the rhizosphere isolates with a mean growth diameter of 12.1 mm. The phenomenon of positive correlation between a sugar present in root exudate and the utilization of the particular sugar by actinomycetes isolated from the same rhizosphere is of utmost importance because of its possible application in the selective stimulation of a particular microorganism or a group of microorganisms.